

Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonization of the Baltic Sea area

J. NILSSON,* R. GROSS,† T. ASPLUND,‡ O. DOVE,‡ H. JANSSON,§ J. KELLONIEMI,‡ K. KOHLMANN,¶ A. LÖYTYNOJA,‡ E. E. NIELSEN,** T. PAAVER,† C. R. PRIMMER,†† S. TITOV,‡‡ A. VASEMÄGI,† A. VESELOV,§§ T. ÖST§ and J. LUMME‡

*Department of Aquaculture, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden, †Department of Fish Farming, Institute of Animal Husbandry, Estonian Agricultural University, EE-51014 Tartu, Estonia, ‡Department of Biology, University of Oulu, POB 3000, FIN-90401 Oulu, Finland, §Salmon Research Institute, Forskarstigen, S-814 94 Älvsjö, Sweden, ¶Department of Fish Culture and Fish Pathology, Institute of Freshwater Ecology and Inland Fisheries, D-12587 Berlin, Germany, **Danish Institute for Fisheries Research, Department of Inland Fisheries, DK-8600 Silkeborg, Denmark, ††Department of Ecology and Systematics, PO Box 17, FIN-00014 Helsinki University, Finland, ‡‡GosNIORKH, Makarova Embankment 26, 199053, St Petersburg, Russia, §§Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Russia

Abstract

Sixty-four samples from 46 salmon populations totalling 2369 specimens were used for polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis of the mitochondrial ND1 region. The final analyses included 3095 specimens from 60 populations in Northern Europe. A subsample was analysed by RFLP of ND3/4/5/6. Representative RFLP haplotypes from different parts of the distribution area were sequenced and the phylogeny of European haplotypes and their relations to the North American lineage was described. The four common European haplotypes derive from the ancestral ND1-BBBA (rooting the European clade to the North American) by one-step substitutions: AAAA < AABA < BBBA > BBBB. The Swedish west-coast populations differ from the geographically close southern Baltic, indicating absence of inward and limited outward gene flow through the Danish straits during the last 8000 years. Within the Baltic Sea, only three ND1 haplotypes were detected and there was no variation for ND3/4/5/6. In the whole southern Baltic and in lakes Vänern, Ladoga and Onega the haplotype AABA dominated. Proposed postglacial colonization routes to the Baltic Sea are discussed in relation to the haplotype distribution pattern.

Keywords: Baltic, colonization, mtDNA, populations, RFLP, salmon, sequencing

Received 30 April 2000; revision received 22 August 2000; accepted 22 August 2000

Introduction

Atlantic salmon (*Salmo salar* L.) is naturally distributed around the North Atlantic Ocean. In Europe salmon spawn in rivers from northern Portugal to the eastern coast of the Barents sea in Russia including Iceland and rivers flowing into the Baltic Sea. In North America, salmon are found from Virginia to Labrador. Ståhl (1987)

was the first to reveal the global genetic structure of Atlantic salmon populations by enzyme electrophoresis. In that work, the division between the North American and European populations was the deepest divergence, but the Baltic and East Atlantic clades were also strongly supported. Later, the divergence of eastern and western Atlantic populations was confirmed by other studies on allozymes (Bourke *et al.* 1997), nuclear rRNA genes (Cutler *et al.* 1991), minisatellites (Taggart *et al.* 1995), microsatellites (McConnell *et al.* 1995) and mitochondrial DNA (mtDNA) markers (Bermingham *et al.* 1991; Birt *et al.* 1991). However, the divergence between Atlantic and Baltic salmon has been less studied.

Correspondence: J. Nilsson, SLU-Department of Aquaculture, S-901 87 Umeå, Sweden. Fax: 46–90123729; E-mail: jan.nilsson@vabr.slu.se

The biogeography of the Baltic Sea region, colonized stepwise by immigrating species during the retreat of the continental ice, which ended less than 10 000 years ago, has been one of the main themes of biological and geological investigations in Nordic countries (Eronen 1983). The geologists have revealed in detail the different freshwater and salty periods in the history of the Baltic Sea, the largest brackish water reservoir in the world (Saarnisto 1970; Hyvärinen & Eronen 1979; Sohlenius 1996). The subfossil molluscs giving their names to different phases of development (*Yoldia*, *Ancylus*, *Litorina*) also indicate the large changes that have occurred in the biotic environment of this sea. However, before these stages there existed large freshwater reservoirs – ice-dammed lakes around the ice margin. During the retreat of the glacier they grew larger and the largest among them was the South Baltic Ice Lake, where predecessors of large eastern European rivers (e.g. Visla, Nemunas and Daugava) drained. The Baltic Ice Lake was formed approximately 12 000 years BP when the ice lakes of eastern Europe merged into one large water body. Before the Baltic Ice Lake became connected to the Atlantic it encompassed what is now the southern and eastern Baltic and existed as a freshwater lake for about 2000 years. The first marine connection between the Atlantic and the Baltic Ice Lake was through the Närke Strait over southern Sweden, which opened around 10 300 years BP (Donner 1995) and remained for less than 1000 years, covering, for example, the present Lake Vänern. The geological history of lakes Saimaa, Onega and Ladoga is also well known.

The aquatic fauna of northern Europe contains several cold-adapted species, known as glacial relicts, as pointed out already by Lovén (1862). This interesting faunal component is best understood among crustaceans (e.g. Väinölä 1995) but contains several fish species too. Since the predecessors and first phases of the present North European water system were proglacial freshwater lakes, it was suggested that glacial relicts were present in those water bodies during the last glaciation (Segerstråhle 1982). The salmon could have belonged to the glacial relict fauna as well.

Based on allozyme studies, Kazakov & Titov (1991) first suggested that salmon from proglacial lakes could have contributed to colonization of North Europe and later Koljonen *et al.* (1999) proposed that salmon in the southern Baltic originated from such lakes. Whether salmon colonized ice-free parts of the Baltic Sea from glacial lakes during the early freshwater phases or whether the colonization occurred later by invasion from the Atlantic is the topic of this study. We have used mitochondrial variation to investigate the origin of Baltic populations of salmon. Polymorphism of mtDNA has been shown to be a powerful tool for the assessment of phylogeographic patterns of freshwater and anadromous fish (Bernatchez & Wilson

1998 and references therein). One of the main advantages of using mitochondrial markers is the lack of recombination, which, together with maternal inheritance, enables identification of maternal lineages and inference of distances between them. Previous studies have shown that Atlantic (Nielsen *et al.* 1996) and Baltic salmon (Nilsson 1997) are variable for the ND1 mitochondrial gene, suggesting this segment as a useful marker. Verspoor *et al.* (1999) described nine haplotypes by restriction fragment length polymorphism (RFLP) analysis of ND1 indicating this region as the most variable hitherto studied in salmon. In this study we have combined RFLP analysis of the mitochondrial ND1 gene with sequencing in order to reveal population structuring and phylogenetic relationships between the lineages. Nielsen *et al.* (1998) showed that in salmon from the North Sea and Atlantic, variation is also present at the mitochondrial ND3/4 and ND5/6 segments. We have analysed these segments from a subsample of the Baltic Sea fish.

Materials and methods

Study area and samples

Sixty-four samples from 46 salmon populations, totalling 2369 specimens, were used for polymerase chain reaction (PCR)–RFLP analysis of the mitochondrial ND1 region. In addition, earlier published RFLP data on 726 specimens from 17 populations (Palva *et al.* 1989; Nielsen *et al.* 1996; Verspoor *et al.* 1999) were included in the analysis. The geographical location of the sampled populations is given in Fig. 1. In Table 1 sample size and the wild or hatchery status are given for each population. A wild origin is used here only to indicate that the fish were from field sampling in rivers.

For analysis of the mitochondrial ND3/4 ($n = 83$) and ND5/6 ($n = 66$) regions we used fish from the Rivers Vindelälven, Skellefteälven and Indalsälven in the Gulf of Bothnia, the Rivers Pielinen and Lieksa from Lake Saimaa, River Svir in Ladoga, the Rivers Shuja and Lizhma in Lake Onega, the Rivers Rolfsån, Sävån and Örekilsälven from the Swedish west-coast and River Spaddagh/Moy in Ireland.

For sequencing, selected specimens from Baltic populations were used: Torneälven (two individuals), Luleälven (two individuals), Skellefteälven (two individuals), Vindelälven (two individuals), Ångermanälven (two individuals), Indalsälven (two individuals), Simojoki (two individuals), Iijoki (two individuals), Neva (two individuals) and Daugava (two individuals). Eastern Atlantic populations were represented by specimens from Swedish Lagan (one individual), Ätran (two individuals) and Rönne å (one individual), Danish Skjern (one individual), Irish Burrishoole (one individual) and Corrib (one individual),

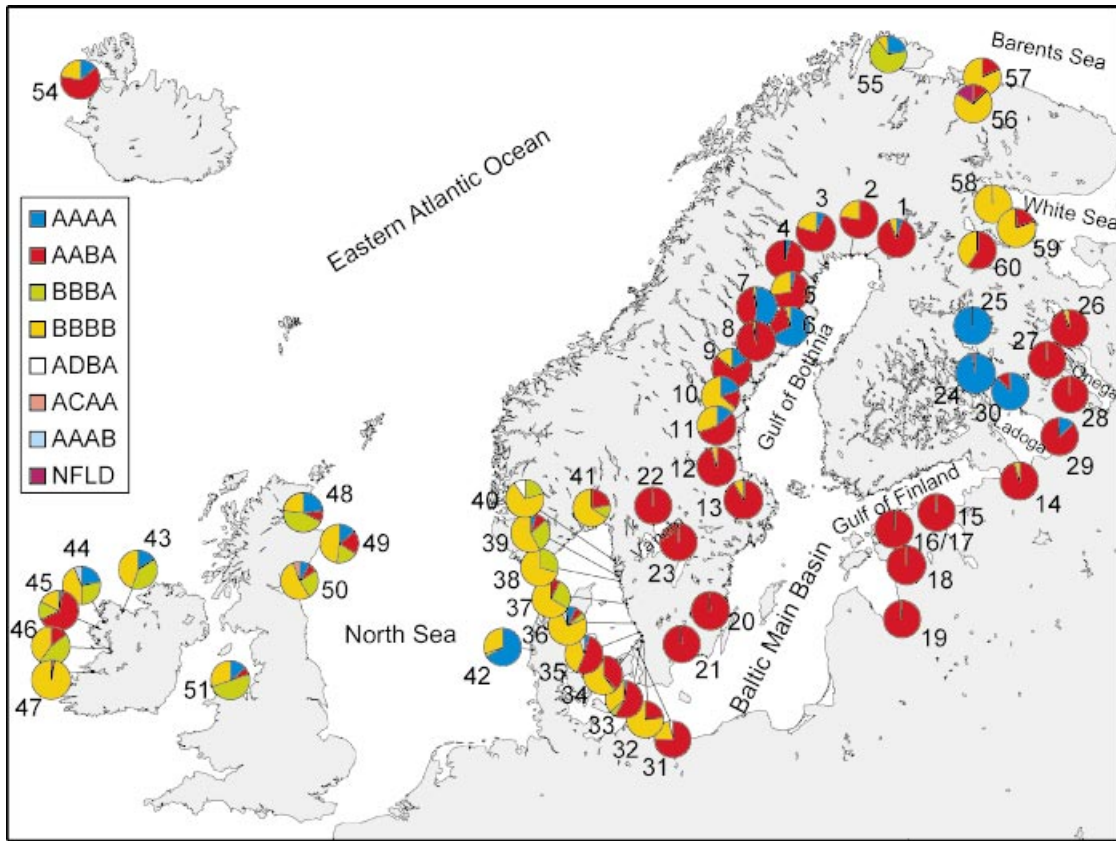


Fig. 1 Map of sampling locations with pie diagrams showing the distribution of haplotype frequencies among studied Atlantic salmon populations. Sample numbers follow those given in Table 1.

Scottish Conon (one individual), Norwegian Tana (one individual) and Icelandic Laxá i Adaldal (one individual) and Ulfarsá (one individual). Additionally, two specimens of landlocked salmon from Lake Saimaa, Finland and six specimens from four populations in Newfoundland, Canada were sequenced.

RFLP analysis of mtDNA

DNA was prepared according to Laird *et al.* (1991) or from chelex extraction (Walsh *et al.* 1991). PCR primers used to amplify the 2 kilobase (kb) fragment containing the ND1 and 16S rRNA genes were: 5'-CCCGCTGTTTACCAAAAAC-3' (forward) and 5'-GGTATGAGCCCGAAAGC-3' (reverse). These primers are modifications of those given by Hall & Nawrocki (1995) for brown trout (*Salmo trutta*) and correspond to the nucleotide positions 2996–3015 and 4986–5002 of the complete sequence of *Salmo salar* mitochondrial haplome (Hurst *et al.* 1998; GenBank accession number U12143). PCR amplifications were performed with 0.5–5 ng total DNA in a 25-µL reaction volume including 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.4 units of *Taq* DNA polymerase, 100 µM of each dNTP, 2.3 mM

MgCl₂ and 0.2 µM of each primer. The PCR profile consisted of a denaturing step of 5 min at 95 °C followed by 30 cycles consisting of 1 min at 95 °C, 1 min at 55 °C and 1 min at 72 °C. The last elongation step was lengthened to 10 min. In a preliminary survey that included 10 salmon from each of the Baltic rivers Skellefteälven, Vindelälven, Ångermanälven and Indalsälven, the amplified fragments were digested with the restriction enzymes *AluI*, *HaeIII*, *HindIII*, *HinfI*, *TaqI*, *AvaII*, *RsaI*, *HpaI*, *HhaI*, *DraI* and *HaeII*. Variation was found only with *HaeIII*, *HinfI*, *RsaI* and *AvaII*. Routine screenings were made using 1.2% agarose gels stained with ethidium bromide. The four observed RFLP haplotypes were found to be the same as those described by Nielsen *et al.* (1996), as could be seen from fragment sizes and was later verified by sequencing, and we have therefore adopted their haplotype designation. In our samples, one additional haplotype (ADBA) was found. In Verspoor *et al.* (1999) a different haplotype designation was used: AAAA = III, AABA = IV, ADBA = VII, BBBA = II and BBBB = I.

RFLP analysis of mitochondrial ND3/4 by *AccI*, *HinfI* and *RsaI*, and ND5/6 by *TaqI*, *AluI* and *MwoI* was performed as described by Nielsen *et al.* (1998), covering

Table 1 Source, origin, year-class and number of samples used for RFLP analysis

No.	Population	Country	Origin	Year-class	<i>n</i>
Baltic Sea/Gulf of Bothnia					
1	Torneälven/Tornio	Sweden/Finland	wild	1994	85
2	Kalixälven	Sweden	wild	1989	35
3	Luleälven	Sweden	hatchery	1995	58
			hatchery	1997	38
4	Byskeälven	Sweden	wild	1999	53
5	Skellefteälven	Sweden	hatchery	1995	50
			hatchery	1996	47
6	Vindelälven	Sweden	wild	1995	108
7	Umeälven	Sweden	hatchery	1992	41
			hatchery	1995	47
8	Lögdeälven	Sweden	wild	1994/95	43
9	Ångermanälven	Sweden	hatchery	1995	63
10	Indalsälven	Sweden	hatchery	1995	48
			hatchery	1997	37
11	Ljungan	Sweden	hatchery + wild	1997	24
			hatchery + wild	1998	25
12	Ljusnan	Sweden	hatchery	1997/98	51
13	Dalälven	Sweden	hatchery	1997	45
			hatchery + wild	1998	55
Baltic Sea/Southern					
14	Neva	Russia	hatchery	1995	45
15	Kunda	Estonia	wild	1996	15
			wild	1997	33
16	Keila	Estonia	wild	1996	25
			wild	1997	34
17	Vasalemma	Estonia	wild	1996	16
			wild	1997	11
18	Pärnu	Estonia	wild	1997	23
19	Daugava	Latvia	hatchery + wild	1998	53
20	Emån	Sweden	wild	1988/99	59
21	Mörrumsån	Sweden	wild	1996	51
Landlocked					
22	L. Vänern/Klarälven	Sweden	hatchery	1997	44
23	L. Vänern/Gullspångsälven	Sweden	wild	1986/87	22
			wild	1989	24
			wild	1996	10
			hatchery	1991	18
			hatchery	1997	38
24	L. Saimaa/Pielisjoki	Finland	hatchery	?†	23
			hatchery	1996	20
			wild	1997	16
25	L. Saimaa/Lieksa	Finland	wild	1997	22
26	L. Onega/Lizhma	Russia	wild	1997	20
27	L. Onega/Shuja	Russia	wild	1997	17
28	L. Onega/Kumsa	Russia	wild	1997	18
29	L. Ladoga/Svir	Russia	hatchery	1996	29
30	L. Ladoga/Sysky	Russia	wild	1999	42
Atlantic Ocean/Scandinavia					
31	Rönne å	Sweden	wild	1992	33
32	Stensån	Sweden	wild	1994	25
33	Lagan	Sweden	hatchery	1995	49
			hatchery	1994	35
			hatchery	1994*	37
34	Genevadsån	Sweden	wild	1990	36
			wild	1994	24

Table 1 Continued

No.	Population	Country	Origin	Year-class	<i>n</i>
35	Fylleån	Sweden	wild	1994	25
36	Ätran	Sweden	hatchery	1992	46
			hatchery	1994*	27
37	Rolfsån	Sweden	wild	1994	27
38	Säveån	Sweden	wild	1990	9
			wild	1992	39
39	Grönån	Sweden	wild	1997	43
40	Örekilsälven	Sweden	wild	1990	36
			wild	1992	37
41	Numedalslågen	Norway	hatchery	1993*	26
42	Skjern	Denmark	wild	1989*	33
Atlantic Ocean/British Isles					
43	Moy/Spaddagh	Ireland	wild	1995‡	50
44	Burrishoole	Ireland	hatchery	1994*	32
45	Delphi	Ireland	hatchery	1995	34
46	Corrib	Ireland	hatchery	1994*	27
47	Shannon	Ireland	hatchery	1995	40
48	Conon	Scotland	hatchery	1994*	34
49	North Esk/Cruick Water	Scotland	wild	1995‡	50
50	Dee	Scotland	hatchery	1993*	111
51	Dee	Wales	wild	1995‡	50
Atlantic Ocean					
52	Adour/Nive	France	wild	1995‡	32
53	Narcea	Spain	wild	1995‡	44
54	Laxa i Dolum	Iceland	wild	1995‡	50
Barents Sea Basin					
55	Tana	Norway	wild	1995‡	50
56	Kola/Pecha	Russia	wild	1995‡	50
57	Tuloma	Russia	wild	adults	21
White Sea Basin					
58	Nilma	Russia	wild	1999	43
59	Pulonga	Russia	wild	1999	50
60	L. Kuito/Pista (landlocked)	Russia	wild	1999	59

*Nielsen *et al.* 1996; †Palva *et al.* 1989; ‡Verspoor *et al.* 1999.

4770 nucleotides (sites from 10594 to 15364 of the standard sequence of Hurst *et al.* (1998), GenBank no. U12143). We named the most common haplotype, i.e. that shared by all ND1 types, as -AAA-AAA, which corresponds to the designation of Nielsen *et al.* (1998) in their Table 2 and Fig. 1, but mislabelled in Table 1.

Sequencing of a mtDNA segment around ND1

DNA isolation and PCR followed standard procedures, PCR primers corresponded to nucleotide positions 3637–3661 (forward) and 4916–4940 (reverse) according to Hurst *et al.* (1998; GenBank no. U12143). Six internal primers were used for sequencing by ABI Prism™ 377 automatic sequencer, first by Dye Terminator Cycle Sequencing Ready Reaction kit and later by dRhodamine Terminator

Cycle Sequencing Ready Reaction kit, following the manufacturer's manuals. Alignment of the accumulating sequence fragments was conducted by aid of PILEUP on the published sequence of *Oncorhynchus mykiss* (GenBank no. L29771; Zardoya *et al.* 1995) utilizing the computer facilities of the Center of Scientific Computing, Espoo, Finland. We obtained a complete sequence of 1227 bp for a total of 38 fish spanning the region from 16S rRNA over tRNA-leu, ND1, tRNA-ile to tRNA-gln. The sequences are deposited in GenBank nos. AF115412 to AF115420.

Data analysis

Genetic distances between haplotypes and their phylogenetic relationships were inferred from the nucleotide sequences. The number of nucleotide substitutions between

haplotypes was calculated according to Jukes & Cantor (1969) and the number of net nucleotide substitutions per site between all pairs of populations (d_A) was calculated according to Nei (1987) using the DNASP VERSION 3.0 program (Rozas & Rozas 1999). The matrix of d_A values was used as input into NEIGHBOUR of the PHYLIP version 3.572c program package (Felsenstein 1994) for Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering of the populations. For each individual population, level of haplotype variation was estimated by calculating the unbiased haplotype diversity (h) and the nucleotide diversity (δ) (Nei 1987). The phylogeny of haplotypes was reconstructed manually.

Results

Population analysis by restriction site variation

RFLP analysis of the ND1 gene revealed a total of five composite haplotypes: AAAA, AABA, ADBA (new), BBBB and BBBA. Exact sizes of the restriction fragments inferred from the sequenced haplotypes are given in Table 2. In the present material, the rare Atlantic haplotypes ACAA and AAAB described by Nielsen *et al.* (1996) were not observed.

Analysis of the ND3/4 ($n = 70$) and ND5/6 ($n = 54$) gene regions did not reveal any variation among salmon from the Baltic Sea. All Baltic, Saimaa and Onega salmon had the composite ND3/4 and ND5/6 haplotype AAA-AAA. Among our samples from the Atlantic, AAA-AAA was

also the dominating haplotype but among the fish with ND1-BBBA we detected two individuals with a novel BBBA-AAA-ACA fragment pattern, not described by Nielsen *et al.* (1998). The exact fragment sizes (based on the complete nucleotide sequence of *Salmo salar* mtDNA) of ND3/4 and ND5/6 haplotypes found in this study are given in Table 2.

The ND1 haplotype frequencies are presented in Table 3 together with estimates of haplotype and nucleotide diversity of the populations and in Fig. 1. In the Baltic Sea, three haplotypes (AAAA, AABA, BBBB) were found but their distribution was not continuous. Gulf of Bothnia populations were characterized by the presence of all three haplotypes, AABA dominating in 10 out of 13 populations. Populations in the southern Baltic Sea (including Gulf of Finland, Gulf of Riga and the Baltic Main Basin) were fixed or almost fixed for the AABA haplotype. The AAAA haplotype was absent in the southern Baltic and BBBB was very rare. Along the Swedish west-coast, AABA was found at high frequency in five rivers closest to the strait between Denmark and Sweden. Further north, the AABA haplotype almost disappeared, and was replaced by the BBBB haplotype, but most clearly vicariated by BBBA.

All five ND1 main haplotypes were found in the Atlantic populations. ADBA was rare and restricted to the Swedish west-coast while the other four were more widely distributed, BBBB having the highest frequency. The strictly Atlantic BBBA haplotype was observed in most Atlantic populations and was common in salmon from

Table 2 Exact sizes (base pairs) of restriction fragments for the PCR-amplified Atlantic salmon ND1 (2009 bp), ND3/4 (2332 bp) and ND5/6 (2471 bp) gene regions, inferred from the sequenced haplotypes and the complete nucleotide sequence of *Salmo salar* mtDNA (Hurst *et al.* 1998, GenBank no. U12143). Variant restriction patterns are designated by capital letters

ND1				ND3/4						ND5/6					
<i>Ava</i> II		<i>Hae</i> III		<i>Hin</i> fl		<i>Rsa</i> I		<i>Ac</i> I	<i>Hin</i> fl	<i>Rsa</i> I	<i>Taq</i> I	<i>Alu</i> I			<i>Mwo</i> I
A	B	A	B	D	A	B	A	B	A	A	A	A	A	C	A
	866			632	982	982		510	1023	640	927	566		1021	960
530		604	604		529		411	411	299	582	917	536	567		571
406	406	560				436	331		269	488	234	402	538	538	309
336			499		395	395	321	321	198	284	146	296	454		259
319	319	193	193	193	103	103	291	291	198	151	108	285	248	248	169
215	215	168	168	168		93	254	254	138	127		223	197	197	122
152	152	151	151	151			222	222	131	42		163	116	116	57
51	51	122	122	122			179		72	18			109	109	12
		84	84	84					4				72	72	9
		72	72										57	57	3
			61										53	53	
													28	28	
													15	15	
													12	12	
													5	5	

Table 3 ND1 gene haplotype frequencies and estimates of haplotypic (h) and nucleotide (π) diversity among Atlantic salmon populations (pooled year-classes)

Population	n	AAAA	AABA	BBBA	BBBB	ADBA	ACAA	AAAB	h	π
<i>Baltic Sea</i>										
Gulf of Bothnia										
Torneälven/Tornio-94	85	0.05	0.89	0.00	0.06	0.00	0.00	0.00	0.197	0.00026
Kalixälven-89	35	0.00	0.77	0.00	0.23	0.00	0.00	0.00	0.363	0.00050
Luleälven-95/97	96	0.07	0.72	0.00	0.21	0.00	0.00	0.00	0.439	0.00066
Byskeälven-99	53	0.04	0.96	0.00	0.00	0.00	0.00	0.00	0.073	0.00006
Skellefteälven-95/96	97	0.04	0.68	0.00	0.28	0.00	0.00	0.00	0.463	0.00073
Vindelälven-95	108	0.68	0.29	0.00	0.04	0.00	0.00	0.00	0.464	0.00048
Umeälven-92/95	88	0.51	0.47	0.00	0.02	0.00	0.00	0.00	0.527	0.00049
Lögdeälven-94/95	43	0.00	0.98	0.00	0.02	0.00	0.00	0.00	0.047	0.00008
Ångermanälven-95	63	0.17	0.68	0.00	0.14	0.00	0.00	0.00	0.491	0.00065
Indalsälven-95/97	85	0.20	0.16	0.00	0.64	0.00	0.00	0.00	0.536	0.00103
Ljungan-97/98	49	0.14	0.55	0.00	0.31	0.00	0.00	0.00	0.594	0.00091
Ljusnan-97/98	51	0.00	0.96	0.00	0.04	0.00	0.00	0.00	0.077	0.00013
Dalälven-97/98	100	0.01	0.91	0.00	0.08	0.00	0.00	0.00	0.167	0.00026
Average, G. Bothnia		0.15	0.69	0.00	0.16	0.00	0.00	0.00	0.341	0.00048
Southern Baltic										
Neva-95	45	0.00	0.96	0.00	0.04	0.00	0.00	0.00	0.087	0.00014
Keila-96/97	59	0.00	0.98	0.00	0.02	0.00	0.00	0.00	0.034	0.00006
Vasalemma-96/97	27	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Kunda-96/97	48	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Pärnu-97	23	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Daugava-98	53	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Emån-98/99	59	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Mörrumsån-96	51	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Average, South. Baltic		0.00	0.99	0.00	0.01	0.00	0.00	0.00	0.015	0.00003
Average, Baltic Sea		0.09	0.81	0.00	0.10	0.00	0.00	0.00	0.217	0.00031
<i>Landlocked</i>										
Lake Vänern										
Klarälven-97	44	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Gullspångsälven -86/87/89/91/96/97	112	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Lake Saimaa										
Pielisjoki-t/96/97	59	0.98	0.02	0.00	0.00	0.00	0.00	0.00	0.034	0.00003
Lieksa-97	22	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Lake Onega										
Lizhma-97	20	0.00	0.95	0.00	0.05	0.00	0.00	0.00	0.100	0.00016
Shuja-97	17	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Kumsa-97	18	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00000
Lake Ladoga										
Svir-96	29	0.14	0.86	0.00	0.00	0.00	0.00	0.00	0.246	0.00020
Sysky-99	42	0.88	0.12	0.00	0.00	0.00	0.00	0.00	0.217	0.00018
<i>Atlantic Ocean</i>										
Scandinavia										
Rønne å-92	33	0.00	0.76	0.00	0.21	0.03	0.00	0.00	0.392	0.00058
Stensån-94	25	0.00	0.24	0.00	0.76	0.00	0.00	0.00	0.380	0.00062
Lagan-94*/94/96	121	0.02	0.56	0.06	0.36	0.00	0.00	0.00	0.553	0.00061
Genevadsån-90/94	60	0.00	0.38	0.02	0.60	0.00	0.00	0.00	0.501	0.00079
Fylleån-94	25	0.04	0.52	0.00	0.40	0.04	0.00	0.00	0.590	0.00090
Åtran-92/94	73	0.07	0.07	0.05	0.74	0.05	0.00	0.01	0.443	0.00059
Rolfsån-94	27	0.00	0.07	0.26	0.67	0.00	0.00	0.00	0.501	0.00049
Säveån-90/92	48	0.00	0.00	0.29	0.71	0.00	0.00	0.00	0.422	0.00034
Grönån-97	43	0.05	0.09	0.28	0.58	0.00	0.00	0.00	0.587	0.00068

Table 3 Continued

Population	<i>n</i>	AAAA	AABA	BBBA	BBBB	ADBA	ACAA	AAAB	<i>h</i>	π
Örekilsälven-90/92	73	0.00	0.00	0.21	0.70	0.10	0.00	0.00	0.467	0.00029
Numedalslågen-93*	26	0.00	0.23	0.12	0.65	0.00	0.00	0.00	0.526	0.00069
Skjern*	33	0.67	0.00	0.03	0.30	0.00	0.00	0.00	0.477	0.00110
Average, Scandinavia		0.07	0.24	0.11	0.56	0.02	0.00	0.00	0.487	0.00064
British Isles										
Moy/Spaddagh-95‡	50	0.16	0.00	0.38	0.44	0.00	0.00	0.00	0.628	0.00087
Burrishoole-94*	32	0.22	0.00	0.28	0.44	0.00	0.00	0.06	0.700	0.00102
Delphi-96	34	0.03	0.65	0.15	0.18	0.00	0.00	0.00	0.544	0.00066
Corrib-94*	27	0.00	0.15	0.44	0.41	0.00	0.00	0.00	0.638	0.00084
Shannon-96	40	0.00	0.03	0.00	0.98	0.00	0.00	0.00	0.057	0.00009
Conon-94*	34	0.24	0.09	0.44	0.24	0.00	0.00	0.00	0.708	0.00097
North Esk-95‡	50	0.14	0.20	0.18	0.48	0.00	0.00	0.00	0.684	0.00099
Dee/Scotland-93*	111	0.09	0.07	0.25	0.54	0.00	0.05	0.00	0.640	0.00078
Dee/Wales-95‡	50	0.14	0.06	0.49	0.31	0.00	0.00	0.00	0.645	0.00082
Average, British Isles		0.11	0.14	0.29	0.44	0.00	0.01	0.01	0.583	0.00078
Adour/Nive-95‡	32	0.00	0.41	0.04	0.56	0.00	0.00	0.00	0.526	0.00082
Narcea-95‡	44	0.19	0.00	0.00	0.81	0.00	0.00	0.00	0.301	0.00075
Laxá i Dolum-95‡	50	0.14	0.63	0.00	0.22	0.00	0.00	0.00	0.528	0.00077
Barents Sea Basin										
Tana-95‡	50	0.23	0.00	0.67	0.10	0.00	0.00	0.00	0.495	0.00075
Kola/Pecha-95‡	50	0.00	0.14	0.00	0.71	0.00	0.00	0.00	0.276	0.00046
Tuloma	21	0.00	0.19	0.00	0.81	0.00	0.00	0.00	0.316	0.00053
White Sea Basin										
Nilma-99	43	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.000	0.00000
Pulonga-99	50	0.00	0.20	0.00	0.80	0.00	0.00	0.00	0.323	0.00053
L.Kuito/Pista-99	59	0.00	0.59	0.00	0.41	0.00	0.00	0.00	0.487	0.00080
Average Atlantic Ocean		0.08	0.21	0.15	0.54	0.01	0.00	0.00	0.478	0.00067

*Nielsen *et al.* 1996; †Palva *et al.* 1989; ‡Verspoor *et al.* 1999.

the British Isles. In salmon from the River Tana, the Atlantic haplotype BBBA dominated (Verspoor *et al.* 1999) but in populations further to the east, in the Barents and White Seas, this haplotype was not found.

Landlocked salmon in Swedish Lake Vänern were fixed for the typical southern Baltic haplotype AABA. In Russian Lake Onega AABA also dominated. In Lake Ladoga AAAA and AABA were found and in the Lake Saimaa (Finland), AAAA was close to fixation with the exception of a single individual with an AABA haplotype.

Haplotype and nucleotide diversities (Table 3) were highest in Atlantic populations, followed by those in Gulf of Bothnia. Southern Baltic Sea salmon showed only marginal variability. As expected, low diversity values were also obtained from all landlocked salmon populations, with the exception of lakes Kuito and Ladoga.

An UPGMA dendrogram clustering the populations (Fig. 2) revealed a major division of samples into two clusters, one of which was dominated by populations with high frequencies of haplotypes AAAA and AABA, consist-

ing mainly of Baltic Sea and the landlocked populations (25 out of 32). Atlantic populations in which BBBA and BBBB were common formed the other cluster (23 out of 24).

Nucleotide sequence variation and interpretation of RFLP patterns

The variable nucleotide sites of the seven different haplotypes are given in Fig. 3 (site numbering according to Hurst *et al.* (1998), GenBank no. U12143, our sequences nos AF115412 to AF115420). The phylogenetic relationship of the haplotypes, together with indication of variable restriction sites, is presented in Fig. 4 as a minimum-spanning network. All ND1 substitutions were phylogenetically informative and used only once. The four common European haplotypes are derived from the ancestral type BBBA (rooting the European clade to the North American) by one-step substitutions: AAAA < AABA < BBBA > BBBB.

To represent AAAA, we sequenced two fish from Lake Saimaa, two from the Gulf of Bothnian rivers Vindelälven

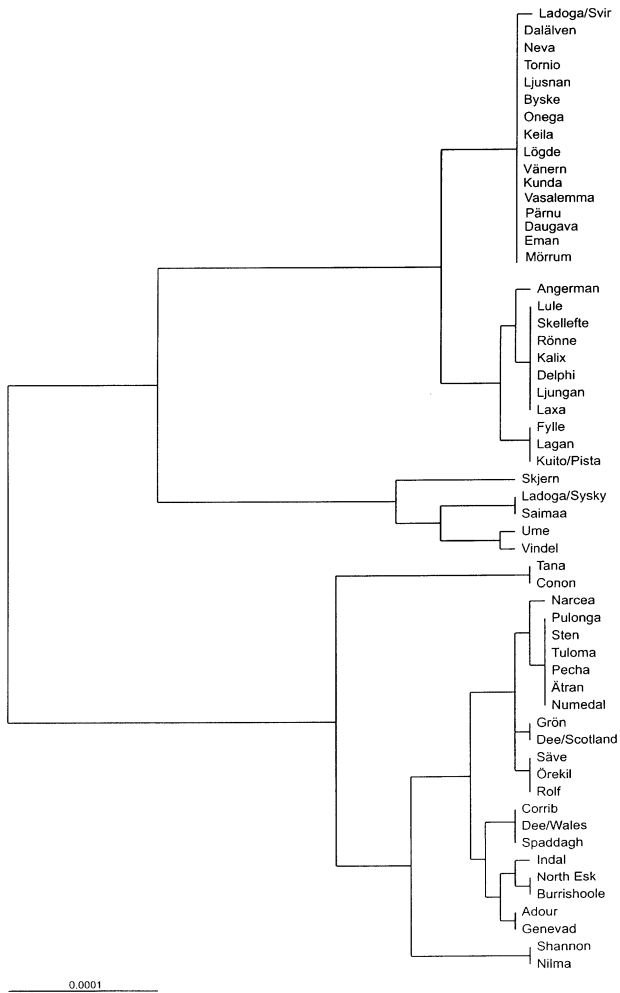


Fig. 2 UPGMA clustering of studied Atlantic salmon populations based on the number of net nucleotide substitutions per site (d_A) at the mitochondrial *ND1* gene.

and Indalsälven, and two from Atlantic rivers, Skjern (Denmark) and Burrishoole (Ireland). Despite representing three discontinuous and distant areas, all six fish had identical sequences.

AABA was the most common haplotype in the Baltic, and 13 fish were sequenced. The Rivers Daugava and Neva represented the southern Baltic area, where AABA was almost fixed. The sequences of all nine individuals from the Gulf of Bothnia were identical with those from the southern Baltic Sea. One AABA haplotype was also sequenced from the Atlantic side (Conon, Scotland), but no additional variation was found.

ADBA, which was found only in rivers along the Swedish west-coast, was sequenced from two fish, and it was ascertained as a derived type from AABA.

BBBA was completely absent from salmon in the Baltic Sea. We sequenced three individuals with this haplotype from Corrib (Ireland), Tana (Northern Norway) and one fish from an anadromous population in Newfoundland, Canada (North-east Brook Trepassey).

BBBB was the only haplotype for which we found nucleotide substitutions additional to those already detected by the four selected restriction endonucleases. BBBB haplotypes from Iceland and Lagan (west-coast of Sweden) were identical to those of the four individuals from Gulf of Bothnia, and the same haplotype was also found in two fish from the landlocked Ouananiche Beck, Newfoundland (population described in Gibson *et al.* 1996). However, one fish from River Ätran (Swedish west-coast) differed from the basic BBBB by one nonsynonymous substitution in the coding region of ND1 (val > ile, site 3900). This type is designated as BBBB².

The Canadian haplotype NFLD1 (Fig. 3) differs from the European ancestral BBBA by 10 substitutions. Only one of the 10 substitutions separating the clades is also a variable restriction site by the enzyme arsenal used to analyse the European populations. Between the European ND1 main types, only one additional substitution exists. With 100 replications the bootstrap support for the rooting of BBBA with NFLD1 and NFLD2 was 100%. In Fig. 4, we have added information of the sequence of the D-loop (Kauppi *et al.* 1997). Figure 4 also presents the 'satellite' types detected by RFLP of the ND3/4/5/6 segment of the mtDNA by Nielsen *et al.* (1998) and in this study.

Haplotype	Sequence position													No. of sequenced individuals					
	3	3	3	3	4	4	4	4	4	4	4	4	4	Baltic Sea	Eastern Atlantic	Western Atlantic			
	7	7	9	9	9	0	0	4	5	5	5	5	7	8	9	9			
	0	3	0	7	8	7	8	2	1	6	6	7	5	2	0	1			
	4	4	0	1	9	9	2	4	8	5	8	8	1	1	2	0			
Hurst	T	A	G	G	A	C	T	C	G	G	C	G	G	G	G	A			
BBBA	G	A	.		2	1
BBBB ²	.	.	A	A	.		1	
BBBB	A	.	4	3	2
AABA	G	.	.	A	A	.	12	1	
AAAA	G	.	.	A	A	G	.	4	2	
ADBA	G	.	.	A	.	.	A	.	.	A	.	.		2	
NFLD2	C	C	.	A	G	T	C	T	.	A	T	.	A	.	A	.			1
NFLD1	C	C	.	A	G	T	C	T	.	A	T	.	A	.	.	.			2

Fig. 3 Variable nucleotide positions along the 1227-bp sequence around the Atlantic salmon mitochondrial ND1 gene that correspond to RFLP haplotypes. The numbering follows the standard sequence of Hurst *et al.* (1998); GenBank Accession No. U12143. Haplotype designations follow Nielsen *et al.* (1996). NFLD is the abbreviation for Newfoundland, Canada. The nucleotide sequences are deposited in GenBank under accession numbers AF115412 to AF115420.

drift and the differences in the distribution of haplotypes between the Gulf of Bothnia and southern areas of the Baltic Sea could be the result of drift. The hypothesis of Atlantic colonization was proposed by Verspoor *et al.* (1999) based on the study of ND1 gene variation in European salmon populations. Their five populations from the Baltic Sea, however, were all from the Gulf of Bothnia, the salmon in which differ in haplotype composition from those in the southern Baltic area. There is also other genetic and ecological evidence that puts the hypothesis of postglacial colonization of the Baltic Sea from the Atlantic under question, at least as the sole origin of the Baltic salmon. Kazakov & Titov (1991) and Koljonen *et al.* (1999) suggested that there is a component of an eastern origin among populations of Baltic salmon. Koljonen *et al.* (1999) showed, based on allozyme data, that the southern Baltic salmon are significantly differentiated from those in the Gulf of Bothnia and explained this with an eastern origin of salmon in the southern Baltic and with an Atlantic or mixed origin in the Gulf of Bothnia. We found concordant differentiation between the southern and northern Baltic populations with the mtDNA data – southern Baltic populations are fixed for the AABA haplotype but additional haplotypes occur with high frequency in the Gulf of Bothnia. This can be interpreted as supporting the hypothesis of an eastern origin for salmon in the southern Baltic area where the Baltic Ice Lake and, draining into it, the large salmon rivers already existed 3000–4000 years before the first marine connection with the Atlantic Ocean opened. Doubts about the hypothesis of an Atlantic immigration can be invoked by the fact that the BBBA haplotype, which is found in most Atlantic populations, is absent from the Gulf of Bothnia. Although this haplotype could have been lost by a founder event combined with drift in Atlantic invaders it is notable that it is also missing among populations in the Barents and White Seas, which are believed to have been colonized by salmon from eastern glacial refugia (Kazakov & Titov 1991). Further, populations that were most probably influenced by invading Atlantic salmon at the Yoldia stage should be those of Lake Vänern and the southern Gulf of Bothnia. We found these populations to have the same haplotype composition as southern Baltic Sea salmon, giving no indication of Atlantic origin. However, in contrast to the mtDNA data, the composition of haplotypes of the nuclear GH-1 gene among salmon from Lake Vänern is much more similar to Atlantic than to Baltic populations [Gross & Nilsson (1999) and unpublished data] which adds confusion to the problem. An additional circumstance that questions the possibility of Atlantic colonization is the presence of the parasite *Gyrodactylus salaris* in the Baltic region. Salmon in the Atlantic is highly susceptible to this parasite but Baltic salmon is tolerant (Bakke *et al.* 1990). The effect of recent unintentional spread of *G. salaris*

to Norwegian rivers demonstrates the difficulties of Atlantic salmon tolerating this parasite. However, on the west-coast of Sweden, *G. salaris* is reported as benign (Malmberg & Malmberg 1993), supporting the idea that some gene flow has occurred outwards from the Baltic.

The importance of eastern glacial refugia as a potential source for postglacial colonization of the Baltic Sea has been suggested also for several other fish species. The closely related brown trout (*Salmo trutta*) was proposed to have colonized the Baltic Ice Lake before the connection to the Atlantic opened (Garcia-Marin *et al.* 1999). Some other North European fish species like perch (*Perca fluviatilis*) (Nesbø *et al.* 1999), European grayling (*Thymallus thymallus*) (Koskinen *et al.* 2000) and chub (*Leuciscus cephalus*) (Durand *et al.* 1999) have been suggested to have colonized the Baltic Sea in part or entirely from eastern or south-eastern glacial refugia. Also whitefish (*Coregonus* sp.) has a mtDNA haplotype distribution that makes an eastern origin of North European populations probable (Bernatchez & Dodson 1994).

Colonization of the Gulf of Bothnia after the isolated Ancylus stage seems least likely. About 8000 years BP the Danish Straits opened and the Ancylus Lake developed into the brackish Litorina Sea. In the event of colonization via the Danish straits, salmon spreading north towards the Gulf of Bothnia must have passed through the entire southern Baltic. In light of the haplotype composition in the southern Baltic this seems to be an unlikely event. The pattern of migratory behaviour of present day wild salmon populations also supports the long isolation of Baltic salmon and speaks against invasion through the Danish Straits. Neither Baltic nor Swedish west-coast wild salmon pass the Danish straits in significant numbers (Christensen & Larsson 1979). At the same time, hatchery salmon stocked into the Baltic Sea by delayed release methods, have disturbed homing behaviour and migrate towards the Atlantic (Glusing & Rasmussen 1996).

The above arguments suggest that the hypothesis of colonization of the Baltic Sea, or particularly of the Gulf of Bothnia, by Atlantic salmon through the Närke Strait or Danish Straits is questionable. In order to explain the presence of haplotypes AAAA and BBBB in the Gulf of Bothnia alternative routes for colonization should be considered. Kazakov & Titov (1991) have indicated that connection between the Barents Sea coast and ice lakes in the White Sea area and Karelia could have occurred in the late glacial period over the Kola peninsula in the region of Lake Imandra. A further spread of salmon to the south over Karelia could create a northeast link to the Gulf of Bothnia. The BBBB haplotype was found at high frequency in the Barents and the White Seas and may have reached the Gulf of Bothnia by following the retreating ice, a route represented in our collection by Lake Kuito in Karelia. However, no source population for the AAAA

haplotype has been found in the White and Barents Sea region. Since the hypothesis of Atlantic colonization directly from the west raises several problems and the link between the Gulf of Bothnia and the White and the Barents Seas lacks strong positive indications the question of the origin of lineages AAAA and BBBB in the Gulf of Bothnia remains to be solved.

Implications of stock transfer

One of the problems with respect to salmon, a species so much influenced by human intervention, is that the original genetic patterns may be blurred by supportive stocking, stock transfer and by loss and recolonization of local populations. Populations in individual rivers may also be affected by naturally occurring genetic drift and founder events in their past history. By covering a significant part of the distribution area of salmon in Europe, we were nevertheless able to detect a large-scale geographical structuring of the mitochondrial variation.

Many Baltic Sea salmon populations now exist mainly as hatchery populations with inherent risks to their genetic integrity. Artificial rearing has made transfer of genetic material among rivers a simple task, and non-native stocking is known to have occurred many times. One example is the stocking of the River Lagan on the Swedish west-coast with salmon from the Gulf of Bothnia. This transfer, and the later use of fish from the River Lagan as stocking material for the River Rönneå and straying to the geographically close and weak population in the River Fylleån, may explain why these west-coast rivers grouped together with Baltic salmon. In the long term, stock transfers are expected to lead to homogenization of variation but such effects cannot be seen in this study. We therefore conclude that attempts to conserve populations are indeed worthwhile.

Future directions

There is still an important gap in the phylogeographic data for salmon. If the salmon of the Baltic Sea originated from proglacial lakes, they should be genetically similar to the salmon of rivers in northern Russia, which formed the large ice lakes during glaciation. Our study area did not include the north-east Atlantic coast. Some allozyme studies indicate that expansion of this study to these directions could be worthwhile. Kazakov & Titov (1991, 1993) studied salmon populations to the east of those treated in Ståhl (1987). In the UPGMA dendrogram of their results, the Barents and White Sea populations clearly differ from the Baltic samples. However, the allele frequencies of some important marker loci indicate significant similarity between the Baltic Sea populations and populations from the southern coast of the White

sea and Barents Sea and differences from populations of the Kola Peninsula. These populations, exhibiting some similarity to Baltic ones, are just the populations which by their history should originate from proglacial lakes. An extended comparison of mitochondrial variation in northern Russian waters with the Baltic Sea data could be informative in order to understand the postglacial colonization by salmon in northeast Europe.

Acknowledgements

We acknowledge financial support to this work from SJFR (to J. Nilsson), Estonian Science Foundation (grants nos 2615 and 4095 to R. Gross) and the Academy of Finland (to J. Lumme). H. Jansson and T. Öst were financially supported by the Swedish Hydropower Companies, The Swedish National Board of Fisheries and the Göte Borgström Foundation. The sequencing work was started with the support of Professor Antti Soivio, and the Game and Fisheries Research Institute in Finland delivered most of the Finnish fish. Professor Matti Eronen kindly explained the postglacial history of Russian Karelia to us. We also thank the numerous people who helped us sample the material and provided technical assistance in the laboratory.

References

- Bakke TA, Jansen PA, Hansen LP (1990) Differences in the host resistance of Atlantic salmon, *Salmo salar* L., stocks to the monogenean *Gyrodactylus salaris* Malmberg, 1957. *Journal of Fish Biology*, **37**, 577–587.
- Bermingham E, Forbes SH, Friedland K, Pla C (1991) Discrimination between Atlantic salmon (*Salmo salar*) of North American and European origin using restriction analyses of mitochondrial DNA. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 884–893.
- Bernatchez L, Dodson JJ (1994) Phylogenetic relationship among Palearctic and Nearctic whitefish (*Coregonus* sp.) populations as revealed by mitochondrial DNA variation. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 240–251.
- Bernatchez L, Wilson CC (1998) Comparative phylogeography of Nearctic and palearctic fishes. *Molecular Ecology*, **7**, 431–452.
- Birt TP, Green JM, Davidson WS (1991) Mitochondrial DNA variation reveals genetically distinct sympatric populations of anadromous and nonanadromous Atlantic salmon, *Salmo salar*. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 577–582.
- Bourke EA, Coughlan J, Jansson H, Galvin P, Cross TF (1997) Allozyme variation in populations of Atlantic salmon located throughout Europe: diversity that could be compromised by introductions of reared fish. *ICES Journal of Marine Sciences*, **54**, 974–985.
- Christensen O, Larsson P-O, eds (1979) *Review of Baltic Salmon Research. ICES Cooperative Research Report no. 89*. ICES, Charlottenlund.
- Cutler MG, Bartlett SE, Hartley SE, Davidson WS (1991) A polymorphism in the ribosomal RNA genes distinguishes Atlantic salmon (*Salmo salar*) from North America and Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 1655–1661.
- Donner J (1995) *The Quaternary History of Scandinavia*. Cambridge University Press, Cambridge.

- Durand JD, Persat H, Bouvet Y (1999) Phylogeography and postglacial dispersion of the chub (*Leuciscus cephalus*) in Europe. *Molecular Ecology*, **8**, 989–997.
- Eronen M (1983) Late Weichselian and Holocene shore displacement in Finland. In: *Shorelines and Isostasy* (eds Smith DE, Dawson AG), pp. 183–207. Academic Press, London.
- Felsenstein J (1994) *PHYLIP (Phylogeny Inference Package)*, Version 3.572c. Department of Genetics, University of Washington, Seattle.
- Garci-Marin J-L, Utter F, Pla C (1999) Postglacial colonisation of brown trout in Europe based on distribution of allozyme variants. *Heredity*, **82**, 46–56.
- Gibson RJ, Williams DD, McGowan C, Davidson WS (1996) The ecology of dwarf fluvial Atlantic salmon, *Salmo salar* L., cohabiting with brook trout, *Salvelinus fontinalis* (Mitchill), in southeastern Newfoundland, Canada. *Polskie Archiwum Hydrobiologii*, **43**, 145–166.
- Glusing H, Rasmussen G (1996) Udsætningsforsøg med Østersø laks. *DFU-rapport* nr. 6–96. Danmarks Fiskeriundersøgelser, Copenhagen.
- Gross R, Nilsson J (1999) Restriction fragment length polymorphism at the growth hormone 1 gene in Atlantic salmon (*Salmo salar* L.) and its association with weight among the offspring of a hatchery stock. *Aquaculture*, **173**, 73–80.
- Hall HJ, Nawrocki LW (1995) A rapid method for detecting mitochondrial DNA variation in the brown trout, *Salmo trutta*. *Journal of Fish Biology*, **46**, 360–364.
- Hurst CD, Bartlett SE, Bruce IJ, Davidson WS (1998) *The complete nucleotide sequence of the mitochondrial DNA of the Atlantic salmon, Salmo salar*. GenBank Accession number U12143. NCBI, Bethesda, MD, USA.
- Hyvärinen H, Eronen M (1979) The quaternary history of the Baltic. The northern part. In: *The Quaternary History of the Baltic. Acta Universitatis Upsaliensis Annus Quingentesimum Celebratis 1* (eds Gudelis V, Königsson L-K), pp. 7–27. University of Uppsala, Uppsala.
- Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. Munro HN), pp. 21–132. Academic Press, New York.
- Kauppi R, Kvist L, Ruokonen M, Soivio A, Lumme J (1997) Lack of variation in mitochondrial DNA of the Baltic salmon (*Salmo salar*) indicates a bottleneck during or long before postglacial recolonisation of the Baltic Sea? *Oulanka Reports*, **17**, 19–23.
- Kazakov RV, Titov SF (1991) Geographical patterns in the population genetics of Atlantic salmon, *Salmo salar* L., on U.S.S.R. territory, as evidence for colonisation routes. *Journal of Fish Biology*, **39**, 1–6.
- Kazakov RV, Titov SF (1993) Population genetics of salmon, *Salmo salar* L., in northern Russia. *Aquaculture and Fisheries Management*, **24**, 495–506.
- Koljonen M-L, Jansson H, Paaver T, Vasin O, Koskiniemi J (1999) Phylogeographic lineages and differentiation pattern of Atlantic salmon in the Baltic Sea with management implications. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1766–1780.
- Koskinen MT, Ranta E, Piironen J *et al.* (2000) Genetic lineages and postglacial colonization of the grayling (*Thymallus thymallus*, Salmonidae) in Europe, as revealed by mitochondrial DNA analyses. *Molecular Ecology*, **9**, 1609–1624.
- Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A (1991) Simplified mammalian DNA isolation procedure. *Nucleic Acids Research*, **19**, 4293.
- Lovén S (1862) Om några I. Vettern och Venern funna crustaceer. *Öfversigt Af Kungliga Vetenskaps-Akademiens Förhandlingar*, **18**, 285–314.
- Malmberg G, Malmberg M (1993) Species of *Gyrodactylus* (Platyhelminthes, Monogenea) on salmonids in Sweden. *Fisheries Research*, **17**, 59–68.
- Martin AP, Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences of the USA*, **90**, 4087–4091.
- McConnell SK, O'Reilly P, Hamilton L, Wright J, Bentzen P (1995) Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 1863–1872.
- McVeigh HP, Bartlett SE, Davidson WS (1991) Polymerase chain reaction/direct sequence analysis of the cytochrome b gene in *Salmo salar*. *Aquaculture*, **95**, 225–233.
- Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Nesbø CL, Fossheim TV, Øllestad A, Jakobsen KS (1999) Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. *Molecular Ecology*, **8**, 1387–1404.
- Nielsen EE, Hansen MM, Loeschke V (1996) Genetic structure of European populations of *Salmo salar* L. (Atlantic salmon) inferred from mitochondrial DNA. *Heredity*, **77**, 351–358.
- Nielsen EE, Hansen MM, Mensberg K-LD (1998) Improved primer sequences for the mitochondrial ND1, ND3/4 and ND5/6 segments in salmonid fishes: application to RFLP analysis of Atlantic salmon. *Journal of Fish Biology*, **53**, 216–220.
- Nilsson J (1997) MtDNA and microsatellite variation in Baltic Atlantic salmon. *ICES Journal of Marine Sciences*, **54**, 1173–1176.
- Palva TK, Lehvälaiho H, Palva ET (1989) Identification of anadromous and non-anadromous salmon stocks in Finland by mitochondrial DNA analysis. *Aquaculture*, **81**, 237–244.
- Rozas J, Rozas R (1999) DnaSP, Version 3 an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics*, **15**, 174–175.
- Saarnisto M (1970) The history of Finnish lakes and Lake Ladoga. *Commentationes Physico-Mathematicae*, **41**, 372–388.
- Segerstråhle SG (1982) The immigration of glacial relicts into Northern Europe in the light of recent geological research. *Fennia*, **160**, 303–312.
- Sohlenius G (1996) The history of the Baltic proper since the Late Weichselian deglaciation as recorded in sediments. *Quaternaria Series A*, no. 3.
- Ståhl G (1987) Genetic population structure of Atlantic salmon. In: *Population Genetics & Fishery Management* (eds Ryman N, Utter F), pp. 121–140. University of Washington Press, Seattle.
- Taggart JB, Verspooer E, Galvin PT, Morán P, Ferguson A (1995) A minisatellite DNA marker for discriminating between European and North American Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 2305–2311.
- Väinölä R (1995) Origin and recent endemic divergence of a Caspian *Mysis* species flock with affinities to the 'glacial relict' crustaceans in boreal lakes. *Evolution*, **49**, 1215–1223.
- Verspooer E, McCarthy EM, Knox D, Bourke EA, Cross TF (1999) The phylogeography of European Atlantic salmon (*Salmo salar* L.) based on RFLP analysis of the ND1/16sRNA region of the mtDNA. *Biological Journal of the Linnean Society*, **68**, 129–146.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR based typing from forensic material. *Biotechniques*, **10**, 506–513.

Zardoya R, Garrido-Pertierra A, Bautista JM (1995) The complete nucleotide sequence of the mitochondrial DNA genome of the rainbow trout, *Oncorhynchus mykiss*. *Journal of Molecular Evolution*, **41**, 924–951.

The data and material of this work were compiled and analysed by Jan Nilsson, Riho Gross and Jaakko Lumme who came to the idea to use the gradually accumulating data from different laboratories for studying the possible post-glacial colonization scenarios of the Baltic Sea by salmon.
