

# Population genetic structure and postglacial colonization of Atlantic salmon (*Salmo salar*) in the Baltic Sea area based on microsatellite DNA variation

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**Abstract:** The genetic structure and phylogeography of Atlantic salmon (*Salmo salar*) across the Baltic Sea basin and neighbouring areas (eastern Atlantic Ocean, North Sea, Barents Sea, White Sea, and two Russian lakes, Onega and Ladoga) were studied to resolve the partly contradictory hypotheses of the species' postglacial colonization history. Thirty-eight populations (total of 2180 individuals) were studied for nine DNA microsatellite loci. Within the Baltic Sea, the anadromous populations formed three clear groups, corresponding to the northern (Gulf of Bothnia), eastern (Gulf of Finland and eastern Baltic Main Basin), and southern regions (western Baltic Main Basin). The genetic differences among these three groups were clearly greater ( $G_{GB}$  5.6%;  $G_{GB}$  being the proportion of diversity components between regions within basins) than were those among population groups in the eastern Atlantic Ocean ( $G_{GB}$  2.2%) from Ireland to the White Sea. The isolation-by-distance model explained part of the differentiation within, but not between, the regions. The results strongly indicate colonization of the Baltic Sea by at least three glacial lineages. Potential refugium areas for each lineage are proposed.

**Résumé :** Nous avons étudié la structure génétique et la phylogéographie du saumon atlantique (*Salmo salar*) de part en part du bassin de la Baltique et dans les régions avoisinantes (est de l'Atlantique, mer de Barents, mer Blanche et deux lacs russes, les lacs Onega et Ladoga) pour résoudre les hypothèses en partie contradictoires sur l'histoire de la colonisation postglaciaire de l'espèce. Nous avons analysé neuf locus ADN microsatellites chez 2180 individus appartenant à 38 populations. Au sein de la Baltique, les populations anadromes forment trois groupes distincts qui correspondent aux régions du nord (golfe de Bothnie), de l'est (golfe de Finlande et bassin principal de l'est de la Baltique) et du sud (bassin principal de l'ouest de la Baltique). Les différences génétiques entre ces trois groupes sont nettement plus importantes ( $G_{GB}$  5,6 %;  $G_{GB}$  représente la proportion d'éléments de diversité entre les régions à l'intérieur des bassins) que celles qui existent entre les groupes de populations de l'est de l'Atlantique ( $G_{GB}$  2,2 %), de l'Irlande à la mer Blanche. Le modèle de l'isolement en fonction de la distance explique une partie de la différenciation au sein des régions, mais non entre elles. Nos résultats indiquent fortement une colonisation de la Baltique par au moins trois lignées glaciaires. Nous proposons des zones possibles de refuge pour chaque lignée.

[Traduit par la Rédaction]

## Introduction

The Atlantic salmon (*Salmo salar*) is one of the most valuable fish species in the Baltic Sea. However, mainly owing to human impact (e.g., construction of hydropower dams that restrict the spawning migration, overexploitation in the

offshore fishery, poaching, and water regulation and pollution), wild populations have declined drastically during the past century. To compensate for the decreased abundance, artificial reproduction in hatcheries and river stocking of reared fish are widely practised, and today, about 80% of offspring are produced in hatcheries. If the releases are based

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on nonnative populations, however, this trend may result in erosion of the genetic structure, loss of local adaptations, and reduced fitness of the remaining wild salmon populations (Hindar et al. 1991; Ryman 1991; Taylor 1991). Even when the released fish originate from the same river, non-natural selective pressures under captive conditions (domestication effect) (Fleming and Einum 1997; Crozier 1998) or loss of genetic variation through genetic drift and inbreeding (which always occurs in populations of restricted size such as hatchery populations) may still pose a threat to the evolutionary potential of the species (Verspoor 1988; Koljonen 1989; Säisä et al. 2003). Management, protection, and reestablishment of salmon populations in the Baltic Sea should therefore be based on knowledge of the genetic population structure and evolutionary history of the populations.

Current knowledge of the salmon population structure in the Baltic Sea and neighbouring areas has been gathered through allozyme (Ståhl 1987; Kazakov and Titov 1993; Koljonen et al. 1999) and mitochondrial DNA (mtDNA) (Nilsson 1997; Verspoor et al. 1999; Nilsson et al. 2001) studies showing that Baltic salmon populations are significantly differentiated from eastern Atlantic populations and that genetic variability is generally somewhat lower than that of Atlantic populations. In addition, the salmon populations from the southern and southeastern Baltic Sea (from the Main Basin and the Gulf of Finland) are significantly differentiated from those of the northern Baltic Sea (Gulf of Bothnia) area (Koljonen et al. 1999; Nilsson et al. 2001); some differentiation also exists within these two regions (Koljonen et al. 1999). Major differences among the salmon populations have most probably arisen through the postglacial colonization of already differentiated lineages from distinct glacial refugia, as the current salmon populations in northern Europe (including the Baltic Sea) have only existed since postglacial times (i.e., not more than 12 000 years). However, there is still no consensus concerning the postglacial salmon colonization scenarios of the Baltic Sea. Three hypotheses have been proposed. First, Baltic salmon may derive from a refugial population that survived in eastern preglacial lakes and may have colonized the Baltic basin before a marine strait connected the Baltic to the North Sea (i.e., before the Yoldia sea stage). This model was first put forward by Kazakov and Titov (1991) on the basis of allozyme data and was later supported by Nilsson et al. (2001) on the basis of mtDNA studies. Second, the salmon may have a western Atlantic origin, as proposed by Verspoor et al. (1999). Their mtDNA study was, however, limited to the Gulf of Bothnia in the Baltic Sea. Third, colonization from both east and west is possible, as suggested by Koljonen et al. (1999).

This lack of consensus is partly attributable to differences in the data sets available to the researchers but also to limitations inherent in the genetic markers used. Major disadvantages of allozyme markers are the small number of polymorphic loci (only five among Baltic salmon populations), the low level of variability commonly observed, and uncertainty about the neutrality of variation (e.g., at the *mMEP-2\** locus), as shown by Verspoor and Jordan (1989) and Jordan and Youngson (1991). Analysis of mtDNA only provides phylogenetic information on a single gene tree that may not

accurately reflect a population tree (Edwards and Beerli 2000; Hey and Machado 2003). Moreover, its usefulness in inferring population relationships may be limited when the temporal scale of divergence has not been sufficient to lead to reciprocal monophyly of mtDNA haplotypes. Under these conditions, much of the geographical variation may comprise the sorting of more ancient divergence among mtDNA haplotypes, and where a severely bottlenecked population has recently colonized an area, there may be little or no phylogenetic signal from the mtDNA sequences (Hewitt 1999). Thus, the study of evolutionary history on small temporal scales may benefit from the use of more rapidly evolving genetic markers such as microsatellites, which have a potential for finer resolution of phylogenetic signals among recently diverged groups of organisms (e.g., Bowcock et al. 1994; Angers and Bernatchez 1998; Brunner et al. 1998). To date, however, only a few studies have used microsatellites to describe the genetic structure of natural populations of Atlantic salmon in Europe, and these studies have focused on a rather limited number of populations from restricted areas either on the Atlantic coast of Europe (Sanchez et al. 1996; Norris et al. 1999; Wennevik et al. 2004) or in the Baltic Sea (Nilsson 1997; Koljonen et al. 2002).

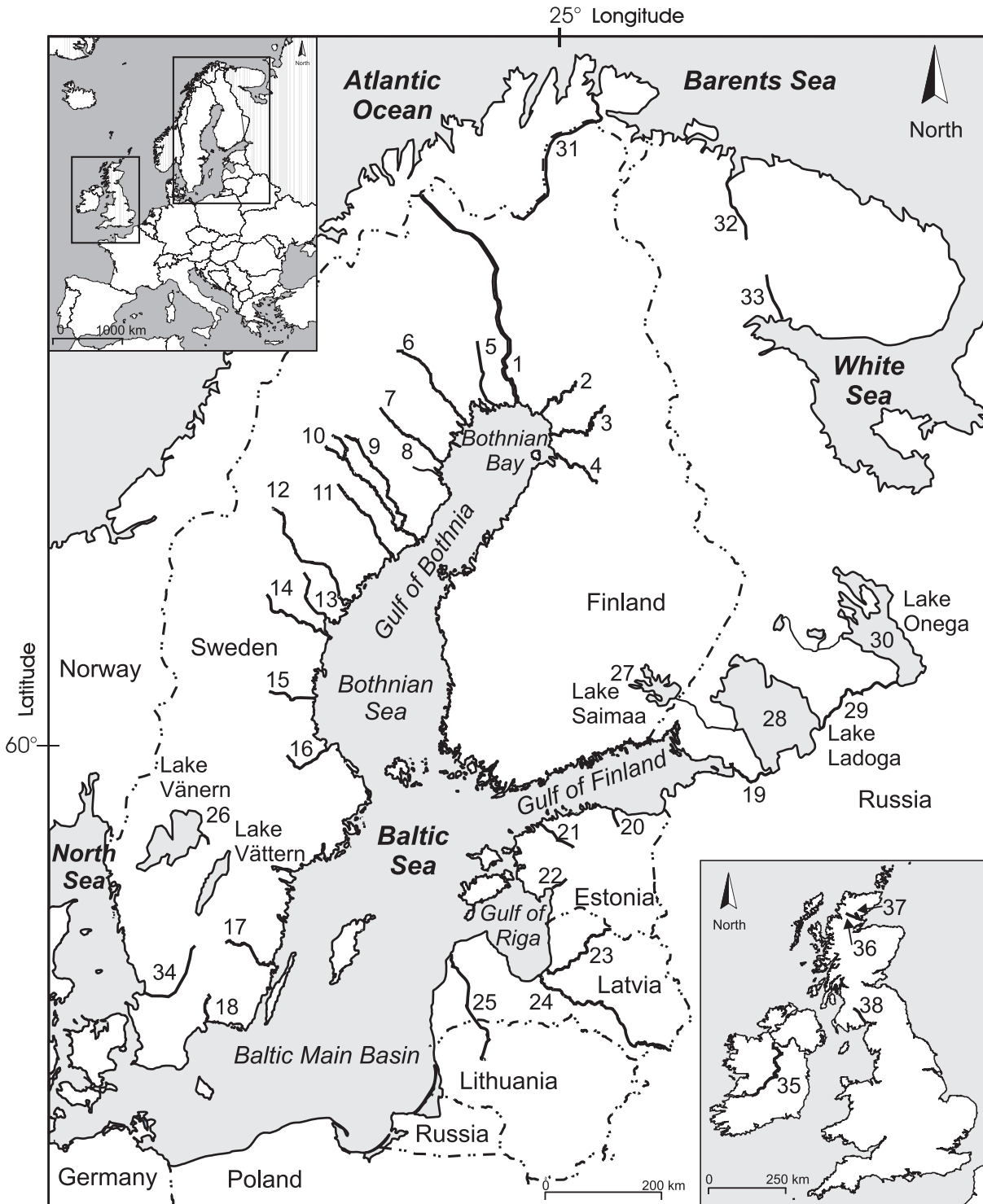
Here, our aim was to solve the population genetic structure and phylogeography of Atlantic salmon across the whole Baltic Sea basin and neighbouring areas and to propose a connective solution for the colonization history based on both our previous understanding of potential colonization routes and on data on allozyme and mtDNA variation as well as on new comprehensive DNA microsatellite data. As most of our sampling locations were the same as those used in earlier allozyme (Koljonen et al. 1999) and mtDNA (Nilsson et al. 2001) studies, we were able to compare the concordance of phylogeographical patterns across multiple genetic markers, which is important for improving the reliability of inferences. A further aim was to clarify the relationships between the main reasons for the observed genetic structure: glacial differentiation, isolation by distance, and genetic drift.

## Materials and methods

### Sample collection

Samples of 33 anadromous Atlantic salmon populations were collected from rivers draining into the Baltic Sea, Barents Sea, White Sea, North Sea, and east coast of the Atlantic Ocean (Fig. 1; Table 1). In addition, samples of five landlocked Atlantic salmon populations were collected from inland waters in the Baltic Sea drainage area: Lake Vänern (Sweden), Lake Saimaa (Finland), Lakes Onega and Ladoga (Russia), and the River Svir (Russia), which flows from Lake Onega to Lake Ladoga. Populations from Lakes Vänern and Saimaa are known to be affected by population size bottlenecks (Vuorinen 1982; Ring and Hanell 1987; Koljonen 1989). One sample from North America, from Big Brook (Michaels River, Labrador, Canada) on the west coast of the Atlantic Ocean, was available from an earlier study (Koljonen et al. 2002) and served as an outgroup for rooting the population tree. The fishes were sampled as juveniles, and some of the samples were of hatchery origin (see Ta-

**Fig. 1.** Sampling locations of the Atlantic salmon (*Salmo salar*) populations studied. Population numbers are as in Table 1.



- |                           |                   |               |                 |             |
|---------------------------|-------------------|---------------|-----------------|-------------|
| 1. Tornionjoki-Torneälven | 8. Skellefteälven | 16. Dalälven  | 24. Daugava     | 32. Kola    |
| 2. Simojoki               | 9. Vindelälven    | 17. Emån      | 25. Venta       | 33. Umba    |
| 3. Iijoki                 | 10. Umeälven      | 18. Mörrumsån | 26. Lake Vänern | 34. Lagan   |
| 4. Oulujoki               | 11. Lögdeälven    | 19. Neva      | 27. Lake Saimaa | 35. Shannon |
| 5. Kalixälven             | 12. Ångermanälven | 20. Kunda     | 28. Lake Ladoga | 36. Oykel   |
| 6. Luleälven              | 13. Indalsälven   | 21. Keila     | 29. River Svir  | 37. Nith    |
| 7. Byskeälven             | 14. Ljungan       | 22. Pärnu     | 30. Lake Onega  | 38. Shin    |
|                           | 15. Ljusnan       | 23. Gauja     | 31. Teno        |             |

**Table 1.** River or lake of origin, sea area, country, propagation, and sample size of Atlantic salmon (*Salmo salar*) samples studied.

Population No.	River or lake	Sea or lake area	Country	Propagation	<i>n</i>
<b>Northern Baltic Sea</b>					
1	Tornionjoki	Gulf of Bothnia	Finland, Sweden	Wild	56
2	Simojoki	Gulf of Bothnia	Finland	Wild	59
3	Iijoki	Gulf of Bothnia	Finland	Hatchery	61
4	Oulujoki	Gulf of Bothnia	Finland	Hatchery	59
5	Kalixälven	Gulf of Bothnia	Sweden	Wild	57
6	Luleälven	Gulf of Bothnia	Sweden	Hatchery	60
7	Byskeälven	Gulf of Bothnia	Sweden	Wild	77
8	Skellefteälven	Gulf of Bothnia	Sweden	Hatchery	57
9	Vindelälven	Gulf of Bothnia	Sweden	Wild	50
10	Umeälven	Gulf of Bothnia	Sweden	Hatchery	50
11	Lögdeälven	Gulf of Bothnia	Sweden	Wild	50
12	Ängermanälven	Gulf of Bothnia	Sweden	Hatchery	60
13	Indalsälven	Gulf of Bothnia	Sweden	Hatchery	65
14	Ljungan	Gulf of Bothnia	Sweden	Wild	51
15	Ljusnan	Gulf of Bothnia	Sweden	Hatchery	53
16	Dalälven	Gulf of Bothnia	Sweden	Hatchery	60
<b>Southern Baltic Sea</b>					
17	Emån	Western Main Basin	Sweden	Wild	54
18	Mörrumsån	Western Main Basin	Sweden	Wild	45
<b>Eastern Baltic Sea</b>					
19	Neva	Gulf of Finland	Russia	Hatchery	60
20	Kunda	Gulf of Finland	Estonia	Wild	61
21	Keila	Gulf of Finland	Estonia	Wild	53
22	Pärnu	Eastern Main Basin	Estonia	Wild	26
23	Gauja	Eastern Main Basin	Latvia	Wild	70
24	Daugava	Eastern Main Basin	Latvia	Hatchery	70
25	Venta	Eastern Main Basin	Latvia	Wild	66
<b>Landlocked populations</b>					
26	Lake Vänern		Sweden	Hatchery	50
27	Lake Saimaa		Finland	Hatchery	58
28	Lake Ladoga		Russia	Wild	94
29	Svir	Lake Ladoga	Russia	Wild	48
30	Lake Onega		Russia	Wild	37
<b>Barents Sea and White Sea</b>					
31	Teno	Barents Sea	Finland	Wild	59
32	Kola	Barents Sea	Russia	Wild	88
33	Umba	White Sea	Russia	Wild	70
<b>Atlantic Ocean, east coast</b>					
34	Lagan	North Sea	Sweden	Hatchery	48
35	Shannon	Atlantic Ocean	Ireland	Hatchery	48
36	Oykel	North Sea	Scotland	Hatchery	50
37	Nith	North Sea	Scotland	Hatchery	50
38	Shin	Atlantic Ocean	Scotland	Hatchery	50
Total					2180

bles 1 and 2). Hatchery populations have been created from local populations and represent the genetic characteristics of the region. Thus, they are not expected to cause bias in a large-scale interregional analysis such as was conducted here. The fitting of hatchery populations into the geographical pattern could also be checked through genetic distance

analysis in which all hatchery populations were located near their wild geographical neighbours.

#### Microsatellite DNA analysis

Total genomic DNA was extracted from muscle tissue samples, adipose fins, or scales according to the method de-

scribed by Taggart et al. (1992) or by using the QIAGEN DNeasy Tissue Kit (QIAGEN). Variation was determined at nine microsatellite loci: *Ssa85* and *Ssa289* (McConnell et al. 1995); *Ssa171*, *Ssa197*, and *Ssa202* (O'Reilly et al. 1996); *SSOSL85*, *SSOSL311*, and *SSOSL417* (Slettan et al. 1995); and *SSOSL438* (Slettan et al. 1996).

Genotypes were assayed by polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis, either with radioactive labelling and autoradiography as previously described by Koljonen et al. (2002) or with fluorescent labelling. For four of the primer pairs (*Ssa85*, *Ssa171*, *Ssa197*, and *Ssa202*), the strand originally named B (reverse PCR primer) in O'Reilly et al. (1996) was labelled, whereas in other primer pairs, the forward PCR primer of each pair was labelled. In the fluorescent method, the primers were labelled with infrared dyes as follows: IRD-700 (*Ssa202*, *Ssa289*, *SSOSL85*, *SSOSL417*, and *SSOSL438*) and IRD-800 (*Ssa85*, *Ssa171*, *Ssa197*, and *SSOSL311*). PCRs were performed in a 10- $\mu$ L reaction volume with 15 ng of genomic DNA, 0.1 pmol of fluorescent-labelled primer, 5 pmol of each forward and reverse primer, 200  $\mu$ mol dNTPs $\cdot$ L $^{-1}$ , 10 mmol Tris-HCl $\cdot$ L $^{-1}$  (pH 9.0), 1.5 mmol MgCl $_2$  $\cdot$ L $^{-1}$  and 0.25 U (1 U  $\approx$  16.67 nkat) of *Taq* DNA polymerase (GE Healthcare, Helsinki, Finland).

PCR products were separated on 25-cm 7% denaturing polyacrylamide gels and detected with a LI-COR automated DNA sequencer (LI-COR Inc., Lincoln, Nebraska). Microsatellite genotypes were analysed with Gene ImagIR<sup>TM</sup> (version 3.52) fragment analysis software (Scanalytics Inc., Fairfax, Virginia). Allele sizes were determined by comparing band mobilities with the M13 DNA sequence ladder (radioactive method) or LI-COR STR marker size standard (fluorescent method). Reference samples with known genotypes were used throughout the analysis to ensure consistent scoring of allele sizes among gels (for details, see Koljonen et al. (2002) and Säisä et al. (2003)).

### Statistical analysis

The number of alleles and the unbiased estimates for expected heterozygosity ( $H_e$ ) (Nei 1978) were calculated with POPGENE 1.32 software (<http://www.ualberta.ca/~fyeh/info.htm>). Exact tests for Hardy-Weinberg equilibrium (Guo and Thompson 1992) and population differentiation were analysed with the GENEPOP 3.2a program package (Raymond and Rousset 1995) with Markov chain parameters, 300 batches, and 3000 iterations. Probabilities of Hardy-Weinberg equilibrium tests for populations were adjusted over loci using the sequential Bonferroni procedure for multiple tests (Rice 1989).

The numbers of alleles in samples of unequal size were compared by allelic richness measure ( $R_s$ ) (El Mousadik and Petit 1996; Petit et al. 1998), which was calculated by the rarefaction approach with FSTAT version 2.9.3 software (Goudet 1995, 2001). The program calculates allelic richness for the smallest number of individuals typed for any locus. To increase the power of the comparison, only samples with more than 30 individuals were included. FSTAT was also used to test the significance of differences in average values of  $R_s$ ,  $H_e$ , and observed heterozygosity ( $H_o$ ) among the groups of populations (1000 permutations, one-sided test of the null hypothesis of no difference).

The populations were tested for a recent reduction in their effective size by the Wilcoxon sign-rank test as implemented in the BOTTLENECK (version 1.2.02) program (Cornuet and Luikart 1996) assuming the two-phase model of mutation (with 5% multistep changes and variance of 12) for microsatellite loci as recommended by Piry et al. (1999). FSTAT version 2.9.3 (Goudet 2001) was used to calculate the  $F_{ST}$  values ( $\theta$ ; Weir and Cockerham 1984). Spatial patterns of differentiation among the anadromous Baltic Sea salmon populations were tested for their fit to the isolation-by-distance model by analysing the regression of pairwise estimates of  $F_{ST}/(1 - F_{ST})$  on geographical distance (Rousset 1997). The significance of the fit was estimated by the Mantel test of the ISOLDE program in the GENEPOP 3.2a software package (Raymond and Rousset 1995) using 10 000 bootstraps. Geographical distances were measured as straight lines over the sea between river mouths. Having no opportunity to migrate, landlocked populations were excluded from the  $F_{ST}$  and isolation-by-distance analyses.

Genetic distances between populations were estimated according to the  $D_A$  distance of Nei et al. (1983) recommended for microsatellite data (Takezaki and Nei 1996), and a phylogenetic tree was constructed with the neighbour-joining algorithm (Saitou and Nei 1987) using the DISPAN program (Ota 1993). The tree file was converted into New Hampshire format with NJBAFD (Takezaki 1998) and the tree was drawn with MEGA 2.1 software (Kumar et al. 2001). The strength of the support for each node in the tree was assessed by bootstrapping 1000 times over loci. The genetic relationships among samples were also examined by multidimensional scaling analysis of the matrix of pairwise  $D_A$  distances with the STATISTICA v. 6.0 software package (Statsoft Inc., Tulsa, Oklahoma). Multidimensional scaling plots permit the genetic relationships among samples to be represented with minimum loss of information and in multidimensional space without imposing a bifurcating evolutionary history. The populations from Lakes Vänern and Saimaa were known to be affected by population size bottlenecks and so were excluded from the distance analysis.

European Atlantic salmon populations were analysed for hierarchical diversity according to Nei (1973, 1977). The following four hierarchical levels were used: (i) total, all anadromous European populations, (ii) main sea basins (Atlantic Ocean and the Baltic Sea), (iii) major population groups within the Atlantic Ocean (eastern Atlantic, White and Barents seas) and the Baltic Sea (northern, eastern, and southern Baltic), and (iv) populations within the groups. Grouping of the populations at different hierarchical levels was based on the clusters from neighbour-joining and multidimensional scaling analyses of genetic distances. All landlocked populations were excluded from the hierarchical analysis because their large genetic differentiation from anadromous populations (owing to complete lack of gene flow) causes bias in the total pattern.

An allele size randomization test (Hardy et al. 2003) implemented in the program SPAGeDi 1.1.b (Hardy and Vekemans 2002) was used to test whether stepwise mutations have contributed to the genetic differentiation among populations (i.e., whether  $R_{ST} > F_{ST}$ ). A significant outcome of the test suggests that populations have diverged for a sufficiently long time for mutations to contribute significantly

**Table 2.** Origin of Atlantic salmon (*Salmo salar*) samples, population propagation history, wild (W) or hatchery (H), sample size (*n*), heterozygosity ( $H_e$ ) with SE.

Population No.	River or lake	Propogation	<i>n</i>	<i>Ssa85</i>	<i>Ssa171</i>	<i>Ssa197</i>	<i>Ssa202</i>	<i>Ssa289</i>	<i>SSOSL85</i>	<i>SSOSL311</i>
<b>Northern Baltic Sea</b>										
1	Tornionjoki	W	56	9	11	15	8	4	8	16
2	Simojoki	W	59	7	9	13	4	5	7	15
3	Iijoki	H	61	9	9	13	5	4	11	14
4	Oulujoki	H	59	9	5	10	7	4	6	12
5	Kalixälven	W	57	10	10	13	6	4	8	14
6	Luleälven	H	60	11	11	14	7	5	9	18
7	Byskeälven	W	77	12	11	15	8	5	10	17
8	Skellefteälven	H	57	10	5	11	7	4	4	12
9	Vindelälven	W	50	9	6	13	6	6	4	9
10	Umeälven	H	50	8	6	13	7	5	6	11
11	Lögdeälven	W	50	9	6	10	5	4	7	10
12	Ängermanälven	H	60	14	8	13	8	6	7	12
13	Indalsälven	H	65	10	9	13	7	5	9	13
14	Ljungan	W	51	11	9	13	7	5	6	14
15	Ljusnan	H	53	11	9	12	7	6	11	14
16	Dalälven	H	60	9	8	15	7	5	7	13
Average										
<b>Southern Baltic Sea</b>										
17	Emån	W	54	10	8	11	7	6	8	14
18	Mörrumsån	W	45	9	7	12	6	5	9	11
Average										
<b>Eastern Baltic Sea</b>										
19	Neva	H	60	12	11	10	8	4	10	15
20	Kunda	W	61	8	6	8	4	5	4	10
21	Keila	W	53	8	8	12	6	3	5	13
22	Pärnu	W	26	6	9	8	4	3	5	10
23	Gauja	W	70	7	10	9	7	3	4	17
24	Daugava	H	70	9	10	10	8	4	3	17
25	Venta	W	66	6	8	10	8	2	3	11
Average										
<b>Lakes</b>										
26	Lake Vänern	H	50	4	3	7	4	4	5	7
27	Lake Saimaa	H	58	1	4	4	4	2	2	3
28	Lake Ladoga	W	94	12	7	9	6	4	6	15
29	Svir	W	48	9	5	6	4	3	4	9
30	Lake Onega	W	37	6	6	10	7	5	6	10
Average										
<b>Barents Sea and White Sea</b>										
31	Teno	W	59	12	12	20	10	4	11	15
32	Kola	W	88	12	15	17	14	4	11	24
33	Umba	W	70	11	8	16	12	6	9	21
Average										
<b>Atlantic Ocean, east coast</b>										
34	Lagan	H	48	8	10	16	9	4	10	14
35	Shannon	H	48	12	10	4	10	3	12	11
36	Oykel	H	50	15	14	22	9	4	13	20
37	Nith	H	50	15	16	20	11	4	9	17
38	Shin	H	50	12	15	23	9	4	9	17
Average										
<b>Total</b>			2180	27	27	30	16	9	20	36

observed number of alleles (each locus and total), mean number over nine loci with SD, total allelic richness ( $R_s$ ), mean  $R_s$ , and mean

<i>SSOSL417</i>	<i>SSOSL438</i>	Total observed	Mean	SD	Total $R_s$	Mean $R_s$	$H_e$	SE
10	4	85	9.4	4.2	80.0	8.9	0.70	0.07
5	3	68	7.6	4.1	63.6	7.1	0.70	0.06
9	4	78	8.7	4.3	71.1	7.9	0.68	0.07
7	3	63	7.0	2.9	58.6	6.5	0.68	0.07
10	6	81	9.0	3.3	74.5	8.3	0.71	0.05
9	7	91	10.1	4.0	83.6	9.3	0.76	0.04
11	8	97	10.8	3.7	84.6	9.4	0.76	0.03
9	3	65	7.2	3.4	93.4	10.4	0.70	0.06
11	4	68	7.6	3.1	64.3	7.1	0.67	0.07
11	3	70	7.8	3.5	65.7	7.3	0.65	0.06
8	6	65	7.2	2.2	64.2	7.1	0.73	0.04
12	6	86	9.6	3.2	79.0	8.8	0.75	0.03
11	4	81	9.0	3.2	74.1	8.2	0.74	0.03
12	6	83	9.2	3.4	80.1	8.9	0.77	0.03
10	6	86	9.6	2.8	79.7	8.9	0.74	0.03
9	4	77	8.6	3.5	71.5	7.9	0.73	0.04
			8.7		74.3	8.3	0.72	
11	5	80	8.9	2.8	75.1	8.3	0.73	0.03
11	7	77	8.6	2.5	75.4	8.4	0.72	0.02
			8.8		75.3	8.4	0.73	
9	4	83	9.2	3.6	78.0	8.7	0.75	0.04
6	3	54	6.0	2.3	48.9	5.4	0.61	0.05
5	4	64	7.1	3.5	60.4	6.7	0.69	0.05
7	4	56	6.2	2.4	—	—	0.71	0.04
7	5	69	7.7	4.2	64.3	7.1	0.68	0.05
5	5	71	7.9	4.3	67.6	7.5	0.70	0.05
6	4	58	6.4	3.1	55.7	6.2	0.70	0.05
			7.2		62.5	6.9	0.69	
4	3	41	4.6	1.5	40.4	4.5	0.63	0.03
4	2	26	2.9	2.1	24.8	2.8	0.47	0.07
7	4	70	7.8	3.7	61.1	6.8	0.72	0.03
5	6	51	5.7	2.1	49.2	5.5	0.62	0.06
5	4	59	6.6	2.1	—	—	0.67	0.04
			5.5		43.9	4.9	0.62	
13	4	101	11.2	5.0	93.1	10.3	0.78	0.04
15	9	121	13.4	5.6	104.4	11.6	0.81	0.03
10	7	100	11.1	4.8	89.2	9.9	0.75	0.04
			11.9		95.6	10.6	0.78	
8	8	87	9.7	3.5	—	—	0.81	0.03
4	8	74	8.2	3.6	—	—	0.78	0.03
14	11	122	13.6	5.4	115.0	12.8	0.83	0.03
13	9	114	12.7	4.9	109.2	12.1	0.84	0.02
12	8	109	12.1	5.6	102.5	11.4	0.80	0.04
		11.2		108.9	12.1	0.81		
23	17	205	22.8	8.2	—	—	0.72	—

to differentiation, which could be the case if populations have originated from different glacial refugia. Locus *Ssa171* was excluded from the test because its variation was due to both di- and tetra-nucleotide repeats. In addition, the sample from the River Simojoki was excluded because of deviations from the regular tetranucleotide repeat pattern at *Ssa197* and the sample from the River Shannon was excluded because of numerous missing genotypes at *Ssa197* and *SSOSL417*.

## Results

### Genetic diversity

A total of 205 alleles were observed across the nine microsatellite loci. The average number of alleles per locus was 22.8, ranging from nine at *Ssa289* to 36 at *SSOSL311* (Table 2). All loci were highly polymorphic in the 38 salmon samples studied with the exception of locus *Ssa85* in the Lake Saimaa landlocked population, which was fixed for allele \*132 (Fig. 2). The number of alleles was highest (24) at locus *SSOSL311* in the Russian River Kola population (Table 2). The total number of alleles over nine loci ranged from 26 for the Finnish Lake Saimaa and 41 for the Swedish Lake Vänern populations to 122 for the Scottish River Oykel population and 121 for the Russian River Kola population. The Baltic Sea area (including the landlocked populations) and eastern Atlantic salmon populations shared 141 (69%) alleles over all loci, whereas a total of 20 and 44 private alleles were observed in the Baltic and Atlantic populations, respectively (Fig. 2). Within the Baltic Sea, the northern Baltic (Gulf of Bothnia) and eastern Baltic (Gulf of Finland, eastern Main Basin, and Russian Lakes Onega and Ladoga) populations shared a similar proportion of alleles with the Atlantic populations (62.7% and 61.9%, respectively) but only 71.5% of alleles with each other owing to the presence of private alleles (29 and 16, respectively) in both population groups (Fig. 2). The allele frequencies of Lakes Saimaa and Vänern and the River Neva were unique. Thus, their pooling with other populations was not justified and they are shown separately in Fig. 2.

Deviations from Hardy–Weinberg equilibrium, at least in one locus and with a probability smaller than or equal to 0.01 after Bonferroni corrections, were observed in nine out of 38 populations, including six anadromous populations from the Rivers Tornionjoki (*SSOSL311*), Oulujoki (*SSOSL311*), Skellefteälven (*Ssa197* and *SSOSL311*), Dalälven (*Ssa171*), Venta (*SSOSL311*), and Shannon (*Ssa85* and *SSOSL311*) and three landlocked populations from the River Svir (*Ssa85*) and Lakes Ladoga (*SSOSL417*) and Vänern (*Ssa85*). The frequent deviation in observed genotype proportions from those expected at the *SSOSL311* locus could have been caused by sampling error, as the highest number of alleles (see above) was observed at this locus. An indication of a recent bottleneck was observed only in the landlocked Saimaa population (one-tailed Wilcoxon sign-rank test for heterozygosity excess,  $P < 0.05$ ). The number of significant ( $P < 0.05$ ) genotypic disequilibrium tests between microsatellite locus pairs, after application of Bonferroni correction for multiple tests, was lower than expected by chance (31 of 1368 tests, 36 tests for 38 populations), supporting the fact that all nine loci used were independent. Significant linkage disequilibrium for multiple loci (nine pairs) was detected only in the

Teno population, which might be a result of tributary substructure, as observed earlier by Elo et al. (1994) in this, the largest Atlantic salmon river in Europe.

Genetic diversity, expressed as the mean allelic richness and expected heterozygosity, was significantly higher ( $P < 0.01$ ) in the Atlantic populations (mean  $R_s = 11.0$  and  $H_e = 0.80$ ) than in the anadromous Baltic Sea populations (mean  $R_s = 7.2$  and  $H_e = 0.70$ ) (Table 2). Within the Baltic Sea, no significant differences in genetic diversity could be observed, either among the three geographical regions (north, east, and south) of the anadromous populations or between the wild and hatchery populations. There were also no differences in mean heterozygosities or in allelic richness between the wild and hatchery groups within the regions. However, the average allelic diversity and heterozygosity in landlocked populations (samples from the River Svir and Lakes Vänern, Saimaa, Onega, and Ladoga) were significantly lower than in the anadromous Baltic Sea populations (mean  $R_s = 4.8$  and 7.6 and  $H_e = 0.62$  and 0.71, respectively;  $P < 0.01$ ).

### Genetic relationships among salmon populations

Pairwise genetic distances ( $D_A$ ) were calculated between all salmon samples to investigate evolutionary relationships in allele frequencies. The greatest genetic distances (average 0.71) were found between North American (Michaels River – Big Brook, Labrador) and European salmon samples, ranging from 0.59 (between the Michaels River and the Barents Sea Rivers Kola and Nith) to 0.85 (between the Michaels River and Lake Saimaa). Within Europe, the greatest distances were observed between population pairs that included the landlocked population of Lake Saimaa, which was known to be a bottleneck population (from 0.41 to 0.65, average 0.50); the shortest distances were between the geographically proximate Baltic Sea populations within the eastern Main Basin (from 0.06 to 0.08) and within the Bothnian Sea (from 0.08 to 0.11).

The unrooted neighbour-joining phenogram depicting the underlying structure of the  $D_A$  distance matrix clustered the European salmon populations into Atlantic and Baltic population groups, separated from each other with 62% bootstrap support (Fig. 3). Within the Atlantic group, the Barents Sea and White Sea populations formed distinct branches. When the North American outgroup population was included, the root was located between the Barents Sea and the eastern Atlantic populations (figure not shown); in other respects, the rooted tree showed the same topology. The Swedish west coast population of the River Lagan, despite its geographical proximity to the Baltic Sea (population No. 34 in Fig. 1), was genetically most similar to the Irish population of the River Shannon, indicating a clear distinction between populations from the Atlantic Ocean and Baltic Sea. Within the Baltic Sea drainage basin, the anadromous populations clustered into northern (Gulf of Bothnia, 81% bootstrap support), eastern (Gulf of Finland and eastern Main Basin, 97% bootstrap support), and southern (western Main Basin, 99% bootstrap support) groups (Fig. 3). The landlocked populations of the two Russian lakes, Ladoga and Onega, formed a distinct subcluster (63% bootstrap support) on the eastern Baltic branch, whereas the salmon of the River Neva (outlet of Lake Ladoga to the Gulf of Finland) seem to be geneti-

**Fig. 2.** Average allele frequencies at nine microsatellite loci in the Atlantic salmon (*Salmo salar*) population groups studied. The groups were defined on the basis of geographical regions and the results of phylogenetic analysis. The surface areas of the circles are proportional to the frequencies of alleles.

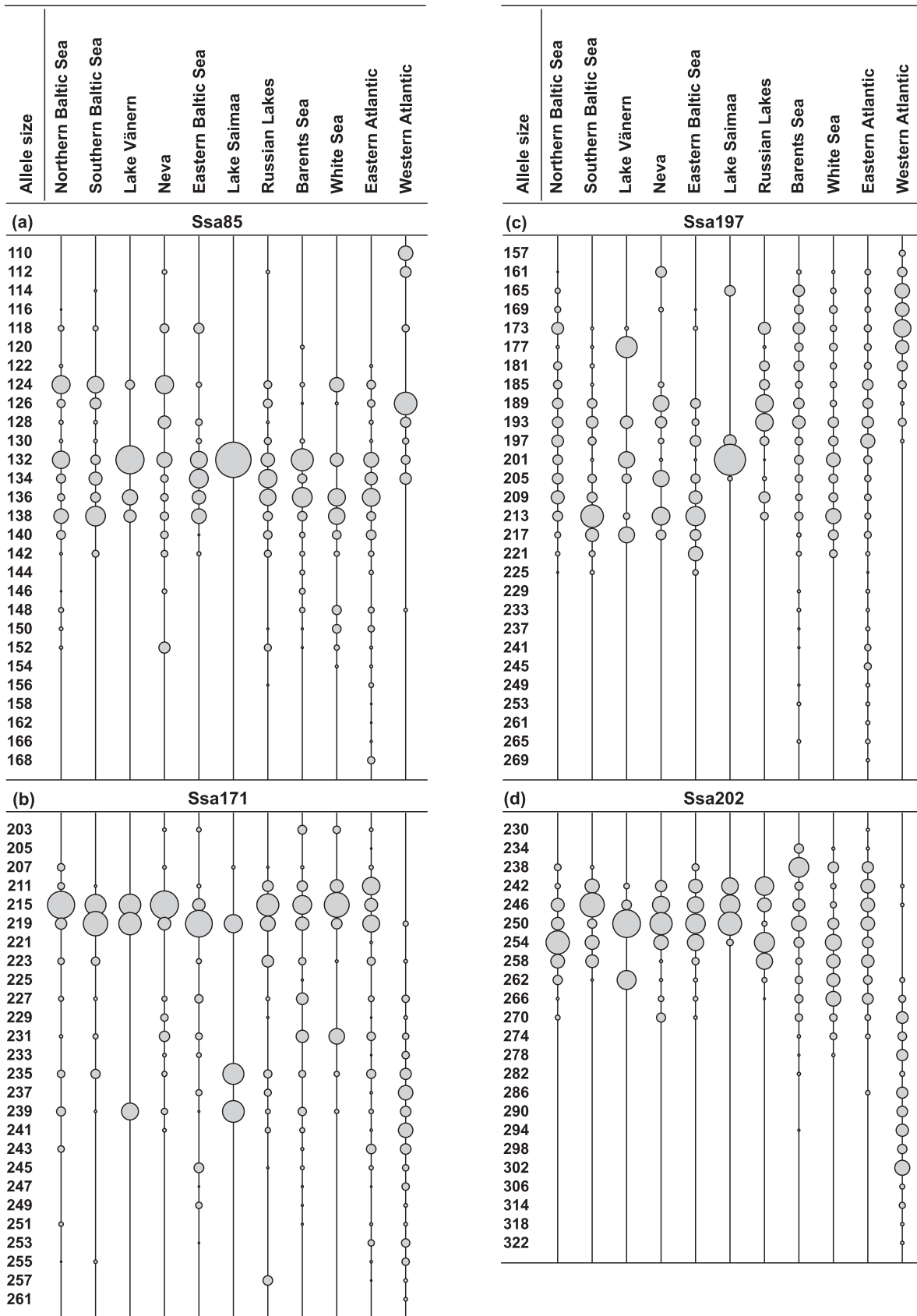
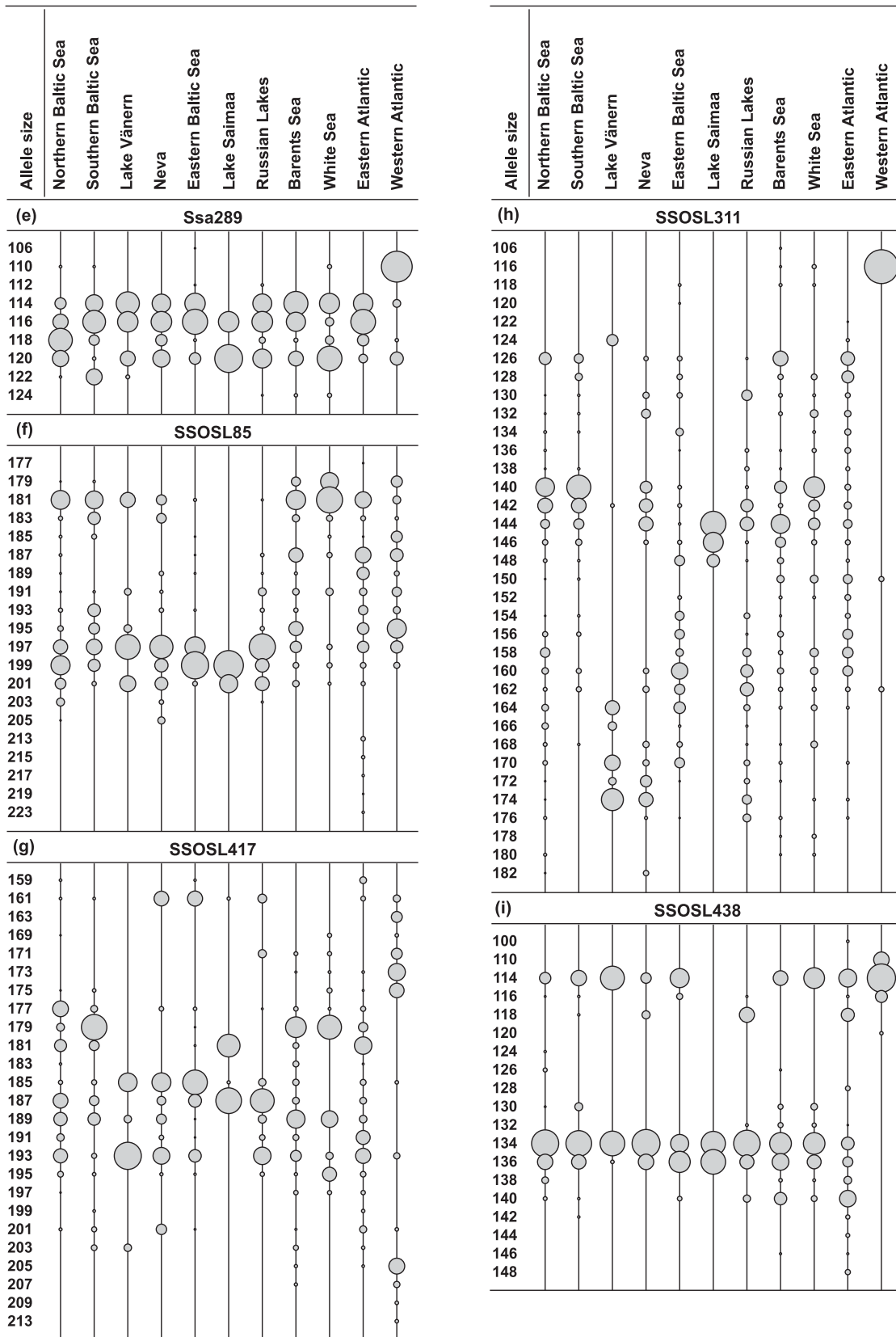
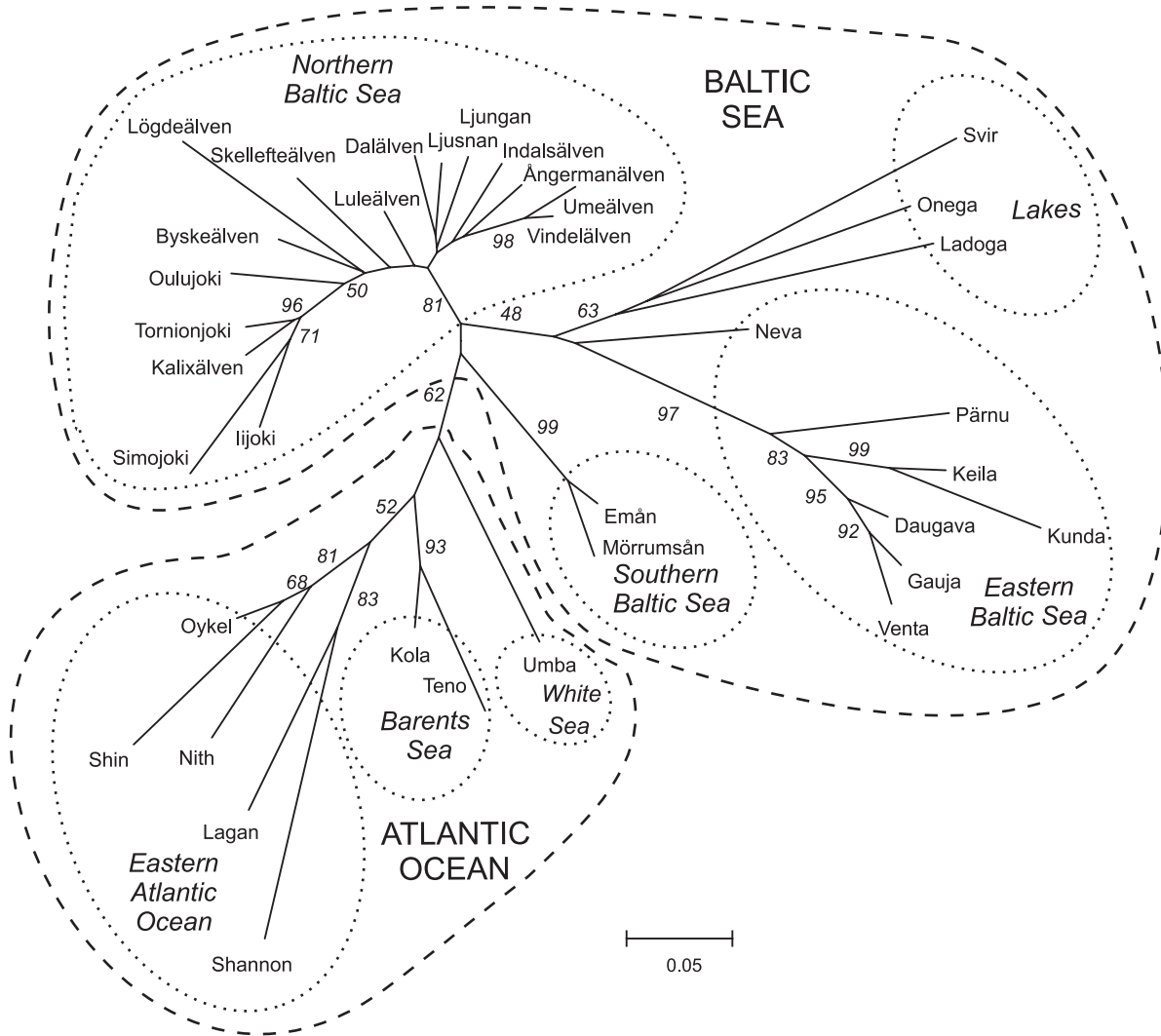


Fig. 2 (concluded).



**Fig. 3.** Unrooted neighbour-joining dendrogram of the European Atlantic salmon (*Salmo salar*) populations studied based on  $D_A$  distances from Nei et al. (1983). Major geographical groupings of populations are indicated by dotted circles. Numbers indicate branches with bootstrap support above 50% in 1000 replicates.



cally intermediate between the landlocked and anadromous populations of this eastern lineage (Fig. 3).

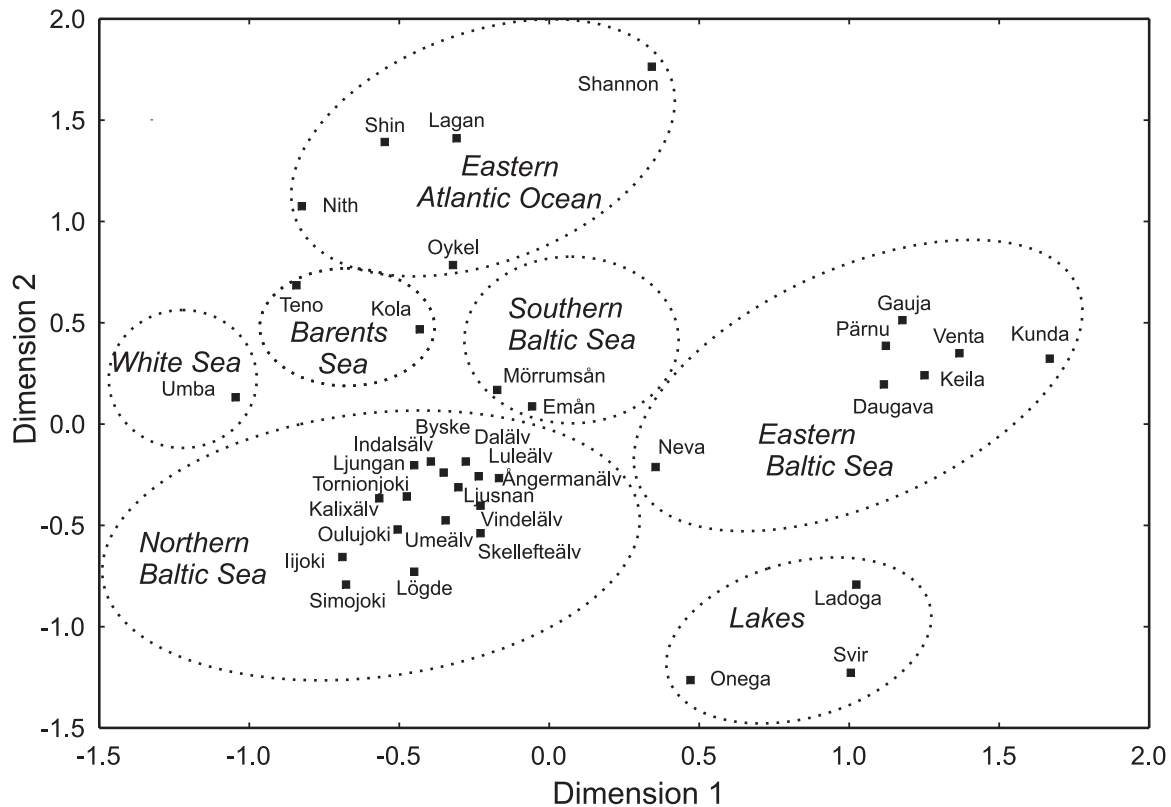
A very similar pattern of population relationships within Europe was also revealed by multidimensional scaling analysis of the  $D_A$  distance matrix (Fig. 4): the Atlantic and Baltic salmon populations were clearly separated from each other and the Baltic populations formed distinct clusters according to the geographical region (north, south, east, or landlocked).

The northern and southern Baltic Sea populations were genetically the most similar to each other (pairwise distances ranging from 0.14 to 0.29, average 0.23) (Table 3). The next most similar to these were the White Sea (0.26) and Barents Sea populations (0.28 and 0.26, respectively). Clearly, larger distances were measured for these northern and southern populations towards both the eastern lake and anadromous populations (0.34–0.37), so the dichotomy, noted earlier between eastern and western groups in both the allozyme (Koljonen et al. 1999) and mtDNA (Nilsson et al. 2001) data, was seen in the microsatellite data, too.

The distance from the southern group towards the eastern anadromous group was somewhat shorter (0.32) than that towards the northern group (0.35). The distance from the southern group towards the eastern Atlantic group was, however, quite large (0.34) and comparable with the distance to the eastern lake populations (Onega, Ladoga, and Svir) (0.37); thus, neither of these seemed to be a potential source population. In contrast, the distance from the southern group to the Barents Sea and White Sea populations was markedly shorter and the same (0.26) (Table 3).

The eastern Baltic Sea group was clearly very different from all other groups (average distance range 0.32–0.40), the most similar being the southern Baltic Sea group and the most distinct the White Sea and eastern Atlantic groups. Likewise, the Russian lake populations differed very clearly, both from each other (average distance 0.31) and from all others (0.32–0.45) (Table 3). The largest genetic distance observed was between the lake populations and the White Sea group (0.45). The eastern Atlantic populations also differed very markedly from the White Sea population (0.34)

**Fig. 4.** Two-dimensional scaling plot of the European Atlantic salmon (*Salmo salar*) populations studied based on the matrix of  $D_A$  distances from Nei et al. (1983). Major geographical groupings of populations are indicated by dotted circles.



**Table 3.** Average genetic distances ( $D_A$ ) (Nei et al. 1983) within (along the diagonal in bold) and between main groups (below the diagonal) of Atlantic salmon (*Salmo salar*) populations.

Population groups	Northern Baltic Sea	Southern Baltic Sea	Eastern Baltic Sea	Lakes Onega and Ladoga	Eastern Atlantic Ocean	Barents Sea
Northern Baltic Sea	<b>0.14</b>					
Southern Baltic Sea	0.23	<b>0.05</b>				
Eastern Baltic Sea	0.35	0.32	<b>0.18</b>			
Lakes Onega and Ladoga	0.34	0.37	0.37	<b>0.31</b>		
Eastern Atlantic Ocean	0.37	0.34	0.40	0.41	<b>0.23</b>	
Barents Sea	0.28	0.26	0.38	0.38	0.25	<b>0.15</b>
White Sea	0.26	0.26	0.40	0.45	0.34	0.18

and were at about the same distance (0.32–0.35) from the southern and northern Baltic Sea groups.

### Hierarchical diversity analysis and spatial differentiation patterns

The overall  $G_{ST}$  for all European salmon populations studied (including the landlocked populations) was 0.14 and for anadromous populations was 0.11 (Table 4). The results of the hierarchical gene diversity analysis for anadromous salmon populations revealed that only 1.9% of the genetic variation occurred between the Atlantic and Baltic basins; 9.4% was due to differentiation among populations within the basins and 88.6% was due to variation within populations (Table 4). Within the Atlantic basin, 2.2% of the differentiation was observed among the Barents Sea, White Sea, and eastern Atlantic regions, 4.1% among populations within the groups, and 93.7% within populations. Within the Baltic

basin, a higher percentage of variation was attributable to differences between the regions (5.6%) and among populations within the regions (5.2%), leaving less of the variation originating within the populations (89.2%). The level of differentiation among eastern Baltic populations (Gulf of Finland and eastern Main Basin) was somewhat higher than that among populations within the northern Baltic Sea ( $G_{ST} = 0.072$  and 0.054, respectively). Only marginal differentiation occurred between the two southern Baltic Sea populations, Mörrumsån and Emån ( $G_{ST} = 0.005$ ).

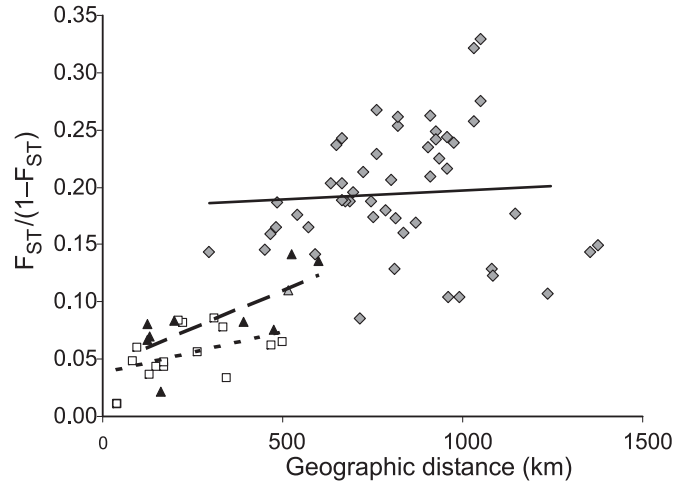
The Mantel test revealed a highly significant ( $P < 0.001$ ) association between geographical and genetic distances among anadromous Baltic salmon populations, and the regression of  $F_{ST}/(1 - F_{ST})$  estimates on geographical distance for pairs of populations explained 46.6% of the total variation for wild populations and 39.9% for hatchery populations. The association was highly significant ( $P < 0.001$ ) for

**Table 4.** Hierarchical gene diversity analysis of European anadromous Atlantic salmon (*Salmo salar*) populations studied.

Level of hierarchy	Gene diversity				Proportion of gene diversity components						
	K	H <sub>T</sub>	H <sub>B</sub>	H <sub>G</sub>	H <sub>S</sub>	G <sub>ST</sub>	G <sub>SB</sub>	G <sub>SG</sub>	G <sub>GB</sub>	G <sub>GT</sub>	G <sub>BT</sub>
Total Europe	33	0.82	0.81	0.77	0.73	0.114	0.094	0.048	0.046	0.065	0.019
Sea basins (B)											
Atlantic Ocean	8		0.85	0.83	0.80		0.063	0.041		0.022	
Baltic Sea	25		0.79	0.75	0.71		0.108	0.052		0.056	
Population groups (G) within basins											
Within the Atlantic Ocean											
Barents Sea (populations 31 and 32)	2			0.81	0.78			0.036			
White Sea (population 33)	1			0.75	0.75			—			
Atlantic Ocean (populations 34–38)	5			0.85	0.81			0.045			
Within the Baltic Sea											
Northern Baltic Sea (populations 1–16)	16			0.76	0.72			0.054			
Eastern Baltic Sea (populations 19–25)	7			0.74	0.69			0.072			
Southern Baltic Sea (populations 17 and 18)	2			0.73	0.72			0.005			

**Note:** K, number of populations within each grouping; H<sub>T</sub>, total diversity; H<sub>B</sub>, diversity for population groups within basins; H<sub>S</sub>, diversity for populations; G<sub>ST</sub>, proportion of diversity components between populations in the whole data set; G<sub>SB</sub>, proportion of diversity components between populations within basins; G<sub>SG</sub>, proportion of diversity components between populations within regions; G<sub>GB</sub>, proportion of diversity components between regions within basins; G<sub>GT</sub>, proportion of diversity components between regions within basins; G<sub>BT</sub>, proportion of diversity components between basins. See Tables 1 and 2 and Fig. 1 for population numbers.

**Fig. 5.** Regression of genetic versus geographical distance in wild anadromous Atlantic salmon (*Salmo salar*) populations from the Baltic Sea. Eastern Baltic Sea (triangles):  $y_{EB} = 1 \times 10^{-4}x + 0.0425$  ( $r^2 = 0.57$ ;  $P < 0.01$ ), northern Baltic Sea (squares):  $y_{NB} = 7 \times 10^{-5}x + 0.0389$  ( $r^2 = 0.21$ ,  $P < 0.01$ ), and interregional (diamonds):  $y_{IR} = 1 \times 10^{-5}x + 0.1818$  ( $r^2 = 0.004$ ,  $P > 0.05$ ).



both wild and hatchery populations. However, the greater the geographical distance between the populations, the weaker was the association, as evidenced by the continuously increasing dispersal of points on the plot. On smaller (regional) geographical scales (i.e., within the northern and eastern Baltic regions), the association between geographical and genetic distances was still significant ( $P < 0.01$ ) and explained 21% and 57% of the variation, respectively, when only wild populations were included (Fig. 5). On a larger (interregional) scale, however, with population pairs originating from different regions of the Baltic Sea, the geographical and genetic distances were unrelated ( $P = 0.11$ ) (Fig. 5), indicating that the level of differentiation between salmon populations from different Baltic Sea regions is unrelated to their geographical distance and that this differentiation can thus most probably be attributed to the different colonization histories of these regions.

**Contribution of stepwise mutations to differentiation**

The results of the allele size randomization tests revealed that at a large scale, multilocus estimates of  $R_{ST}$  values were significantly higher than  $F_{ST}$  values ( $R_{ST} = 0.17$  and  $F_{ST} = 0.13$  for all Baltic and Atlantic populations,  $P < 0.01$ ;  $R_{ST} = 0.16$  and  $F_{ST} = 0.11$  for all Baltic drainage populations,  $P < 0.05$ ) and shifts in average allele sizes contributed significantly to population differentiation. This is to be expected if populations had diverged for a sufficiently long time and is consistent with the hypothesis that Atlantic salmon colonized northern Europe from different refugia.

To further test the different colonization hypotheses of the Baltic Sea, anadromous populations from different regions (northern, eastern, and southern) of the Baltic Sea were compared with the potential source populations from the North Sea (Oykel, Nith, and Lagan) and Russian lakes (Ladoga and Onega). No significant contribution of stepwise mutations to population differentiation was revealed for eastern Baltic and Russian landlocked populations ( $R_{ST} = 0.14$

and  $F_{ST} = 0.12$ ,  $P = 0.13$ ), northern Baltic and North Sea populations ( $R_{ST} = 0.06$  and  $F_{ST} = 0.07$ ,  $P = 0.57$ ), or southern Baltic and North Sea populations ( $R_{ST} = 0.07$  and  $F_{ST} = 0.08$ ,  $P = 0.70$ ), suggesting a common origin for the populations for the specific population groups. In contrast, a significant contribution of stepwise mutations (and accordingly different origin) was revealed for eastern Baltic and North Sea populations ( $R_{ST} = 0.15$  and  $F_{ST} = 0.10$ ,  $P < 0.05$ ), northern Baltic and Russian landlocked populations ( $R_{ST} = 0.11$  and  $F_{ST} = 0.08$ ,  $P < 0.05$ ), and southern Baltic and Russian landlocked populations ( $R_{ST} = 0.34$  and  $F_{ST} = 0.15$ ,  $P < 0.01$ ). Within the Baltic Sea, the difference in average  $R_{ST}$  and  $F_{ST}$  values between the northern and eastern anadromous groups was close to significance ( $P = 0.08$ ). When the populations from these two regions were compared pairwise, a significant ( $P < 0.05$ ) contribution of shifts in allele average allele sizes to population differentiation was revealed in 23 out of 30 comparisons between wild Gulf of Finland (Rivers Kunda and Keila) and Gulf of Bothnia populations. A distinct origin can also be inferred for the Umba River population from the White Sea, which had significantly higher  $R_{ST}$  than  $F_{ST}$  values in most (21 out of 25) pairwise comparisons with the Baltic Sea anadromous populations. Also, the allele sizes of Lake Saimaa salmon differed significantly from those of Lake Onega ( $P = 0.01$ ) and indicatively from those of Lake Ladoga ( $P = 0.08$ ).

## Discussion

### Genetic diversity and differentiation

Analysis of the microsatellite DNA variation in 38 northern European Atlantic salmon populations revealed a level of genetic diversity (205 alleles at nine loci, average  $H_e = 0.72$ ) comparable with that found in the range-wide survey of microsatellite variation in 29 North American and European salmon populations (266 alleles at 12 loci, average  $H_e$  in Europe 0.73; King et al. 2001). However, the more dense sampling of northern European populations in our study enabled us to show that the diversity in Europe is distributed unevenly: the Baltic salmon populations are, on average, significantly less variable than the eastern Atlantic populations, and the diversity of landlocked populations (Lakes Vänern, Saimaa, Onega, and Ladoga) is in turn significantly lower than that of anadromous Baltic Sea populations. A similar trend had been noted in earlier studies of allozyme (Ståhl 1987) and mtDNA (Nilsson et al. 2001) markers and is most probably due to past population size bottlenecks and post-glacial founder effects. Here, only in the Lake Saimaa population was a recent reduction in effective population size detected.

Analysis of genetic distances between the population pairs revealed a clear grouping of populations according to geographical location (i.e., the eastern Atlantic (including the Barents and White seas) and the Baltic Sea basins). This is consistent with earlier findings of allozyme (Ståhl 1987; Bourke et al. 1997; Koljonen et al. 1999) and mtDNA (Verspoor et al. 1999; Nilsson et al. 2001) studies and supports the concept of effective isolation of Baltic salmon populations from their Atlantic counterparts. The proportion of variation owing to differentiation among populations within the basins was similar for microsatellite and allozyme data

(9.4% and 9.1%, respectively) but only about half of that for mtDNA (20.9%). This is consistent with the fact that the effective population size of the mtDNA data is approximately half of that of the autosomal genome, which promotes faster differentiation of populations for mtDNA than for microsatellite or allozyme markers.

The anadromous salmon populations within the Baltic Sea formed three distinct clusters corresponding to the northern (Gulf of Bothnia), eastern (Gulf of Finland and eastern Baltic Sea), and southern (western Main Basin) regions. The northern and eastern Baltic population clusters were also revealed by allozyme (Koljonen et al. 1999) and mtDNA (Nilsson et al. 2001) markers, but in the previous studies, the southern Baltic populations (Mörrumsån and Emån) clustered with the eastern Baltic Sea populations. Differences between the three geographical groups were considerable and explained 5.6% of the total microsatellite variation or approximately half (51.9%) of the interpopulation diversity in the Baltic Sea (10.8%), which is of similar magnitude as based on allozyme markers (50.7%; Koljonen et al. 1999). Estimated from the mtDNA data set in Nilsson et al. (2001), the differences between lineages explained a smaller proportion (about one third) of the interpopulation diversity in the Baltic Sea, but the level of overall population differentiation was much higher, accounting for 41.1% of the total variation.

### Colonization of the Baltic Sea

The whole Baltic Sea area was covered either totally or partly by ice during the phases of the last Weichselian (Wisconsinian) glaciation (from about 100 000 to 13 000 years before present (BP)). The Weichselian ice sheet also covered the two largest lakes in Europe, Onega and Ladoga in Russia, although the outer margin of the glacier was less than 100 km southeast of Lake Onega (Saarnisto et al. 1995).

Three hypotheses have been proposed for the origin of Baltic salmon: from the east from refugia in preglacial lakes before the Yoldia stage (Kazakov and Titov 1991; Nilsson et al. 2001), from the west from Atlantic populations via the Närke Strait (a large strait that connected the Swedish west coast with the northern Baltic Sea) at the beginning of the Yoldia stage (Verspoor et al. 1999), and from both directions (Koljonen et al. 1999). The hypothesis of a single western colonization of all Baltic Sea populations (Verspoor et al. 1999) was based on the mitochondrial *ND1* gene variation in European salmon populations. In that study, however, only the northern Baltic Sea group was represented (Rivers Tornionjoki, Simojoki, Luleälven, and Dalälven); those of the eastern or southern groups were not sampled. The similarity of the northern Baltic Sea group to the Atlantic populations is in any case fully compatible with results from all of the markers, microsatellites, allozymes, and mtDNA (Koljonen et al. 1999; Nilsson et al. 2001).

### Eastern Baltic Sea area

It has been proposed that the anadromous eastern Baltic Sea group (Gulf of Finland and eastern Main Basin) and the landlocked populations in the two Russian lakes, Onega and Ladoga, belong to the Ice Lake lineage, which colonized the Baltic Sea area from an eastern refugium during the Baltic Ice Lake stage (Koljonen et al. 1999; Nilsson et al. 2001; Gross et al. 2003). Current data fully support the close relat-

edness of these subgroups and also their clear divergence from the other groups. Some differentiation between the eastern lake and anadromous populations occurred and can be explained by random genetic drift. However, the possibility remains that this eastern Baltic salmon originates from more than one single eastern freshwater refugium.

Potential refugial areas for this eastern group have very likely existed at least in lakes in the upper Volga area. The ice margin was quite near the Lake Onega basin, which remained above sea level during deglaciation and has been a freshwater basin since Late Weichselian times. No sea connection has existed between the White Sea and Baltic basin. In contrast, the Lake Ladoga basin was below sea level at the Baltic Ice lake stage (Saarnisto et al. 1995; Saarnisto and Saarinen 2001). Lake Onega first drained south to the River Volga system and then to the Lake Ladoga basin. Later, it discharged for a short period (about 100 years) towards the White Sea, causing the water level to drop. This happened soon after deglaciation of the lake, at about 12 750 years BP, and before the lake started to drain towards the Baltic Ice Lake (M. Saarnisto, Geological Survey of Finland, Betonimiehenkuja 4, 02150 Espoo, Finland, personal communication). The first outlet of Lake Onega to the Lake Ladoga basin was probably the Vieljärvi threshold, but it soon turned towards the present, more southerly River Svir, clearly before 10 000 years BP. The Lake Ladoga basin deglaciated at about the same time as Lake Onega, 14 200 – 13 300 years ago (Saarnisto et al. 1995; Mangerud et al. 2004).

#### **Northern Baltic Sea**

The Northern Baltic salmon group showed the shortest genetic distance to the Atlantic group and also a very similar set of alleles with it, suggesting an origin different from that of the eastern Baltic Sea group. Koljonen et al. (1999) proposed invasion of salmon from the Atlantic through the Närke Strait, across southern Sweden, during the Yoldia stage. Nilsson et al. (2001), however, noted that one mtDNA haplotype common in the Atlantic was missing from the Baltic Sea. Also, the population in Lake Vänern, which once formed part of the Närke Strait, was fixed for the typical eastern Baltic haplotype. Some similarity between Lake Vänern salmon and the eastern Baltic populations was seen in the microsatellite data, too. Still, the composition of *GH-1* gene haplotypes in Lake Vänern was more similar to Atlantic than to Baltic salmon populations (Gross et al. 2003). These discrepancies might, however, be explained by the known bottleneck event. In all, several mitochondrial haplotypes absent from the southern and eastern Baltic populations are shared with the northern Baltic Sea group and Atlantic populations (Nilsson et al. 2001). The similarity of the northern group to the Atlantic populations is also clearly shown by both allozyme (Koljonen et al. 1999) and current microsatellite data. Thus, the entry from the Atlantic via the Närke Strait route about 11 500 calendar years BP remains the most likely colonization hypothesis for the northern Baltic Sea group, although the original colonization lineage may later have admixed to some extent with other lineages. The Närke Strait has been proposed as a colonization route for brown trout (*Salmo trutta*) (Bernatchez and Osinov 1995; García-Marín et al. 1999) and also for several other marine

fish and mammal species (e.g., the ringed seal (*Phoca hispida*)) (Forstén and Alhonen 1975; Ukkonen 1993).

#### **Southern Baltic Sea**

Here, the southern Baltic populations of Mörrumsån and Emån form a distinct cluster that, in the dendrogram, is located between the Atlantic and other Baltic population clusters, with the shortest genetic distance within the Baltic Sea being to the northern Baltic Sea group. In all, its genetic distances are shortest to the White Sea and Barents Sea populations. There is no evidence of mutational contribution to the differentiation, but because differentiation is very clear and the mtDNA haplotype composition of this southern Baltic group is, in contrast with allozyme and microsatellite data, identical to that of the eastern Baltic and, further, differs considerably from that of both the Swedish west coast populations and the northern Baltic Sea group (Nilsson et al. 2001), these southern populations are here regarded as a distinct lineage.

The southern Baltic salmon populations were geographically closest to Atlantic waters after the Närke Strait route was closed for migration at 11 000 years BP (Björck et al. 2002) and the Danish Straits opened. However, this Atlantic colonization direction is unlikely because the mtDNA is similar to that of the eastern populations. Moreover, it has been shown that neither Baltic nor Swedish west coast wild salmon pass the Danish Straits in significant numbers (Christensen and Larsson 1979). It is more likely that this area was colonized from a southern refugium during the Baltic Ice Lake stage, as it was deglaciated very early in Baltic Sea history and preglacial ice-dammed lakes are also known to have existed in the Neman, Vistula, Odra, and Elbe drainage basins (Marks 2002), possibly serving as glacial refugia for salmon. The Atlantic salmon populations of these drainages have become extinct, and so no comparison with a living potential source population is possible. An older, Atlantic-type of origin is further supported by the marked similarity with northern Atlantic stocks of the Barents Sea and White Sea area. Southern refugia have been important for colonization of the Scandinavian Peninsula by several freshwater fish (e.g., perch (*Perca fluviatilis*), bullhead (*Cottus gobio*), and European grayling (*Thymallus thymallus*)) (Nesbø et al. 1999; Koskinen et al. 2000; Kontula and Väinölä 2001).

#### **Lake Saimaa**

Here, the Saimaa population is genetically most similar to the northern Baltic salmon group, which is consistent with allozyme (Koljonen et al. 1999) and mtDNA (Nilsson et al. 2001) data. Before uplift, the waters of the Saimaa area drained northwards to the Bothnian Sea. This ancient connection could explain the similarity of Saimaa salmon to the northern Baltic Sea group instead of to the geographically closer Russian lake populations.

#### **Colonization of Atlantic populations**

On the basis of mtDNA studies, it has been suggested that the Atlantic drainages of the British Isles, Iceland, Scandinavia, and the Barents Sea along the Kola Peninsula have all been colonized by salmon from Iberian and North Sea glacial refugia (Verspoor et al. 1999; Consuegra et al. 2002). Asplund et al. (2004) proposed that the salmon in the Kola

Peninsula rivers draining to the White Sea originated primarily from the eastern Barents Sea, which was free of ice during the late Pleistocene glacial maximum (Svendsen et al. 1999; Mangerud et al. 2002, 2004) and could have been populated by salmon that survived the early Weichselian glacial maximum in the proglacial Komi Ice Lake (Mangerud et al. 2001). The Karelian and south coast populations of the White Sea were proposed to originate from a late Weichselian freshwater refugium (Asplund et al. 2004). Our microsatellite data are, in general, consistent with these hypotheses. In our data, the eastern Atlantic populations from Ireland, Scotland, and the Swedish west coast form a relatively compact group distinct from the Karelian White Sea, River Uмба population. The two Barents Sea populations are genetically intermediate between the Atlantic and White Sea salmon. Allele size permutation tests also supported the hypothesis of a different lineage in the White Sea area.

Kazakov and Titov (1991) suggested an alternative colonization route through a connection between the Barents Sea coast and ice lakes in the White Sea area and Karelia over the Kola Peninsula in the Lake Imandra region during the late glacial period. Nilsson et al. (2001) found an mtDNA haplotype that was common among the populations of the Barents and White seas and was also present in the Baltic Sea. However, a common refugium for the White Sea and eastern Baltic groups during the last glaciation seems unlikely, as microsatellite data show that their genetic distance is the highest within Europe. Their similarity in mtDNA data might be attributed to their connections before the last glaciation, when they were part of the ancient Scandinavian Atlantic salmon population. Similar preglacial connections might explain the similarity of the southern and eastern groups within the Baltic Sea, even though they spent the last glaciation in different refugia. The information from mtDNA is more probably related to preglacial history, and several layers of colonization events can be assumed to have occurred. The ancient Scandinavian Atlantic salmon may have included at least four of the current groups: the southern and eastern Baltic Sea groups, the Russian lake populations, and the White Sea salmon. The fish of the eastern Atlantic group on the Norwegian coast and Barents Sea and the northern Baltic Sea group are thus more recent arrivals in Scandinavian waters.

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