Molecular Ecology (1999) **8**, 1387–1404

Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization

C. L. NESBØ,* T. FOSSHEIM,* L. A. VØLLESTAD† and K. S. JAKOBSEN*
**Div. of General Genetics, †Div. of Zoology, University of Oslo, PO Box 1031 Blindern, N-0315 Oslo, Norway*

Abstract

We used the widely distributed freshwater fish, perch (*Perca fluviatilis*), to investigate the postglacial colonization routes of freshwater fishes in Europe. Genetic variability within and among drainages was assessed using mitochondrial DNA (mtDNA) D-loop sequencing and RAPD markers from 55 populations all over Europe as well as one Siberian population. High level of structuring for both markers was observed among drainages and regions, while little differentiation was seen within drainages and regions. Phylogeographic relationships among European perch were determined from the distribution of 35 mtDNA haplotypes detected in the samples. In addition to a distinct southern European group, which includes a Greek and a southern Danubian population, three major groups of perch are observed: the western European drainages, the eastern European drainages including the Siberian population, and Norwegian populations from northern Norway, and western side of Oslofjord. Our data suggest that present perch populations in western and northern Europe were colonized from three main refugia, located in southeastern, northeastern and western Europe. In support of this, nested cladistic analysis of mtDNA clade and nested clade distances suggested historical range expansion as the main factor determining geographical distribution of haplotypes. The Baltic Sea has been colonized from all three refugia, and northeastern Europe harbours descendants from both eastern European refugia. In the upper part of the Danube lineages from the western European and the southern European refugia meet. The southern European refugium probably did not contribute to the recolonization of other western and northern European drainages after the last glaciation. However, phylogenetic analyses suggest that the southern European mtDNA lineage is the most ancient, and therefore likely to be the founder of all present perch lineages. The colonization routes used by perch probably also apply to other freshwater species with similar distribution patterns.

Keywords: D-loop, glacial refugia, mtDNA, *Perca fluviatilis*, phylogeography, postglacial colonization, RAPD

Received 1 November 1998; revision received 4 March 1999; accepted 23 March 1999

Introduction

Rapid and dramatic climatic changes have taken place in the last 2.5 million years (Myr), causing large ice sheets to expand (ice ages) or contract (interglacials) (Andersen & Borns 1994). In the last 0.9 Myr the glaciations have occurred in cycles of approximately 0.1 Myr, characterized by long glaciations and short interglacials (Andersen & Borns 1994). From genetic data of animal populations in Europe and North America, it is evident that present populations reflect the conditions during glaciations in the Pleistocene and the colonization following the glacier withdrawals (Avise 1992; Hewitt 1996; Bernatchez & Wilson 1998; Taberlet 1998; Taberlet *et al*. 1998). Freshwater fishes constitute an important group of organisms in zoogeographic studies, as they are only able to disperse in water bodies of low salinity (Bianco 1990). Due to the barriers formed by land and seawater, gene flow between populations

Correspondence: K. S. Jakobsen. Fax: + 47 22 85 45 05; E-mail: k.s.jakobsen@bio.uio.no

is limited, making the distribution pattern of genetic markers conservative, mainly reflecting dispersal history. Studies of freshwater fishes in North America have revealed strong correlation of genetic lineage distribution and postglacial migration from different refuge areas (reviewed in, e.g. Bernatchez & Wilson 1998). In Europe, phylogeography and postglacial migration patterns of freshwater fishes are largely unknown. However, most studies of European freshwater and anadromous fishes suggest glacial impacts on the geographical distribution of intraspecific polymorphism (e.g. Persat & Berrebi 1990; Bernatchez *et al*. 1992; Bernatchez & Dodson 1994; Osinov & Bernatchez 1996; Stepien *et al*. 1998; García-Marín *et al*. 1999; Hansen *et al*. 1999). For instance, by analysing allozyme loci García-Marín *et al*. (1999) found that present brown trout (*Salmo trutta*) populations in northern Europe probably represent descendants from three refuge areas; two located in western and one located in southeastern Europe.

While previous studies of the widely distributed freshwater fish, perch (*Perca fluviatilis*), using isoenzymes have demonstrated extremely low levels of gene diversity (e.g. Gyllensten *et al*. 1985; Heldstab & Katoh 1995; Marsden *et al*. 1995), nuclear and mitochondrial DNA (mtDNA) markers showing correlation to population history have recently been identified (Nesbø *et al*. 1998b; Refseth *et al*. 1998). In addition to its wide distribution and opportunistic and mobile behaviour (Craig 1987), there are also other reasons for perch being one of the best model species for studying the postglacial history of freshwater fishes. First, the perch tolerates a wide range of temperatures, and was among the earliest colonisers throughout the Pleistocene interglacials (Jerzmanska & Raczynski 1991, but see Shechrebukha 1993). Second, in contrast to the commonly studied salmonid species (Riffel *et al*. 1995; Largiadèr *et al*. 1996) the effect of stocking (dispersal by humans) is not a problem when considering perch. This implies that the distribution of genetic markers within and among perch populations can be expected to reflect both the distribution of refuge areas for freshwater fishes as well as the recolonization pattern of formerly glaciated areas in northern Europe.

Studies of Scandinavian perch populations utilizing mtDNA D-loop sequence polymorphisms detected two major phylogeographic areas in southern Scandinavia (Refseth *et al*. 1998). It was suggested that this pattern was caused by colonization of southern Scandinavia in different waves from two glacial refugia (Refseth *et al*. 1998). In addition to the mtDNA lineages observed in the Scandinavian peninsula, a third lineage was observed in the Baltic Sea (Nesbø *et al*. 1998b; Refseth *et al*. 1998), indicating an even more complex history of this area.

The aim of this study was to obtain information on the phylogeography of perch throughout Europe, and possibly to reveal glacial refuge areas and postglacial recolonization routes for this and, indirectly, other species of freshwater fishes. We have sampled perch from widespread localities in order to survey as much as possible of the intraspecific genetic variation. Moreover, we have focused on areas that could serve as glacial refuge areas for euthermic freshwater fishes (southern France, Danube, and Russian rivers). Fifty-five European populations of perch and one Siberian population have been analysed, representing both unglaciated and recolonized drainages. All perch samples were analysed for mtDNA D-loop sequence variation. However, as data from just one locus are not necessarily representative for the whole genome (e.g. Pamilo & Nei 1988), we have also used RAPD (random amplified polymorphic DNA) markers to complement the mtDNA data. By combining phylogeographic relationships and inter- and intrapopulation genetic variation, we suggest possible refugia for perch during the most recent glaciations in Europe.

Materials and methods

Sample preparation, DNA isolation, amplifications and sequencing

Tail fins were removed from 313 perch collected from 34 populations (Table 1), and stored in 96% ethanol. On average nine individuals were sampled from each locality. Ten yellow perch (*Perca flavescens*) were sampled from L. Michigan (North America). DNA was isolated using the DNA Direct kit (Dynal AS; Rudi *et al*. 1997). All samples were analysed for mtDNA D-loop sequence variation. A subsample was analysed for RAPD polymorphism ($N = 178$).

The following primers were used to amplify and sequence 365 bases from the 5′ end of the mtDNA D-loop (corresponding to base 65–430 in Accession no. 14724): HV2: TTCCCCGGTCTTGTAAACC (Nesbø *et al*. 1998a), CSB-D: GGAACCAAATGCCAGGAA (Nesbø *et al*. 1998a), Sequencing primer: Texas Red, GTCCCTCACCTTCAATAACC (Nesbø *et al*. 1998b). The reaction conditions and amplification profile were as described in Nesbø *et al*. (1998a,b). The mtDNA sequences were run either manually using S^{35} labelling, or on a Vistra DNA Sequencer 725 (Vistra DNA systems, Molecular Dynamics & Amersham Life Science, UK) using Texas Red-labelled primers. For manual sequencing, biotinylated PCR products were directly sequenced by the magnetic solid-phase approach utilizing Dynabeads M-280 Streptavidin (Dynal AS, Oslo, Norway; Hultman *et al*. 1989).

The utility of RAPDs to generate phylogenetic informative DNA fingerprints among fish species has been demonstrated in, e.g., Borowsky *et al*. (1995) (see also Fritch & Rieseberg 1996). Three RAPD primers, A4, A11 and C18, giving a total of 19 variable bands, were selected from the

Table 1 Sampling sites and distribution of mtDNA haplotypes. mtDNA haplotypes in populations from Refseth *et al*. (1998) and Nesbø *et al*. (1998b) used in AMOVA are also included. The major drainages to which the populations belong are indicated

No.	Population	Region*	*N*	Drainage/AMOVA†		Haplotypes (*N*)
1a‡	L. Toke	SN	5	Toke	1	A1 (5)
1b	L. Steinbutjønn	SN	5	western side of Oslofjord	1	A1 (5)
2a‡	L. Sandungen	SN	5	western side of Oslofjord	1	A (5)
2b‡	L. Eikern	SN	7	Drammen	1	A (3), A4 (3)
3‡	Glomma R.	SN	6	Glomma	2	A (2), F (2), A7 (1), A9 (1)
4‡	L. Storsjøen	SN	5	Glomma	2	F (5)
5‡	L. Mälaren	SS	5	Baltic Sea	3	A7 (1), F (4)
6‡	Gulf of Bothnia	NS	20	Gulf of Bothnia¶	3	A7 (2), C (14), F1 (4)
7‡	Anadromous A	NS	20	Gulf of Bothnia¶	3	A (2), A7 (4), C (8), F (3), F1 (2), G (1)
8‡	Anadromous B	NS	17	Gulf of Bothnia¶	3	A (4), C (6), C1 (1), F (2), F1 (2), G (2)
9‡	L. Ängersjön	NS	19	Gulf of Bothnia¶	3	A5 (1), C (13), C1 (4), F1 (1)
10	Angara R.	R	10	Baikal	4	C (10)
11	L. Rybinsk	R	14	Volga§	5	A (1), C (7), C2 (1), C3 (2), G1 (1), J (2)
12	L. Krylovo	R	5	Vilia/Neman¶	6	C (5)
13	L. Hencza	P	5	Neman§	6	C (4), C1 (1)
14	L. Dubrovskoje	R	10	Dnipr§**	6	C (10)
15	L. Vourasjavre	NN	10	Karasjåkka/Tana	7	A (4), A2 (6)
16	L. Vesijärvi	F	10	Kyminijoki¶	8	A2 (1), C (4), C1 (2), F (3)
17	L. Peipsi	ES	9	Emjajogi/Narva¶	9	A6 (4), A7 (1), A8 (1), C (2), J1 (1)
18	Emjajogi R.	ES	5	Emjajogi	9	A (1), A7 (1), A8 (1), C (1), J2 (1)
19	Matsalu Bay	ES	10	Baltic sea	3	A8 (3), F (4), F1(1), F7 (1), E2 (1)
20	Pilico R.	P	10	Vistula¶	10	F (9), G1 (1)
21	L. Kortowo	P	5	Pregola/Vistula¶	10	F (3), F1 (2)
22	L. Lyngsø	D	10	Gudenå/Elbe¶	11	F (10)
23	L. Bautzen	G	10	Elbe¶	11	F (3), F1 (4), F6 (3)
24	L. Ijsselmeer	NE	11	Rhine	12	E (2), F (3), F1(4), F4 (2)
25	Rhine R.	FR	7	Rhine	12	F (7)
26	Moselle R.	FR	7	Moselle/Rhine	12	F (7)
27	Mause R.	FR	5	Mause/Rhine	12	F (5)
28	L. Zürich	SW	10	Rhine	12	F (10)
29	L. Aegeri	SW	10	Rhine	12	E (3), F5 (7)
30	Rhone R.	FR	9	Rhône	13	F (9)
31	Saone R.	FR	9	Saône	13	F (9)
32	L. Leman	FR	10	Rhône	13	F (8), F4 (2)
33	L. Fermur	FR	10	Rance/Seine	14	F (10)
34	L. Windermere	E	10	Thames	15	F (10)
35	Lee R.	IR	7	Lee	16	F4 (7)
36	L. Corbara	IT	10	Tiber	17	E1 (1), F (3), F1 (4), K (1), M (1)
37	L. Trasimeno	IT	10	Tiber	17	F (10)
38	Danube R.	AU	20	Danube**	18	F (11), M (9)
39	L. Race	SL	10	Drava/Danube**	18	C (1), M1 (9)
40	L. of Doirane	GR	10	Axios	19	K1 (9), K2 (1)
41	Siret R.	RO	10	Siret**	20	F1 (1), C (4), C2 (4), J (1)
42	Prut R.	RO	11	Prut**	20	C (6), C2 (2), J (2), M (1)
Total sequenced here						313 (34 populations; pop. nos 1b, 10–42)
Sequences from Refseth *et al*. (1998) not included above						56 (12 populations)
Total analysed						488 (56 populations)

*SN, southern Norway; SS, southern Sweden; NS, northern Sweden; R, Russia; NN, northern Norway; F, Finland; ES, Estonia; P, Poland; D, Denmark; G, Germany; NE, Netherlands; FR, France; SW, Switzerland; E, England; IR, Ireland; IT, Italy; AU, Austria; SL, Slovenia; GR, Greece; RO, Romania. The Neman and Dnipir were probably connected during and immediately after the last glaciation (Zhukov 1965). The Danish river Gudenå was a tributary to Elbe in postglacial times from ≈ 13 000–7500 BP (Nielsen 1975). The perch from the French river Rance are probably recent invaders which have been spread by the establishment of artificial dams and reservoirs (Dr Eric Feunteun, Université de Rennes, personal communication).

†Number refers to the group assigned to in the AMOVA analysis when analysing between drainage variation. Population 1b and 2a represent minor Norwegian drainages, hence the geographical region is included instead.

‡mtDNA data obtained from Refseth *et al*. (1998) or Nesbø *et al*. (1998b). One individual from pop. no. 15 was sequenced in Refseth *et al*. (1998). Pop. nos 1a, 2a, 2b, 3, 4 and 5 are representatives from the three southern Scandinavian phylogeographic regions detected in Refseth *et al*. (1998). For haplotype distribution of the other southern Scandinavian populations used in the AMOVA and nested cladistic analysis of geographical distances see Refseth *et al*. (1998).

§Drainages that enter the Caspian Sea.

¶Drainages that enter, or postglacialy entered, the Baltic Sea. The Gulf of Bothnia is the northern part of the Baltic Sea.

**Drainages that enter the Black Sea.

Table 2 Observed frequency of polymorphic RAPD bands within the four phylogeographic groups and the three suture zones detected from the distribution of mtDNA haplotypes. The populations investigated for RAPD variation in each group are indicated. The RAPD bands are given as approximate size (bp) of the amplified PCR product. Bands observed in only one population within a group are indicated by the population number according to Table 1 and Fig. 1A

Band	Group I (39, 40)	Group II (10–14, 42)	Group III (21–34)	Group IV (1a, 2a, 2b)	Danube (38)	Estonia (17, 18)	Baltic Sea* (4–9, 16, 19)
A4							
500	0.50[40]	—	—	—	—	—	—
950	0.40[40]	0.43	0.08	—	—	0.11	0.17
1250	—	0.17	—	—	—	—	—
1300	1.00	0.88	0.99	0.38	1.00	1.00	0.96
1350	0.30[39]	0.18	0.07	0.88	0.30	0.00	0.36
A11							
750	0.40[39]	0.08[42]	—	—	1.00	—	—
800	0.90	1.00	1.00	1.00	0.57	1.00	1.00
850	—	0.60	0.11	0.13	0.14	0.89	0.55
1000	0.70	0.60	0.76	0.88	0.57	0.78	0.91
C18							
570	1.00	0.14	0.07	1.00	0.88	0.11	0.15
600	—	0.86	0.93	—	0.12	0.89	0.85
840	—	0.17	0.14	—	—	0.22	0.34
870	—	—	0.07	—	0.38	—	—
880	—	0.03[12]	0.78	—	0.50	0.56	0.20
900	0.60	0.11	0.10	0.44	0.13	0.22	0.30
920	0.40	0.69	0.04	0.63	—	0.33	0.08
950	—	0.03[14]	0.03[21]	—	—	0.11	0.41
1000	—	0.05[10]	—	—	—	—	—
1080	0.88	0.94	0.99	1.00	1.00	1.00	0.98

*Pop. no. 4 sampled from the eastern side of the Oslofjord was included in the Baltic Sea 'group' because only this population from the southen Scandinavian suture zone was analysed for RAPD polymorphism.

Operon A and C kits (Operon Technologies, Alameda, CA, USA) based on prescreening of perch representing most major European drainages. Lynch & Milligan (1994) showed that it is important to increase the number of individuals to achieve the same statistical power for dominant RAPD markers compared to codominant markers. Hence, because our objective was to score similarity between geographical regions, we chose to use a relatively low number of markers on a large number of populations. PCR conditions and amplification profile were as described in Nesbø *et al*. (1998b). PCR products were separated on 1.4% agarose gels and visualized by ethidium bromide staining. To confirm reproducibility of the RAPD phenotypes obtained, at least one individual of each phenotype observed within a population was amplified twice. Representatives of RAPD phenotypes from different gels were run together in order to score bands between gels. The RAPD phenotype of each individual was expressed as a binary vector. Individuals displayed only one of the RAPD C18 570 bp and C18 600 bp bands, and the frequency always added up to 1 (see Table 2). Therefore, only the C18 600 bp band was included in the analyses (Lynch & Milligan 1994).

Data analysis

The samples obtained in this study did not cover the Scandinavian peninsula and the northern part of the Baltic Sea. As perch in Scandinavia has been investigated in two previous studies (Nesbø *et al*. 1998b; Refseth *et al*. 1998), we included mtDNA D-loop haplotypes and RAPD phenotypes from these studies in the present study of European perch; 109 mtDNA D-loop sequences obtained from 18 populations investigated by Refseth *et al*. (1998), and 66 mtDNA D-loop sequences and 24 RAPDs from four populations investigated by Nesbø *et al*. (1998b), giving a total $N = 488$ (56 populations) for the sequence data and a total $N = 202$ (35 populations) for the RAPD data.

A haplotype cladogram was estimated according to Templeton *et al*. (1992). This cladogram estimation procedure is specifically designed to estimate intraspecific gene trees where most of the nodes (haplotypes) are present in the population under investigation, and are only a few mutations apart. High levels of site and length variation heteroplasmy associated with a tandemly repeated array flanking the *trnP* gene were reported by Nesbø *et al*. (1998a).

Hence, the variable positions 111–137 were initially excluded, and a cladogram was estimated based on the remaining 15 unique haplotypes. The haplotypes defined by mutations in the repeated array were then added to the cladogram and all such mutations were considered to be one mutational step from the internal haplotype in the respective clade; i.e. from haplotype A, C, F, G, E, J, K or M. The resulting cladogram was then converted into a nested design as described in Templeton *et al.* (1987) and Templeton & Sing (1993). The units defining the nested branches of the cladogram are called *n*-step clades, where *n* indicates the number of mutational steps necessary to define the clade. 0-step clades refer to haplotypes. Given the *n*-step clades, the $n + 1$ clades are defined by the union of all *n*-step clades that can be joined together by moving back one mutational step from the terminal *n*-step clades. Hence, haplotypes are grouped into 1-step clades (i.e. 1–1, 1–2, 1–3, etc.), and these clades are further grouped into 2-step clades (i.e. 2–1, 2–2 and 2–3).

The root and relative age of the haplotypes were evaluated by phylogenetic analyses using yellow perch as outgroup (Nesbø *et al*. 1998a). Both distance- and character-based analyses were performed in PAUP* (Swofford 1998). Divergence times were estimated from the mtDNA sequences using the formulas in Tajima (1983) and Templeton (1993). Generally, a 2% mtDNA divergence rate per Myr has been assumed for bony fishes (see e.g. Faber & Stepien 1997); however, divergence rates as low as 0.5% have been suggested (Cantatore *et al*. 1994). Moreover, in some Perciform species higher intraspecific divergence rates have been suggested in the 5′ end of the D-loop, e.g. 6–7%/Myr for the same segment as used here for cichlids (e.g. Meyer *et al*. 1990; see also Strecker *et al*. 1996), and about 12% in the D-loop of swordfish (*Xiphias gladius*; Alvarado Bremer *et al*. 1995). Comparison of divergence within the mtDNA *cytb* gene and the D-loop in mackerel (*Scomber scombrus*) suggested a five-times higher divergence rate in the 5′ D-loop compared to the *cytb* gene (i.e. 2–10% depending on the *cytb* rate assumed; Nesbø *et al*. unpublished). Somewhat lower mutation rates (1.4-times higher than *cytb*) were observed among closely related species of rainbow fish (Melanotaeniidae; Zhu *et al*. 1994). Hence, to provide an estimate of divergence we used both 2% and 6% divergence per Myr.

To separate population structure from population history, we used the nested character-based analysis described by Templeton *et al*. (1995). Four measures of the geographical distance based on the nested cladogram described above are used; Dc (x), Dn (x), Itc (y) and Itn (y), where x and y refer to a specific n-step and n + 1-step clade, respectively. The Dc (x) is the average distance for all members of clade x from their geographical centre, i.e. it measures how geographically widespread the individuals bearing haplotypes from clade x are. The Dn (x) is average distance for all members of clade x from the geographical centre of the nesting clade y; i.e. it measures how far individuals that bear clade x haplotypes are from all individuals that bear clade y haplotypes (which include clade x and other clades that are evolutionary close to x). The Itc (y) and Itn (y) are the average Dc or Dn values for all interior clades within the nesting clade y, minus the average Dc or Dn value for all terminal clades within nesting clade y. Hence, these indices give a measure of the distribution of old vs. young clades. The geographical centre of clade x was calculated by averaging the latitude and longitude over all observations from clade x (Templeton *et al*. 1995). Geographical distances in kilometres between individuals and clade centres were calculated as great circle distances. The statistical distributions of the distance measures were determined by recalculating all distances after each of 1000 random permutations of clades against sampling locality. For further statistical methodology see Templeton *et al*. (1995). The geographical locations of sampling sites in latitudes and longitudes can be obtained upon request from the authors.

The extent of geographical structuring of genetic variation was assessed by quantifying the intergroup component of total variance by Φ statistics using the analyses of molecular variance model (AMOVA; Excoffier *et al*. 1992) in the Arlequin version 1.1 program package (Schneider *et al*. 1997). The groups defined when analysing between drainage variation are presented in Table 1. mtDNA haplotype distances were calculated from the haplotype cladogram in Fig. 1B. Distances between RAPD phenotypes were calculated by treating the phenotypes as haplotypes using Euclidean squared distances (Huff *et al*. 1993; Gabrielsen *et al*. 1997; Nesbø *et al*. 1998b).

Gene diversity (mtDNA) was calculated according to Nei (1987). Between-population nucleotide divergence (D_A; Nei 1987), based on the same haplotype distance matrix as in AMOVA, was used to construct a population UPGMA dendrogram in PHYLIP ver. 3.572 (Felsenstein 1995). Confidence of the branches was obtained by bootstrapping haplotypes within populations. Gene diversity was estimated from the RAPD data using the formulas in Lynch & Milligan (1994), including monomorphic bands. Notably, if there are allelic relationships among bands, this would bias the gene diversity estimates downwards as allele frequencies would be overestimated, particularly in the most polymorphic populations (Lynch & Milligan 1994). Between-population distances were calculated as Euclidean squared distances from a presence–absence matrix of bands within populations. Principal coordinate analysis (PCO) was performed in NTSYS-pc (Rholf 1990). Spatial autocorrelation statistics were computed using Mantel statistics (Mantel 1967) in the R-package (Legred & Vaudor 1991).

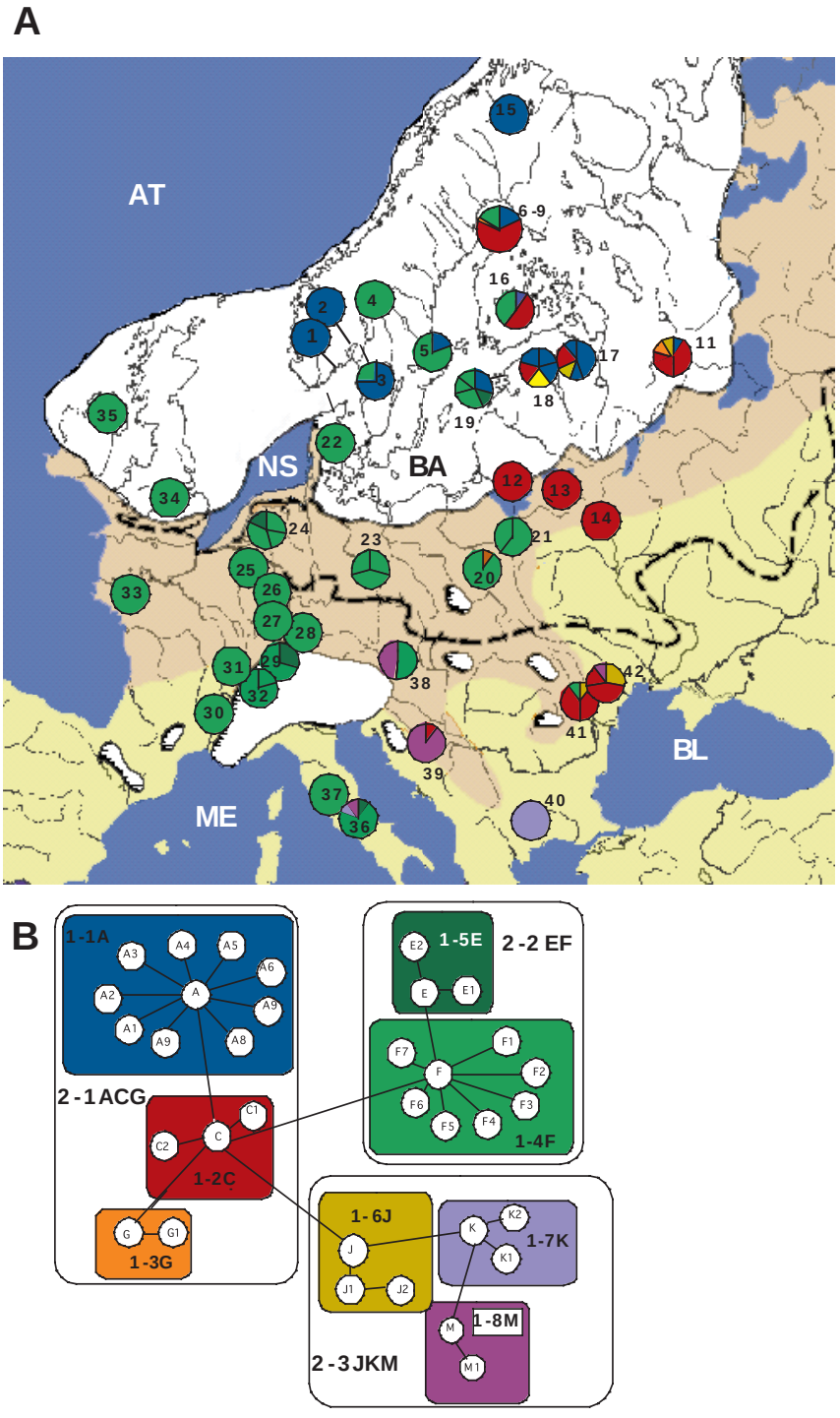

Fig. 1 A. The distribution of mtDNA haplotypes in Europe. Only first clade level (i.e. 1–1, 1–2, etc.) is displayed. The colours of the sector diagrams correspond to the colours used in the minimum-spanning network in B. Numbers refer to population numbers in Table 1. The Siberian population (Angara R.) is not shown. However, in this population the C-haplotype common in eastern Europe was observed (Table 1). The white regions illustrate the extent of the ice during the last glaciation (the Weichselian glaciation), the ice covering the Alps was probably smaller than indicated by the map. The North Sea has been a controversial region; the map probably shows conditions 22–21 000 years ago, as 20–18 000 years ago the Scandinavian and Devensian ice sheet over Britain probably did not merge, and the North Sea area was land (Andersen & Borns 1994). The Atlantic Ocean was probably covered with ice possibly all the way south to Portugal during winter (not shown; Andersen & Borns 1994). Vegetation zones and positions of periglacial lakes (notably even larger lakes were situated further east outside the map) during the maximum extent of the Weichselian ice sheet are approximately simplified from fig. 2–19 in Andersen & Borns (1994); orange zones were polar desert or tundra, yellow zones were tundra, steppe or parkland. The most severe climate occurred in western Europe (Andersen & Borns 1994). The winter temperatures were approximately 20 °C colder than the present time, and precipitation was less than half of the present level in a wide zone adjacent to the ice sheet in northwestern Europe. The southern limit for permafrost conditions during late Weichselian times lies in southern France. AT, Atlantic Ocean; NS, North Sea; BA, Baltic Sea; BL, Black Sea; and ME, Mediterranean Sea. The stippled line refers to the maximum extent of the ice sheet during the Saalian glaciation. The map was modified from fig. 11.42 in Banarescu (1992). B. Minimum-spanning network derived from the 35 haplotypes found in the European perch populations.

Results

mtDNA sequence variation

In total, 313 perch were sampled from 34 populations (Table 1). Twenty-one variable sites were observed, defining 29 mtDNA D-loop haplotypes. With inclusion of data from Refseth *et al*. (1998) and Nesbø *et al*. (1998b) 35 different haplotypes were observed among a total of 56 populations (Table 3). The haplotypes and their distribution among the populations are presented in Table 3 and Table 1, respectively.

Table 3 mtDNA D-loop haplotypes observed among European perch*

		Repeated array																					
		1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	4	
	Previous	0	1	1	2	2	3	3	3	3	3	8	3	3	4	6	6	8	9	0	8	2	
Haplotype	designation†	3	0	3	0	9	0	1	4	5	7	6	1	3	4	2	8	6	9	0	2	4	*N*‡
A	A	A	T	T	T	C	G	C	A	A	T	A	T	A	C	G	T	T	C	C	C	T	52
A1	B															A							16
A2	J						T																7
A3	F												C										12
A4	E																C						3
A5	O			A																			1
A6							T	T	G	C													4
A7	D						T	T															10
A8							T	T	G														5
A9	G			A			T	T															1
C	K																		T				95
C1	M						T	T											T				8
C2								T											T				7
C3				A															T				2
F	C	T																	T				155
F1	L	T					T	T											T				25
F2	H	T					T	T	G										T				7
F3	I	T					T	T	G	C									T				5
F4		T						T											T				12
F5		T												G					T				7
F6		T	C		C		T												T				3
F7		T					T												T				1
E		T									—								T				5
E1		T					T	T			—								T				1
E2		T									—								T		T		1
G	N										—								T				3
G1				A							—								T				2
J																			T	T			5
J1								T											T	T			1
J2								T										C	T	T			1
K												C							T	T			1
K1					C							C							T	T			9
K2												C							T	T		C	1
M												C			T				T	T			11
M1						T						C			T				T	T			9
Total																							488

*Position refers to the complete perch control region (acc no. Y14724).
†Haplotype designation used in Refseth *et al*. (1998) and Nesbø *et al*. (1998b).
‡Total *N* when sequences from this study were combined with the data sets from Refseth *et al*. (1998) and Nesbø *et al*. (1998b).

Construction of haplotype phylogeny; southern European perch are phylogenetically most closely related to American yellow perch

The relative age of the haplotypes was evaluated from phylogenetic trees using yellow perch as outgroup. Only haplotypes not defined by mutations in the repeated array were considered. The analyses suggested the M-haplotype observed in the Danube as most ancient. The most parsimonious tree obtained is presented with bootstrap values in Fig. 2. A single mutation separates the M-haplotype from the other perch haplotypes, and the bootstrap values are consequently low (Fig. 2). The same topology was inferred from neighbour-joining analysis of Tamura & Nei (1993) distances (data not shown). Mean corrected divergence (Tamura & Nei 1993; gamma $\alpha = 0.45$) between perch and yellow perch was 6.4%.

A haplotype cladogram for all 35 observed haplotypes

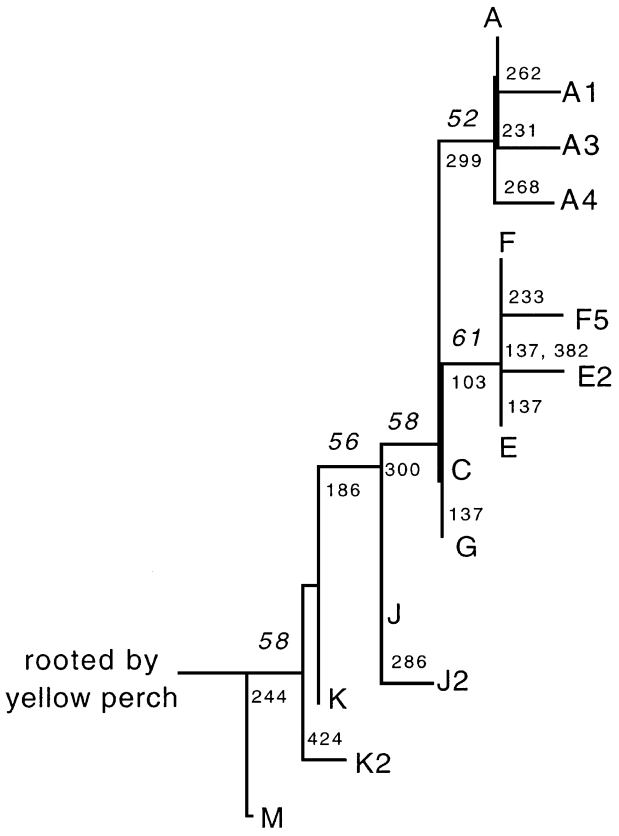

Fig. 2 Maximum parsimony tree of perch mtDNA haplotypes obtained using the branch and bound search option in PAUP 4.0. The haplotypes defined by mutations in the repeated array are excluded. Bootstrap values after 1000 heuristic bootstrap replicates are indicated in italics. The mutation at each branch is given (see Table 1 and Fig. 2A). The yellow perch sequence was used as out-group (Acc. no. Y14728). Tree length = 31, consistency index = 0.9677, homoplasy index = 0.0323, retention index (RI) = 0.9706 and rescaled consistency index = 0.9393.

was estimated using the algorithms described by Templeton *et al*. (1987, 1992); Templeton & Sing (1993) (Fig. 1B). The confidence of the cladogram, i.e. the probability of all haplotypes being connected in a parsimonious fashion, was estimated to ≥ 98% by the algorithm of Templeton *et al*. (1992). Apart from high levels of homoplasy in the repeated array (Nesbø *et al*. 1998a) a single homoplasy was observed in haplotype E, E1, G and G1 (Table 3). For these haplotypes, either the A to T transversion at position 103, located in the *trnP* gene, or alternatively the deletion at position 137, has occurred twice (see Fig. 2). Both nucleotides are positioned in the region flanking the highly variable tandem repeat. However, Nesbø *et al*. (1998a) suggested that the mutation at position 103 has occurred only once during the evolution of the perch D-loop because all other mutations in the repeat can be accounted for by a unidirectional 5′ to 3′ (relative to the sequence in Table 3) slippage mechanism. Hence, in the cladogram presented in Fig. 1B the deletion at position 137 is considered a homoplasy. From this cladogram, the haplotypes could be nested into eight 1-step clades, and these could further be grouped into three 2-step clades.

Geographical distribution of mtDNA haplotypes

The distribution of mtDNA haplotypes is presented in Fig. 1A and Table 1. Most regions are characterized by possessing phylogenetically close haplotypes (compare Fig. 1A and 1B), i.e. within regions haplotypes are in general no more than one mutation from the most frequent haplotype observed within that region. However, three main suture zones, where haplotypes from different clades mix (Taberlet *et al*. 1998), can be identified in addition to the southern Scandinavian contact zone described in Refseth *et al*. (1998; pop. nos 3, 4). One such zone is located in the upper Danube (pop. no. 38). This population displayed a high frequency of haplotypes phylogenetically close to haplotypes common in both western Europe and southern Europe. The second suture zone involves populations in regions draining (present or historically) into the Baltic Sea (pop. nos 5–9, 16, 19), characterized by possessing haplotypes from both the 2–1ACG and 2–2EF clades. Finally, the Estonian inland populations possess haplotypes from the 1–1A lineage common in southern and northern Norway and the 1–2C and 1–6J lineage common in eastern Europe. In addition to these main suture zones, some populations possess haplotypes (at low frequency) from clades common in neighbouring regions (i.e. pop. nos 11, 20, 36, 39, 41, 42).

Between-population nucleotide divergence is presented as an UPGMA dendrogram in Fig. 3. Four population groups are suggested: southern European populations (group I: pop. nos 39, 40), eastern European populations including the Siberian population (group II: pop. nos 10–14, 41, 42), western European populations (including the Vistula drainage in Poland and Italian populations; group III: pop. nos 20–37), and Norwegian populations from the western side of Oslofjord and northern Norway (group IV: pop. nos 1–2, 15). The same groups were obtained applying neighbour joining. However, the clustering of group II–IV was [pop. no. 42 ((II, IV) (III)]. Notably, only the southern European cluster, found at the root of the tree, obtained high bootstrap support. Furthermore, most of the between-population variation is observed in this region. Populations suggested to represent suture zones are found within all groups except the southern European group. Excluding these populations gave the same topology; however, high bootstrap support was obtained for all groups; 98% for group I and group II, 94% for group III and 100% for group IV.

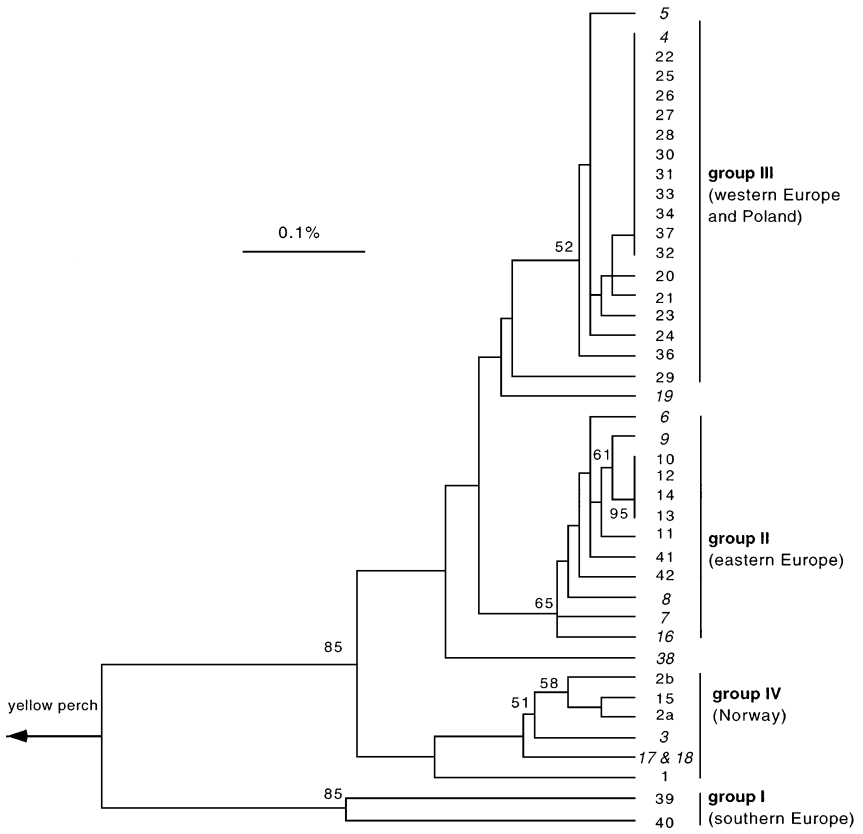

Fig. 3 UPGMA phenogram clustering perch populations according to the mtDNA distance matrix resulting from net average number of nucleotide substitutions per site between populations (D_A; Nei 1987). Bootstrap values are given at branches supported in more than 50% of 100 trees obtained by resampling haplotypes within populations. Numbers refer to the population numbering in Table 1. Names on the clusters refer to their geographical distribution. Populations suggested to represent suture zones are given in italics. The yellow perch (*Perca flavescens*) population was used as an outgroup.

Cladistic analysis of geographical distances suggests range expansions as the main force determining mtDNA haplotype distribution

A nested cladistic analysis of geographical distances was used to test the null hypothesis of no geographical association of haplotypes, and to test whether significant associations are probably due to restricted gene flow or historical events (i.e. fragmentation, range expansion, long-distance colonization). Figure 4 presents the clade and nested clade distances, significance of the distances after permutating haplotypes against sampling site, and the inference reached by the key given in the appendix of Templeton *et al*. (1995), using the M haplotype as root haplotype. As one of the populations, Angara R. (pop. no. 10), is located in Siberia about 4000 km from the geographically nearest European population in this study (pop. no. 11), the analysis of geographical distances was performed both with and without this population. With few exceptions, the haplotypes showed significantly small geographical ranges (i.e. significantly small Dc and Dn values, Fig. 4). The results, shown in Fig. 4, suggest that population history has played an important role in determining the geographical distribution of perch haplotypes, as most clades showed distance patterns reflecting contiguous range expansion. We also performed the analysis excluding the southernmost populations (i.e. pop. nos 36, 37, 39–42). This resulted in much the same pattern, with contiguous range expansion as the main factor determining haplotype distribution. However, some new patterns were also detected. Past fragmentation was inferred for the 1–4F clade observed in western Europe. In addition, the distance patterns obtained with the J and C haplotypes, observed in eastern Europe, suggested range expansion for these perch groups. This is probably a result of excluding most populations not affected by the last glaciation (see Fig. 1A). Past fragmentation was obtained for the 3-step clade (i.e. all 2-step clades combined). As the southern populations were excluded, this is likely to represent the same fragmentation as detected for the 2–1ACG clade (Fig. 4). Finally, we performed analyses with two different topologies of the haplotype cladogram: (i) using the widespread C-haplotype as root haplotype (Castelloe & Templeton 1994); and (ii) nesting haplotypes according to shared mutations in the repeated array (i.e. portioning the 1–1A and 1–4F clade into two and three 1-step clades, respectively (see Table 1), resulting in four 2-step clades nested into one 3-step clade). Using topology (i) changed none of the inferences reached in the above analyses. This was also the case for topology

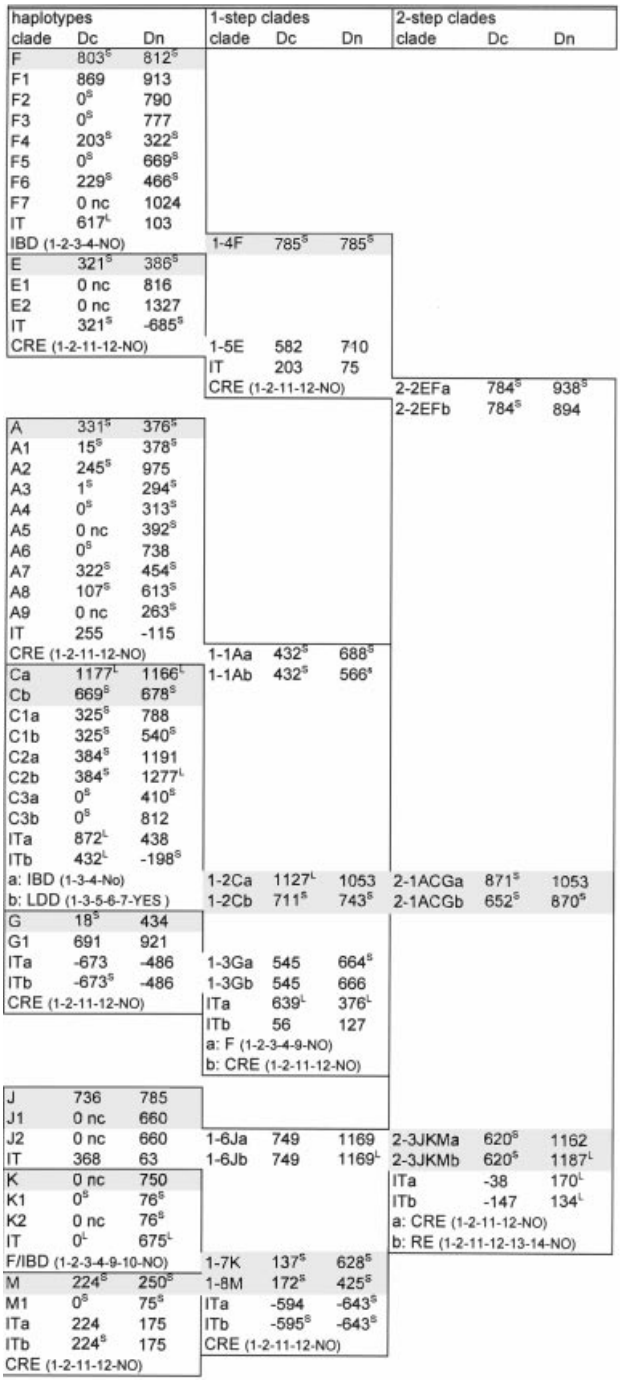

Fig. 4 Results of the nested geographical analysis of the perch mtDNA haplotypes following the nomenclature of Templeton (1998). The nested design is given in Fig. 2C. Interior clades are shaded. The clade Dc and Dn values are given in kilometres after the clade names. Dc(x) is the average distance of individuals displaying clade x haplotypes to the geographical of centre clade x. Dn(x) is the average distance of individuals displaying clade x haplotypes to the nesting clade centre. The average difference between tip and interior clades is given in the row labelled IT. S or L denotes that the distance measure was significantly small or large at the 5% level. At the bottom of each box representing a clade with significantly small or large distances, the biological inference is given. The numbers in parenthesis refer to the answers to the questions in the key given in Templeton *et al.* (1995). CRE, contiguous range expansion; IBD, restricted gene flow due to isolation by distance; LDD, restricted gene flow, but some long-distance dispersal; FR, fragmentation; RE, range expansion; nc, not calculated due to only one observation of the haplotype; a, analysis including Angara R. (pop. no. 10); b, analysis without Angara R.

(ii), except that past fragmentation was inferred for all 2-step clades combined. This possibly represents the same fragmentations as inferred from the 2–1ACG clade and for all clades combined when analysing north European populations.

mtDNA sequence divergence within and between populations

Low sequence divergence was observed among the mtDNA haplotypes. Maximum sequence divergence was 1.3%, while mean divergence across all haplotypes was 0.7% (SD = 0.3). Maximum divergence among haplotypes found in western and northern Europe was 1%. However, within these regions lower sequence diversity was observed, e.g. as low as 0.2% in western Europe. Gene diversity within populations varied extensively, and ranged from 0.00 to 0.87 (mean = 0.34, SD = 0.33), with 13 populations displaying zero values (Table 4). As a group, the western European populations (group III) showed the lowest level of diversity (mean = 0.16, SD = 0.29). In addition, almost all sequence diversity among these populations was confined to the repeated array. The highest level of gene diversity was found in the Baltic Sea (mean = 0.66, SD = 0.19) and the eastern European drainages (group II; mean = 0.49, SD = 0.36). For the two southern European populations (group I), the gene diversity was low (0.20 in both populations).

The RAPD data reveal similarity between eastern and western European populations

To obtain information on nuclear variation we analysed a subsample of 178 perch using three RAPD primers (A4, A11 and C18) showing scorable polymorphic bands within and among the European samples. The observed frequency of polymorphic bands within each of the groups detected by mtDNA (Fig. 3), as well as in regions suggested to be suture zones, is shown in Table 2. For all three RAPD primers, relatively high variation was found in populations from eastern Europe, the Baltic Sea region and southern European (Table 4).

The first three axes in a PCO of between-population

Table 4 Gene diversity calculated from the two markers employed for perch from different populations in Europe

No.*	Population	Region	mtDNA	*N*	RAPD
1a	L. Toke	SN	0.00	6	0.06
1b	L. Steinbutjønn	SN	0.00		—
2a	L. Sandungen	SN	0.00	6	0.06
2b	L. Eikern	SN	0.60	5	0.10
3	Glomma R.	SN	0.87		—
4	L. Storsjøen	SN	0.00	5	0.12
5	L. Mälaren	SS	0.40	5	0.08
6	Baltic sea	NS	0.48	7	0.09
7, 8	Anadromous	NS	0.79	11	0.11
9	L. Ängersjön	NS	0.51	9	0.09
10	Angara R.	R	0.00	5	0.09
11	L. Rybinsk	R	0.75	5	0.12
12	L. Krylovo	R	0.00	6	0.09
13	L. Hencza	P	0.40	5	0.07
14	L. Dubrovskoje	R	0.00	10	0.05
15	L. Vourasjavre	NN	0.53		—
16	L. Vesijärvi	F	0.71	5	0.11
17,18	L. Peipsi	ES	0.85	9	0.11
19	Matsalu Bay	ES	0.86	5	0.12
20	Pilico R.	P	0.20		—
21	L. Kortowo	P	0.60	4	0.13
22	L. Lyngsø	D	0.00	6	0.04
23	L. Bautzen	G	0.73	6	0.10
24	L. Ijsselmeer	NE	0.69	7	0.05
25	Rhine R.	FR	0.00	5	0.09
26	Moselle R.	FR	0.00	5	0.09
27	Mause R.	FR	0.00	5	0.08
28	L. Zürich	SW	0.00	5	0.08
29	L. Aegeri	SW	0.47	6	0.06
30	Rhone R.	FR	0.00	5	0.04
31	Saone R.	FR	0.00	5	0.04
32	L. Leman	FR	0.36	5	0.07
33	L. Fermur	FR	0.00	5	0.07
34	L. Windermere	E	0.00	5	0.04
35	Lee R.	IR	0.00		—
36	L. Corbara	IT	0.75		—
37	L. Trasimeno	IT	0.00		—
38	Danube R.	AU	0.52	8	0.11
39	L. Race	SL	0.20	5	0.13
40	L. of Doirane	GR	0.20	5	0.07
41	Siret R.	RO	0.73		—
42	Prut R.	RO	0.64	6	0.12
Total			0.83	202	0.09

*Numbers refer to the population numbers given in Table 1. Geographically close populations showing no (pop. nos 17 and 18) or little differentiation (pop. nos 7 and 8) and similar gene diversity estimates ≠, were pooled to increase precision. Number of individuals analysed for mtDNA polymorphism is given in Table 1. Populations not investigated for RAPD variation are indicated by —.

distances extracted 48% of the total variation (Fig. 5). In agreement with the mtDNA data, populations suggested to represent suture zones are mainly observed in the centre of the diagram (i.e. the Baltic Sea, Estonia, Danube). However, compared to the mtDNA data set, the RAPD data revealed a higher level of diversity within the groups suggested by the population UPGMA dendrogram (Fig. 3), especially within group II, as well as a lower level of between-group divergence. Most western European populations (group III) were separated from the other populations along the first axis. However, some of the drainages from northwestern Europe (pop. nos 21, 23, 25) are found in the middle of the diagram, along with the suture zone populations. The eastern European populations

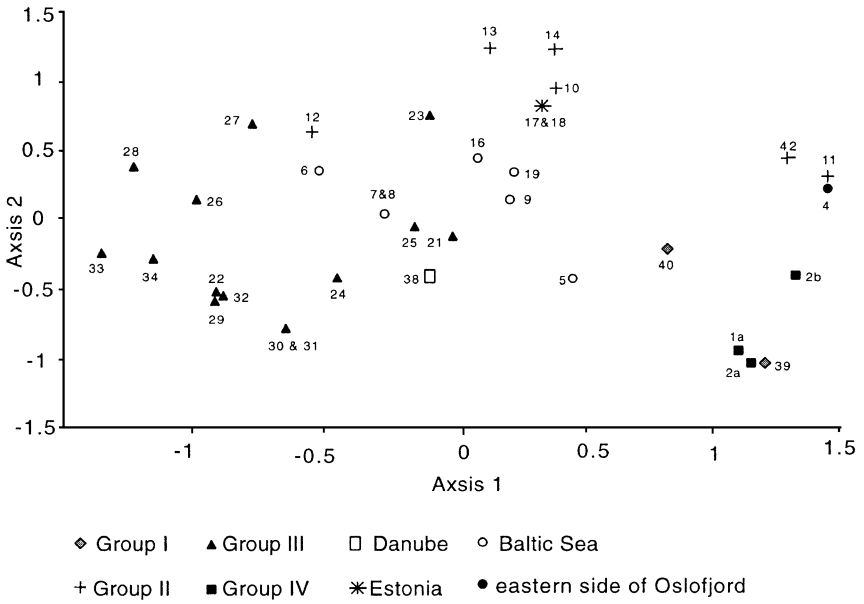

Fig. 5 Principal coordinate analysis (PCO) of 35 populations based on Euclidean squared distances obtained by scoring presence and absence of bands within populations. Axis 1 extracted 23% of the variance, and axis 2 extracted 13% of the variance. Numbers refer to population number in Table 1 and Fig. 1A. Symbols indicate population group or suture zone suggested by mtDNA.

(group II) form two 'clusters': populations (pop. nos 10, 12–14) displaying low mtDNA polymorphism (i.e. only C-haplotypes; Table 1) were most similar to western European populations; and populations with higher mtDNA diversity (pop. nos 11, 42) cluster together with the southern European populations (group I) along the first axis. In particular, one of the populations from the Neman drainage (pop. no. 12) showed high similarity to western European populations. Notably, while mtDNA haplotypes observed in the populations from southern Europe and the western side of Oslofjord were highly divergent (Fig. 1C), these populations cluster together along both axes in the PCO. However, this might be an artefact of sharing 'null bands' (see Table 2), as all bands observed in populations from the western side of Oslofjord also were observed in eastern Europe. Supporting this idea, Refseth *et al*. (1998) observed reduced variable number of tandem repeat (VNTR) polymorphism in lakes on the western side of Oslofjord presumably due to a severe bottleneck during colonization. Hence, excluding the Scandinavian populations and the populations suggested to be suture zones, the PCO suggests a clinal structure of the RAPD variation from southern Europe, via the eastern to the western European populations. This was also observed by spatial autocorrelation analysis using Mantel statistics (data not shown).

AMOVA reveals high level of structuring for both markers

To quantify between- and within-population variation, AMOVA was performed on both data sets and the results are presented in Table 5. It should be noted, however, that as historical range expansion was detected as the main factor determining mtDNA haplotype distribution, the Φ-statistics, at least within regions, will underestimate the level of differentiation (Templeton 1998). For the mtDNA data set, a global Φ_{ST} value of 0.66 was obtained. Grouping the populations into 20 drainage systems, 48% of the mtDNA variation was found between drainages, and only 20% of the variation accounted for within-drainage variation. Classifying the populations according to the four groups suggested from analysis of between-population divergence results in an even higher between-group component (73%). High levels of structuring were also observed for the RAPD data set (global $\Phi_{ST} = 0.48$). However, as compared to the mtDNA data set, higher values were obtained for the within-group and within-population components, which is in agreement with the PCO in Fig. 5. Grouping all populations into drainages, the between-drainage variation accounted for 29% of the total RAPD variation. Analysing the groups defined from the distribution of mtDNA haplotypes, 36% of the total variation was explained by the between-group component for RAPD.

Discussion

The levels of genetic variation and mtDNA divergence times suggest recent divergence of perch populations Low differentiation was observed among the perch mtDNA D-loop haplotypes. All 35 haplotypes observed could be connected through single-mutational steps, and the haplotypes were, at most, six mutations apart. To provide approximate estimates of divergence times among perch haplotypes, we applied both the 2% mtDNA divergence rate per Myr and a 6% rate as suggested for the D-loop in some Perciformes (see the Materials and

Table 5 Results from hierarchical analysis of molecular variance (AMOVA)*

	Among populations											
	Among groups				Within groups				Within populations			
Structure analysed†	*Va*	%	*P*	Φ_{CT}	*Vb*	%	*P*	Φ_{SC}	*Vc*	%	*P*	Φ_{ST}
mtDNA												
All populations					0.55	66	< 0.001		0.27	33		0.66
By drainage	0.40	48	< 0.001	0.48	0.17	20	< 0.001	0.39	0.27	32	< 0.001	0.68
Group I-II-III-IV	0.83	73	< 0.001	0.73	0.15	15	< 0.001	0.50	0.15	13	< 0.001	0.87
RAPD												
All populations					1.04	48	< 0.001		1.14	52		0.48
By drainage	0.64	29	< 0.001	0.29	0.45	20	< 0.001	0.28	1.14	51	< 0.001	0.49
Group I-II-III-IV	0.88	36	< 0.001	0.36	0.61	25	< 0.001	0.39	0.95	39	< 0.001	0.61

**Va*, *Vb*, and *Vc* are the variance components due to differences among groups, among populations within groups and within populations within a group, respectively. % indicates the proportion of the total variance explained by *Va*, *Vb* or *Vc*.
Φ_{ST} is the correlation of random haplotypes within populations relative to that of random pairs of haplotypes drawn form the complete data set analysed; Φ_{CT} is the correlation of random haplotypes within a group of populations relative to that of random pairs of haplotypes drawn form the complete data set analysed; Φ_{SC} is the correlation of the molecular diversity of random haplotypes within a population relative to that of random pairs of haplotypes drawn from the region. Significance of the *V* and Φ estimates is obtained by permutation of the original data. For further statistical methodology see e.g. Schneider *et al*. (1997).
†Groups refer to four population groups detected from the mtDNA haplotypes (Fig. 3).

methods). The θ (= $N_e\mu$ = 0.0039) used was estimated from the total data set. Hence, for clades associated with regions with larger or smaller long-term N_e the most recent common ancestor (mrca) may be considerably older or younger, respectively (e.g. Harding 1996). Maximum coalescent time among perch haplotypes was 0.19 Myr (SD = 0.07) and 0.57 Myr (SD = 0.21) when using the M-haplotype as root haplotype and rates at 6% and 2%, respectively. Hence, applying a 6% rate, the mrca of all present perch lineages is suggested to have lived during the Saalian glaciation, while a 2% rate suggests that the mrca date back approximately five glaciation cycles. Coalescent time of tip haplotypes and 1-step clades (i.e. 1–2 mutations) was estimated to 0.05–0.24 Myr (SD = 0.04 – 0.14). The mean of these estimates correlates to the two last glaciation cycles. A recent mrca, at least for clades with a northern distribution, is corroborated by the RAPD data, because relatively low divergence was observed across all sampling sites for these markers (Table 2), as well as by the extremely low levels of allozyme diversity observed in previous studies of northern European perch (e.g. Gyllensten *et al*. 1985; Heldstab & Katoh 1995). Hence, regardless of exact timing, our results suggest that perch populations in northern Europe and Siberia (at least in Angara R.) date back to a relatively recent ancestral population which went through a severe bottleneck in connection with one of the Pleistocene glaciations. Similarly, major population bottlenecks in connection with earlier ice ages are also suggested for other European animal species (Hewitt 1996; Brunner *et al*. 1998).

Diversity among and within European river systems reflects postglacial colonization history

High levels of congruence were observed between the two molecular markers employed in this study. The estimates of gene diversity varied considerably among populations. However, within the same population comparable values were generally obtained for both markers (Table 4). Where discrepancies occur, it is mainly due to 'fixation' of one mtDNA haplotype while higher levels of diversity were observed for the RAPD markers (see Table 4). The AMOVA analyses revealed considerable structuring between drainages for both markers, in the range of 39–49%, while within-drainage or group structuring was low. Particularly among western European drainages, little divergence was observed for both markers. Similarly, Heldstab & Katoh (1995) found low differentiation in western Europe using isoenzymes when comparing populations from Rhône, Saône and Po. The almost complete lack of within- and between-population variation in western Europe indicates a probable postglacial origin of the perch populations in most nonglaciated as well as glaciated parts of western Europe. The Baltic Sea, which was covered by the last ice sheet, harbours the populations with the highest level of intrapopulation variation, possibly reflecting colonization from several different refugia. Southern and eastern European drainages not directly affected by the last ice sheet (Fig. 1A), showed relatively high intrapopulation variation as well. The southern region also displayed the highest level of between-population

variation (Fig. 3), suggesting a longer time of divergence compared to the northern populations.

Low levels of differentiation were found between perch from the Angara R. (pop. no. 10) and from the other European sampling sites. Within the Angara R. sample only the mtDNA C-haplotype common in eastern Europe was observed. The RAPD markers also revealed a low level of divergence (see Fig. 5). Similar patterns, where Siberian and European populations show high similarity, are observed in whitefish (*Coregonus* sp.; Bernatchez & Dodson 1994) and ruffe (*Gymnocephalus cernuus*; Stepien *et al*. 1998). Bernatchez & Dodson (1994) attributed this to recolonization of Siberia by a whitefish group that dispersed recently in Eurasia. This also seems to be the most likely explanation for the geographical distribution of perch haplotypes. However, as only one population was sampled from this area, occurrence of genetically distinct Siberian populations cannot be excluded.

Range expansion as the main historical factor explaining the geographical distribution of perch mtDNA haplotypes

The distribution of perch mtDNA haplotypes was strongly correlated with geography (Fig. 1A), and almost all clades showed significantly small geographical ranges (Fig. 4). Hence, separation of perch into isolated refugia during glaciations with subsequent range expansions into northern areas during deglaciation can probably account for the observation of three major groups of perch in northern Europe. This was strongly supported by the nested cladistic analysis of geographical distances, which suggested contiguous range expansion for almost all mtDNA haplotype clades (see Fig. 4), indicating colonization into new areas as the main factor determining geographical haplotype distribution.

Perch from northern Norway and the western side of Oslofjord in southern Norway may descend from the same refugium

Populations located on the western side of Oslofjord all displayed haplotypes from the 1–1A clade, and were probably colonized shortly after deglaciation from an early perch population inhabiting the Baltic Ice lake (Refseth *et al*. 1998). Haplotypes from the clade 1–1A are also found in populations from Estonia, Russia (Volga), northern Norway and the Baltic Sea. Hence, the refugium harbouring this perch group, R4 (named after the population group it colonized), could have been located in one of the large ice-dammed lakes east of the ice sheet (Fig. 1A, Andersen & Borns 1994). Supporting this idea, perch inhabiting these lakes during the last glaciation could have been among the first to enter the Baltic ice lake (see, e.g. figs 2–46 and 2–53 in Andersen & Borns 1994). Colonization of northern Norway probably occurred through the periglacial lake system (i.e. an area near a glacier) formed during the deglaciation of this area 10–11 000 BP (see Donner 1995). The low frequency of the clade 1–1A haplotypes in present populations in the area suggested to harbour this refugium (pop. no. 11, see Fig. 1A), and the occurrence of 1–2C and 1–6J haplotypes in Estonia (pop. nos 17, 18), could be due to introgression of an extensively expanding perch group from a southern refugium.

Extensive dispersal from a large southeastern refugium

All eastern European populations possessed high frequency of haplotypes nested within the 1–2C clade. In addition, C-haplotypes are present in the Slovenian population, and the populations within and surrounding the eastern side of the Baltic Sea. Apart from the population from the Volga drainage (pop. no. 11), high diversity was observed in the populations from the southern Black Sea drainages (pop. nos 41, 42). The Black/Caspian Sea formed a continuous freshwater system, isolated from the Mediterranean, during the Saalian glaciation and the early phases of the last glaciations (Bianco 1990; Arkhipov *et al*. 1995). Hence, origin of the 1–2C clade from this area is conceivable. This was supported by the RAPD data, which indicated that pop. nos 42 and 11 were similar to the southern European populations. The range expansion suggested for this clade when excluding the southernmost populations is likely to reflect postglacial dispersal from this area. Perch from this refugium (R2) probably colonized the eastern European drainages, and reached as far east as Siberia (Angara R.), and as far west as the Baltic Sea.

Recolonization of western European drainages from a common glacial refugium

All western European populations display haplotypes from the 2–2EF clade, suggesting dispersal from one common glacial refugium into western European rivers. As the F haplotype and its derivates dominate in the Vistula, Elbe, Rhine, Rhône, Saône and Thames, and also occur at high frequency in the upper Danube, our data support postglacial contact between these river systems, at least for short periods of time, e.g. via periglacial rivers formed during deglaciation (Gibbard 1988; Arkhipov *et al*. 1995). Furthermore, lack of vegetation immediately following deglaciation produced marked flood events (Gibbard 1988), which could have facilitated dispersal between presently isolated rivers. The fragmentation detected for the 1–4F clade when excluding the southern populations probably reflects the isolation into present river systems. From the topology of the haplotype cladogram in Fig. 1B,

it is unlikely that the refugium (R3) harbouring this perch group was located in the Danube. Although the F-haplotype observed in western Europe was observed at high frequency in the upper Danube, the M-haplotype also found at this location is highly divergent from F (1% divergence). Hypothetically, it is conceivable that these divergent mtDNA groups could have been retained by chance without historical separation. However, there is little empirical support for such a scenario (Avise 1992; Bernatchez & Dodson 1994). A more likely explanation is that the western European drainages were recolonized by perch that dispersed extensively in western Europe, and subsequently intergraded with the Danubian group. Among the western European rivers the highest levels of both mtDNA and nuclear variation were observed in the northern drainages (Vistula, Elbe and Rhine; Table 4), suggesting a north–south dispersal route (Hewitt 1993, 1996). However, the higher level of nuclear diversity in northern rivers could be due to introgression from other refugia via, for instance, the Baltic Sea. In the Vistula drainage one individual displaying the G1 haplotype from the 2–1ACG clade was observed, indicating introgression at least in the easternmost range of the western European perch group. Furthermore, the most southern locations in France could have sustained perch populations during the last ice age as suggested for, e.g., *Barbus* sp. (Persat & Berrebi 1990) and brown trout (García-Marín *et al*. 1999). Hence, the exact location of the R3 refugium is somewhat unclear. High frequency of clade 2–2EF haplotypes was also observed in the two Italian populations. These lineages may have entered Italian drainages through postglacial connections during the melt of the ice cap that covered the Alps. One of the populations, L. Corbara, also possessed haplotypes from the 2–3JKM clade, possibly reflecting the haplotype composition of Italian populations prior to the last deglaciation. The haplotype interior to F, the C-haplotype, is found at high frequency in eastern Europe suggesting a pre-Weichselian eastern European origin of this perch group as well. This is also supported by the distribution of RAPD bands (Table 2, Fig. 5), which suggested high similarity between eastern and western European populations. Hence, the fragmentation inferred for the 2–1ACG clade (when including the Angara population), and for all clades when excluding the southernmost populations (or by analysing the alternative topology no. 2), may reflect isolation of the R3 and R4 perch lineages, possibly in connection with recolonization of northern Europe from eastern Europe after one of the earlier glaciations (e.g. the Saalian glaciation, see Fig. 1A).

A southern European origin of present perch lineages?

Phylogenetic analyses suggested the M-haplotype observed in the Danube to be the most ancient haplotype. The relatively high between- and within-population mtDNA and RAPD diversity observed within the southern region (Figs 3 and 5), as well as preliminary results from microsatellites (Fossheim T, Nesbø CL and Jakobsen KS, unpublished) support this idea. Hence, we find it most likely that the range expansion detected for clade 2–3JKM was from the Danube and into the Black Sea, possibly in connection with one of the earlier Pleistocene glaciations. However, the bootstrap support for the M-haplotype as root was low, and few populations were analysed from this area. Hence, more populations from southern Europe need to be analysed to obtain a more comprehensive picture of the history in this region. Banarescu (1992) argues that the Baltic Sea and northern European drainages were colonized from a Danubian refugium (R1) after the last glaciation. Our data contradict this with respect to the most recent postglacial dispersal. However, a Danubian origin of the whole present perch lineage, after a bottleneck during earlier glaciations, is plausible.

Glacial refugia and the dynamics of the recolonization of Europe

The refuge areas for perch suggested from our data are depicted in Fig. 6. Black lines indicate phylogenetic relationships among mtDNA haplotypes presumed to originate from the putative refugia, while coloured arrows indicate postglacial dispersal. R1 presumably served as a founder population for present perch lineages in Europe after one of the earlier Pleistocene glaciations, but did not contribute to the most recent recolonization of populations in glaciated areas investigated here. After the last glaciation northern European regions were colonized by perch originating from three refugia (R2, R3 and R4). Perch from these refugia have all reached the Baltic Sea. From the distribution of haplotypes in southern Scandinavia, perch from R4 entered the Baltic Sea first probably during the Baltic Ice Sea stage about 15–10 000 BP (Sholenius 1996; see also Refseth *et al*. 1998). Perch from R2 and R3 probably entered at the Ancylus lake phase (9500–8000 BP). As the highest frequency of the clade 1–2C haplotypes occurs in the eastern and northern part of the Baltic Sea and in Finland, perch from R2 probably entered from the east, perhaps via the Dnipr–Neman connection (Zhukov 1965). Perch from R3 entered from the south, indicated by the high number of clade 2–2EF haplotypes in the southern part of the Baltic Sea region and south-eastern Scandinavia. As contiguous range expansion and almost no long-distance colonization was inferred from the distribution of mtDNA haplotypes, the recolonization was probably mainly achieved through expanding population fronts via individual short distance movements from the refuge areas.

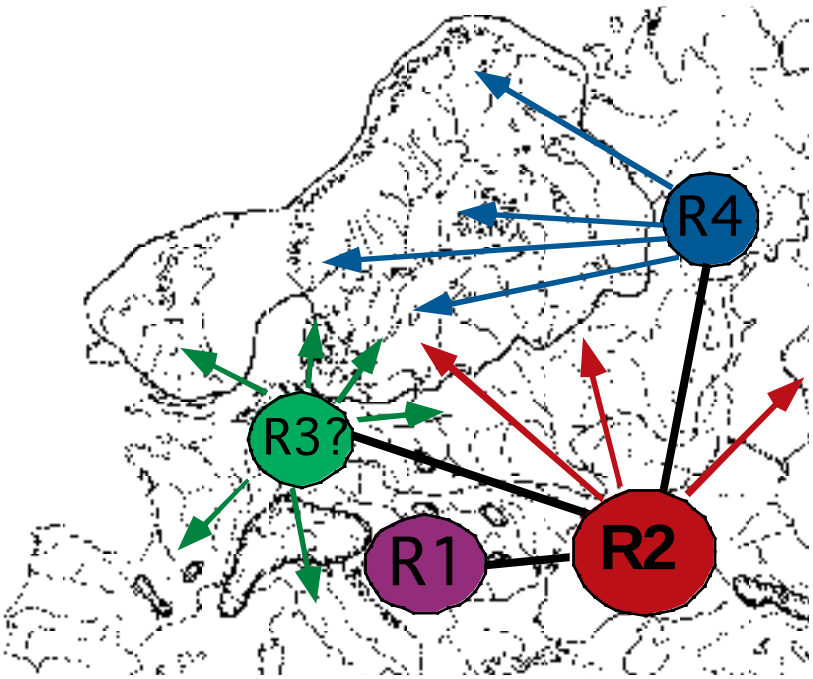

Fig. 6 Map showing a schematic overview of the suggested refugia. Colours refer to the cladogram in Fig. 1C. Black lines indicate the phylogenetic relationship among haplotypes presumed to originate from the different refugia (see Fig. 2). Coloured arrows indicate postglacial dispersal. The question mark indicates that the position of the R3 refugium is somewhat unclear. The map was modified from fig. 11.42 in Banarescu (1992).

General patterns of postglacial recolonization of Europe

In conclusion, the distribution of present mtDNA haplotype clades in northern European populations of perch seems to be closely linked to the most recent glaciation. The importance of eastern Europe as opposed to the Mediterranean refugia has also been observed for small mammals (Bilton *et al*. 1998). In addition, several similarities can be drawn to studies of salmonid species, such as recolonization of northern and western Europe by an extensively expanding stock (Nielsen *et al*. 1996; Brunner *et al*. 1998; García-Marín *et al*. 1999; Hansen *et al*. 1999). Moreover, studies of brown trout indicate that the Danubian refugium (R1), although harbouring the most ancient groups, did not contribute markedly to the recolonization of northern glaciated areas (Bernatchez *et al*. 1992). The high level of heterogeneity observed for the Baltic Sea area has also been observed in brown trout (García-Marín *et al*. 1999). Three of the refugia suggested for perch (R1, R2 and R3; Fig. 6) have also been proposed for brown trout (García-Marín *et al*. 1999). R4 was not considered by García-Marín *et al*. (1999). However, Osinov & Bernatchez (1996) considered a refugium located east of the ice sheet as highly probable. The distribution of mtDNA haplotypes among Siberian and European whitefish also corresponds well with the suggested refugia for perch (Bernatchez & Dodson 1994). This was also the case for three native populations of ruffe, which showed a high level of divergence between a Danubian and an eastern European–Siberian population group (Stepien *et al*. 1998). Hence, the refugia suggested here are very likely to have been shared by other European freshwater species with similar distribution patterns.

Acknowledgements

The authors are very grateful to A. O. Kasumyan, V. Ostroumov, T. Paaver, C. D. Triantaphyllidis, H. Persat, L. Oprea, I. Winfield, L. Lucentini, M. Katoh, M. M. Hansen, P. Galvin, P. Brzuzan, M. Rask, T. Mehner, T. Saat, S. Weiss, B. Sket, E. Feunteun, P. Laffaille, M. Przybylski, J. Markowski, J. Freyhof, T. A. Rygg, W. Dekker and P. Steinmann for providing, or assisting in providing, perch material from all over Europe, and E. Marsden for providing the yellow perch samples. We thank D. Ehrich and T. Paaver for translating Russian papers. The authors are particularly grateful to T. Paaver, C. D. Triantaphyllidis, J.-D. Durand and H. Persat for valuable information and discussions of some questions in this article. We thank A. R. Templeton for providing the program for calculating the significance of the minimum spanning network and for estimating θ. We are grateful to U. H. Refseth, K. Østbye, J. E. Stacy, T. E. Nesbø, T. Paaver, three anonymous referees and L. Bernatchez for helpful comments and suggestions. This work was supported by grant no 107622/420 from the Norwegian Research Council (NFR) to K. S. J.

References

Alvarado Bremer JR, Baker AJ, Mejuto J (1995) Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) population in the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 1720–1732.

Andersen BG, Borns HW Jr (1994) The Ice Age World. Scandinavian University Press, Oslo.

Arkhipov SA, Ehlers J, Johnson RG, Wright HE Jr (1995) Glacial drainages towards the Mediterranean during middle and late Pleistocene. *Boreas*, **24**, 196–206.

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos*, **63**, 62–76.

Banarescu P (1992) *Zoogeography of freshwaters*, Vol. 2. AULA-Verlag, Wiesbaden.

Bernatchez L, Dodson JJ (1994) Phylogenetic relationships among Paleartic and Neartic 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 Neartic and Paleartic fishes. *Molecular Ecology*, **7**, 431–452.

Bernatchez L, Guyomard R, Bonhomme E (1992) DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. *Molecular Ecology*, **1**, 161–173.

Bianco PG (1990) Potential role of the paleohistory of the Mediterranean and Paratethys basins on early dispersal of Euro-Mediterranean freshwater fishes. *Ichthological Exploration of Freshwaters*, **1**, 167–184.

Bilton DT, Mirol PM, Mascheretti S, Fredga K, Zima J, Searle JB (1998) Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonisation. *Proceedings of the Royal Society of London, Series B*, **265**, 1219–1226.

Borowsky RL, McClelland M, Cheng R, Welsh J (1995) Arbitrarily primed DNA fingerprints for phylogenetic reconstruction in vertebrates: The *Xiphophorous* model. *Molecular Biology and Evolution*, **12**, 1022–1032.

Brunner PC, Douglas MR, Bernatchez L (1998) Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Artic charr *Salvelinus alpinus* (Telostei: Salmonidae) from central Alpine lakes. *Molecular Ecology*, **7**, 209–223.

Cantatore P, Roberti M, Pesole G, Ludvico A, Milella F, Gadaleta MN, Saccone C (1994) Evolutionary analysis of cytochrome *b* sequences in some Perciformes: evidence for a slower rate of evolution than in mammals. *Journal of Molecular Evolution*, **39**, 589–597.

Castelloe J, Templeton AR (1994) Root probabilities for intraspecific gene trees under neutral coalescent theory. *Molecular Phylogenetics and Evolution*, **3**, 102–113.

Craig JF (1987) *The Biology of Perch and Related Fish*. Croom Helm Ltd, London.

Donner J (1995) *The Quaternary History of Scandinavia*. Cambridge University Press, New York.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Faber JE, Stepien CA (1997) The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the Percidae. In: *Molecular Systematics of Fishes* (eds Kocher TD, Stepien CA), pp. 125–139. Academic Press, San Diego.

Felsenstein J (1995) PHYLIP (Phylogeny Inference Package), Version 3.572. Native-mode Executables for PowerMac.

Fritch P, Rieseberg LH (1996) The use of random amplified polymorphic DNA (RAPD) in conservation genetics. In: *Molecular Genetic Approaches in Conservation* (eds Smith TB, Wayne RK), pp. 54–73. Oxford University Press, New York.

Gabrielsen TM, Bachman K, Jakobsen KS, Brochmann C (1997) Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. *Molecular Ecology*, **6**, 831–842.

García-Marín J-L, Utter FM, Pla C (1999) Postglacial colonisation of brown trout in Europe based on distribution of allozyme variants. *Heredity*, **82**, 46–56.

Gibbard PL (1988) The history of the great northwestern European rivers during the past three million years. *Philosophical Transactions of the Royal Society of London*, **318**, 559–602.

Gyllensten U, Ryman N, Ståhl G (1985) Monomorphism of allozymes in perch (*Perca fluviatilis* L.). *Hereditas*, **102**, 57–61.

Hansen MM, Mensberg KLD, Berg S (1999) Postglacial recolonization patterns and genetic relationships among whitefish (*Coregonus* sp.) populations in Denmark, inferred from mitochondrial DNA and microsatellite markers. *Molecular Ecology*, **8**, 239–252.

Harding RM (1996) New phylogenies: an introductory look at the coalescent. In: *New Uses for New Phylogenies* (eds Harvey PH, Leigh Brown AJ, Maynard Smith J, Nee S), pp. 15–22. Oxford University Press, Oxford, UK.

Heldstab H, Katoh M (1995) Low genetic variation in perch (*Perca fluviatilis* L.) from three major European drainage systems in Switzerland. *Aquatic Sciences*, **57**, 14–19.

Hewitt GM (1993) Postglacial distribution and species substructure: lessons from pollen, insects and hybrid zones. In: *Evolutionary Patterns and Processes* (eds Lees DR, Edwards D), pp. 98–123. Harcourt Brace & Company Publishers, London, UK.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Huff DR, Peakall R, Smouse PE (1993) RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.). *Theoretical and Applied Genetics*, **86**, 927–934.

Hultman T, Ståhl S, Hornes E, Uhlén M (1989) Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *Nucleic Acids Research*, **17**, 4937–4946.

Jerzmanska A, Raczynski P (1991) Middle Pleistocene freshwater fishes from the interglacial deposits near Belchtów, Poland. *Folia Quaternaria*, **61/62**, 109–123.

Largiadèr CR, Scholl A, Guyomard R (1996) The role of natural and artificial propagation on gene diversity of brow trout (*Salmo trutta* L.) of the upper Rhône drainage. In: *Conservation of Endangered Freshwater Fishes in Europe* (eds Kirchhofer A, Hefti D), pp. 181–197. Birkhäuser Verlag, Basel.

Legred P, Vaudor A (1991) *The R package: Multidimensional Analysis, Spatial Analysis*. Département de Sciences Biologiques, Université de Montréal, Montréal.

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, **3**, 91–99.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Marsden JE, Kassler T, Philip D (1995) Allozyme confirmation that North American yellow perch (*Perca flavescens*) and Eurasian yellow perch (*Perca fluviatilis*) are separate species. *Copeia*, **4**, 977–981.

Meyer A, Kocher TD, Basasibwaki P, Wilson AC (1990) Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature*, **347**, 550–553.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nesbø CL, Arab MO, Jakobsen KS (1998a) Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*). *Genetics*, **148**, 1907–1919.

Nesbø CL, Magnhagen C, Jakobsen KS (1998b) Genetic differentiation among stationary and anadromous perch (*Perca fluviatilis*) in the Baltic sea. *Hereditas*, **129**, 241–249.

Nielsen AV (1975) Landskabets tilblivelse. In: *Danmarks Natur. Landskabernes Opståen* (eds Nørrevang A, Meyer TJ), pp. 251–344. Politikens Forlag, Copenhagen. (In Danish).

Nielsen EE, Hansen MM, Loeschcke V (1996) Genetic structure of European populations of *Salmo salar* L. (Atlantic salmon) inferred from mitochondrial DNA. *Heredity*, **77**, 351–358.

Osinov AG, Bernatchez L (1996) 'Atlantic' and 'Danubian' phylogenetic groupings of brown trout *Salmo trutta* complex: Genetic divergence, evolution, and conservation. *Journal of Ichthology*, **36**, 723–746. (Translated from *Voprosy Ikhtiologii*, **36**, 762–786.)

Pamilo P, Nei M (1988) Relationship between gene trees and species trees. *Molecular Biology and Evolution*, **5**, 568–583.

Persat H, Berrebi P (1990) Relative ages of present populations of *Barbus barbus* and *Barbus meridionalis* (Cyprinidae) in southern France: preliminary considerations. *Aquatic Living Resources*, **3**, 253–263.

Refseth UH, Nesbø CL, Vøllestad LA, Fjeld E, Stacy JE, Jakobsen KS (1998) Genetic evidence for different migration routes of freshwater fish into Norway revealed by analysis of current perch (*Perca fluviatilis*) populations in Scandinavia. *Molecular Ecology*, **7**, 1015–1027.

Rholf FJ (1990) NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 1.60. Exeter Software, New York.

Riffel M, Storch V, Screiber A (1995) Allozyme variability of brown trout (*Salmo trutta* L.) populations across the Rhenian–Danubian watershed in soutwest Germany. *Heredity*, **74**, 241–249.

Rudi K, Kroken M, Dahlberg OJ, Deggerdal A, Jakobsen KS, Larsen F (1997) A rapid, universal method to isolate PCR-ready DNA using magnetic beads. *Biotechniques*, **22**, 506–511.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) Arlequin, Version 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland. Available from http://anthropologie.unige.ch/arlequin.

Shechrebukha A (1993) On the relations between fossil and recent taxa in the genus Perca. *Veshnik Zoologii*, **4**, 39–40. (In Russian).

Sholenius G (1996) The history of the Baltic proper since the late Weichselian deglaciation as recorded in sediments. *Quarternarya*, **3**, 3–34.

Stepien CA, Dillon AK, Chandler MD (1998) Genetic identity, phylogeography, and systematics of ruffe *Gymnocephalus* in the North American Great Lakes and Eurasia. *Journal of Great Lakes Research*, **24**, 361–378.

Strecker U, Meyer CG, Strumbauer C, Wilkens H (1996) Genetic divergence and speciation in an extremely young species flock in Mexico formed by the genus *Cypriodon* (Cyprinodontidae, Telostei). *Molecular Phylogenetics and Evolution*, **6**, 143–149.

Swofford DL (1998) *PAUP* (Phylogenetic Analysis Using Parsimony): Preliminary Test Version*. Sinauer, Sunderland, MA.

Taberlet P (1998) Biodiversity at the intraspecific level: the comparative phylogeographic approach. *Journal of Biotechnology*, **6**, 91–100.

Taberlet P, Fumagalli L, Wust-Saucy A-G, Cosson J-F (1998) Comparative phylogeography and postglacial colonisation routes in Europe. *Molecular Ecology*, **7**, 453–464.

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics*, **105**, 437–460.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Templeton AR (1993) The 'Eve' hypothesis: a Genetic critique and reanalysis. *American Anthropologist*, **95**, 51–72.

Templeton AR (1998) Nested clade analysis of phylogeographical data: testing hypotheses about gene flow and population history. *Molecular Ecology*, **7**, 381–397.

Templeton AR, Sing CF (1993) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analysis with cladogram uncertainty and recombination. *Genetics*, **134**, 659–669.

Templeton AR, Boerwinkle E, Sing CF (1987) A cladistic analysis of the phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. *Genetics*, **117**, 343–351.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of the phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.

Templeton AR, Routman E, Phillips CA (1995) Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics*, **140**, 767–782.

Zhu D, Jamison BGM, Hugall A, Moritz C (1994) Sequence evolution and phylogenetic signal in control region and cytochrome *b* sequences of rainbow fish (Melanotaeniidae). *Molecular Biology and Evolution*, **11**, 672–683.

Zhukov PI (1965) *Fishes of Belarus*. Nauka i Tehnika, Minsk. (in Russian).

This research is part of investigations aimed at understanding population genetics and phylogeography of European freshwater species with emphasis on the effects of the Pleistocene glaciations. Evolutionary geneticist K. S. Jakobsen and zoologist L. A. Vøllestad initiated the perch project in 1992. The work was carried out at the DNA Laboratory for Ecology, Systematics and Evolution (DESE) at the University of Oslo, and represents a part of Camilla L. Nesbø's PhD project and Tonje Fossheim's Master Thesis.
