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Genetic differentiation and relationship between genetic and geographical distance in Danish sea trout (*Salmo trutta* L.) populations

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Twenty-four samples of anadromous brown trout from four river systems/geographical regions were studied by PCR-RFLP analysis of the ND-1 and ND-5/6 regions of the mitochondrial genome. A total of 14 haplotypes was observed, and these could be divided into three phylogenetic groups. Populations within river systems/regions tended to be more closely related to each other than to populations from other river systems/regions. Also, a significant correlation was observed between geographical and genetic distances between populations. These results contrast with results from other studies of mainly resident and landlocked populations, where no correspondence was observed between genetic relationships and geographical location of populations. Gene flow connecting anadromous populations is probably the reason for the observed isolation-by-distance patterns, whereas in isolated resident and landlocked trout populations drift leads to random genetic divergence of populations. Tests for nonrandom geographical distribution of phylogenetic groups of haplotypes showed that drift and gene flow are probably the predominant factors affecting the distribution of haplotypes. There were, however, also some indications of clines in frequencies of phylogenetic groups of haplotypes.

Keywords: gene flow, genetic differentiation, isolation by distance, mitochondrial DNA, phylogeography, *Salmo trutta*.

Introduction

Patterns of genetic differentiation and gene flow in various organisms are highly influenced by both macro- and microgeographical conditions experienced by the organisms, and their migratory abilities. Many species of salmonid fishes undertake long migrations between foraging areas in lakes or in the sea and spawning grounds in rivers. Even though these species often exhibit very precise homing, some gene flow usually occurs among populations.

In species of Pacific salmon (*Oncorhynchus* spp.) it has been found that genetic and geographical distances between populations are generally positively correlated (Allendorf & Waples, 1996), suggesting an isolation-by-distance effect. However, in the case of the Atlantic salmon (*Salmo salar* L.) and, in particular, the brown trout (*Salmo trutta* L.) several studies, mainly based on allozyme electrophoresis, have suggested that no correlation exists between geographical and genetic distances between populations (cf. Ryman, 1983; Ferguson, 1989; Nielsen *et al*., 1996). Also, hierarchical gene-diversity analyses applied to brown trout allozyme data often have shown that more genetic diversity is distributed among individual tributary populations than among river systems, indicating strong microgeographical differentiation (e.g. Ryman, 1983; Ferguson, 1989; Riffel *et al*., 1995). However, many of these studies include populations which are effectively reproductively isolated from each other because of waterfalls, impassable dams, or other barriers, and thus drift may lead to erratic changes of allele frequencies without the homogenizing force of gene flow. The observation of no correlation between geographical and genetic distances between populations also contrasts with tagging experiments with anadromous trout, which show that strayers, i.e. individuals spawning in a 'wrong' river, are most likely to ascend

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a river in close proximity to the natal river (e.g. Svärdson & Fagerström, 1982).

Two distinct life history types are observed in brown trout, i.e. resident and migratory trout. The anadromous form of migratory brown trout, sea trout, is found in the Atlantic and Baltic region of the species' range of distribution. Sea trout and resident trout often coexist and only little, if any, genetic divergence has been observed between these sympatric life history forms, suggesting extensive interbreeding (Hindar *et al*., 1991). However, the presence of sea trout is potentially important, as they can mediate gene flow among populations from different river systems. Thus, Ferguson *et al*. (1995) have found that anadromous populations are generally more variable than resident populations. They ascribe this result to gene flow among anadromous populations, effectively rendering each sea trout population part of a large metapopulation. Also, because gene flow may in principle take place among all sea trout populations, the genetic structure may differ considerably from that of populations of purely resident and landlocked trout in isolated rivers and lakes, and the assumption of no correlation between genetic and geographical distances may not necessarily be valid. In spite of this, only a few studies have been aimed specifically at resolving the genetic structure of sea trout populations.

This paper focuses on the genetic structure of Danish sea trout populations, with waterway distances between sampling sites ranging from 1 to $\approx$650 km. The study was based on PCR-RFLP analyses of mitochondrial DNA segments, and the main aim was to assess the distribution of genetic diversity among trout populations within and among river systems, and to investigate if there was a correlation between estimated gene flow and geographical distances between populations. Finally, we wanted to get an impression whether the distribution of mtDNA haplotypes in Danish trout populations reflected primarily drift and gene flow or if more specific phylogeographical patterns could be observed, resulting from postglacial recolonization from different refugia.

Materials and methods

The studied localities

The study was based on samples from 24 localities distributed among four geographical regions: the Odder River System (data included from Hansen & Mensberg, 1996) and the Gudenaa River System, situated in Eastern Jutland, both of which flow into the Kattegat Sea, the Karup River System, which flows into the Limfjord in Northern Jutland, and Bornholm Island, situated in the Baltic Sea (data included from Hansen & Loeschcke, 1996a) (see Fig. 1; sample locality codes are listed in Table 1). The localities from Bornholm Island, the Odder River System, and most localities from the Gudenaa River System had never been stocked with trout. The Karup River System had previously been stocked with hatchery trout, but a study by Hansen *et al*. (1995) showed that the genetic contribution of these fish was negligible. However, some of the localities were still being stocked with trout of local origin (see Table 1). At some of the sampled localities the original populations were assumed to have been extirpated as a result of permanent organic pollution over several years. The present populations most likely represented recent recolonization events following improvement of environmental conditions. At some other localities, the trout populations were isolated from the other parts of the river system by impassable dams (GU1b and KA5). The status of the sampled populations, i.e. stocked, recently recolonized and/or isolated is indicated in Table 1.

All fish were caught by electrofishing. Most samples comprised trout aged $0+-3+$. However, the samples KA4 and KA6 consisted of adult anadromous trout. The tributaries GU1a and GU2 were sampled twice at an interval of some years in order to assess temporal changes in mtDNA haplotype frequencies.

MtDNA RFLP analysis

DNA was extracted from muscle or adipose fin tissue according to the method of Taggart *et al*. (1992). The ND-1 and ND-5/6 mtDNA segments were PCR-amplified, using the primers of Cronin *et al*. (1993), and analysed with the restriction endonucleases: *Ava*II, *Hae*III, *Alu*I, *Hpa*II, *Hin*fI and *Dde*I (ND-1), and *Ava*II, *Hae*III, *Hin*fI, *Taq*I and *Xba*I (ND-5/6). In previous studies of Danish trout populations variability was detected with this set of enzymes (Hansen & Loeschcke, 1996a; Hansen *et al*., 1997a). For more technical details, including PCR reaction conditions, we refer to these two papers.

Statistical treatments

Presence or absence of restriction sites in haplotypes was assessed from the presence or absence of frag-

ments in individual restriction enzyme profiles. The genetic relationships among haplotypes were analysed by constructing a tree according to the Wagner parsimony principle (Farris, 1970).

Genetic variability within populations was quantified by nucleon diversity and nucleotide diversity (Nei & Tajima, 1981). Homogeneity of haplotype frequencies between samples was tested according to the algorithm of Roff & Bentzen (1989), using the program MONTE from the REAP package (McElroy *et al*., 1991). Genetic differentiation was estimated by a two-level hierarchical analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992), using the program WINAMOVA vers. 1.53, developed by these authors. The first level of the hierarchy was the four geographical regions (Bornholm, the Karup, Gudenaa and Odder Rivers) and the second level consisted of populations within these regions. Average number of nucleotide substitutions per site between haplotypes (Nei & Tajima, 1981) was used

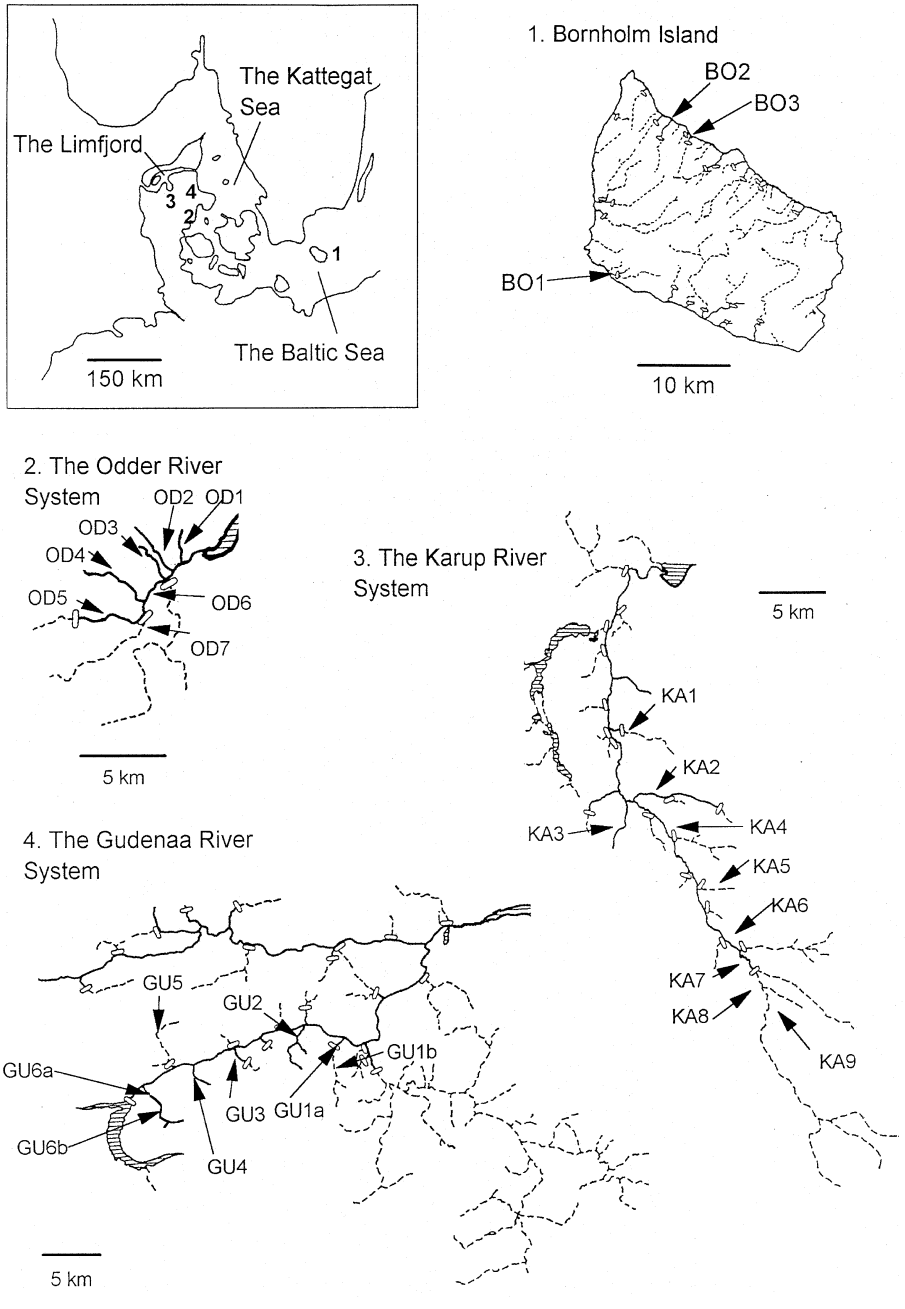

Fig. 1 Map showing the geographical location of the localities sampled for *Salmo trutta* in Denmark. Bars across rivers denote impassable barriers and inaccessible reaches of the river systems.

as molecular distance between haplotypes. The genetic relationships among the sampled populations were summarized by estimating sequence divergence between populations (Nei & Miller, 1990), using the program DA from the REAP package (McElroy *et al*., 1991), and constructing a neighbour-joining dendrogram (Saitou & Nei, 1987), using the program NEIGHBOR from the PHYLIP package (Felsenstein, 1993).

Female gene flow between populations ($N_f m_f$, where N_f is the effective population number of females, and m_f is migration rate for females) was estimated from the relationship between Φ_{ST} (or F_{ST}) and $N_f m_f$: $\Phi_{ST} = 1/(1+2N_f m_f)$ (Takahata & Palumbi, 1985). Correlation between geographical and genetic distances between samples was tested by Mantel tests (Mantel, 1967), using the program ISOLDE from the GENEPOP 3.1 package (Raymond & Rousset, 1995). Φ_{ST} between pairs of populations was used as a genetic distance, and geographical distances were estimated as the shortest waterway distance between each pair of populations. Isolation-by-distance patterns were further analysed by plotting log ($N_f m_f$) against log (geographical distance), as described by Slatkin (1993).

A permutation test, modified from a test procedure suggested by Templeton *et al*. (1995), was used for testing relationships between the phylogeny of haplotypes and their geographical distribution, with a null hypothesis that frequencies of haplotypes belonging to different phylogenetic groups did not differ among populations. Closely related haplotypes were merged into phylogenetic groups, and for each population the number of individuals exhibiting haplotypes belonging to these groups was pooled. Next, a chi-squared value was calculated, based on observed and expected numbers of individuals exhibiting haplotypes of the different groups. The significance of the chi-squared statistic was estimated by permuting haplotypes among phylogenetic groups, and the *P*-value was simply equal to the proportion of outcomes by chance resulting in a higher chi-squared value than the original statistic. The tests were performed using a program written in TURBO PASCAL, and each test was based on 1000 permutations. Table-wide significance levels were

Table 1 Locality codes, sampled localities, corresponding geographical regions, year of sampling, and status of the sampled populations of *Salmo trutta* (i.e. recently recolonized, accessible or inaccessible to gene flow, and stocked or nonstocked)

Locality code	Locality	Geographical region	Year of sampling	Recently recolonized	Restricted gene flow	Stocked
BO1	Vellens River	Bornholm	1993	—	—	—
BO2	Tejn R.	Bornholm	1993	—	—	—
BO3	Dyndals R.	Bornholm	1993	—	—	—
OD1	Fiskbaek R.	Odder R.	1992	+	—	—
OD2	Assedrup R.	Odder R.	1992	—	—	—
OD3	Kragebaek R.	Odder R.	1992	—	—	—
OD4	Asbaek R.	Odder R.	1992	—	—	—
OD5	Stampemølle R.	Odder R.	1992	—	—	—
OD6	Odder Main R.	Odder R.	1992	—	—	—
OD7	Odder Main R.	Odder R.	1992	+	—	—
KA1	Trevad Møllebaek R.	Karup R.	1992	+	—	+
KA2	Sejbaek R.	Karup R.	1992	+	—	+
KA3	Haderis R.	Karup R.	1992	+	—	+
KA4	Karup Main R.	Karup R.	1992	—	—	+
KA5	Rabis R.	Karup R.	1992	—	+	—
KA6	Karup Main R.	Karup R.	1993	+	—	+
GU1a	Tjaerbaek R.	Gudenaa R.	1989/94	—	—	—
GU1b	Tjaerbaek R.	Gudenaa R.	1994	—	+	—
GU2	Brandstrup R.	Gudenaa R.	1994/95	—	—	—
GU3	Kjelbaek R.	Gudenaa R.	1994	—	—	—
GU4	Gullev R.	Gudenaa R.	1994	+	—	—
GU5	Møbaek R.	Gudenaa R.	1993	—	—	+
GU6a, 6b	Skibelund R.	Gudenaa R.	1994	—	—	—

applied to all tests of the study, using the sequential Bonferroni technique (Rice, 1989).

Results

Fourteen different haplotypes were detected (Table 2). All restriction morphs and haplotypes have been described previously by Hansen & Loeschcke (1996a) and Hansen *et al*. (1997a). The parsimony tree (Fig. 2) showed that the haplotypes could be divided into three phylogenetic clades. Within each clade haplotypes were separated from each other by one or two mutations, whereas the three clades were connected to each other by at least three mutational steps. The precise relationship among the clades could not be resolved. For instance, clades II and III might as well be connected through haplotypes 4 and 13 or 5 and 13, which were also separated by three mutational steps.

The number of haplotypes detected in individual populations ranged between 2 and 10, nucleon diversity values between 0.31 and 0.85, and nucleotide diversity values between 0.0008 and 0.0087 (Table 2). Genetic variability was high in most of the Gudenaa River, Odder River and Bornholm populations with a mean number of haplotypes of 6.0 (range: 3–10) and mean nucleon and nucleotide diversities of 0.70 (range: 0.31–0.87) and 0.0068 (range: 0.0022–0.0091), respectively (Table 2). Karup River trout exhibited less variation with a mean number of haplotypes of 3.8 (range: 2–5), mean nucleon and nucleotide diversities of 0.60 (range: 0.47–0.69) and 0.0021 (range: 0.0008–0.0034), respectively (Table 2). The two populations which were inaccessible to gene flow (KA5 and GU1b) both exhibited decreased variability compared to other populations from the same river systems (Table 2). Haplotype 6 was found at relatively high frequencies in the Gudenaa River populations, but was not detected elsewhere. Haplotypes 11, 12 and 13 were found only in the Bornholm region.

Significant genetic differentiation, in terms of Φ-statistics, was observed (Table 3). The hierarchical analyses showed that a major part of the molecular variance was distributed among regions, although differentiation among populations within regions was also strong. The pairwise tests for homogeneity of haplotype frequencies between samples resulted in many significant outcomes. There was clearly a tendency towards fewer significant results when samples from the same river system/region were compared than when samples from different river systems/regions were tested against each other (Table 4). Both in populations GU1a and GU2 the tests involving samples taken in different years revealed significant temporal heterogeneity of haplotype frequencies ($P<0.01$ and $P<0.05$, respectively). The dendrogram describing the relationships among populations (Fig. 3) showed that populations from the same regions tended to cluster, though some examples of the opposite were also observed.

The Mantel test for correlation between geographical and genetic distance (Φ_{ST} values between pairs of populations), involving all populations, yielded a significant outcome ($P<0.001$), whereas tests for correlation within each of the Karup, Gudenaa and Odder river systems all yielded nonsignificant results. Gene flow ($N_f m_f$) between all populations was estimated from pairwise Φ_{ST} values, but in a few cases Φ_{ST} was zero and, consequently, $N_f m_f$ could not be defined. These pairwise comparisons were not included in further analyses. The plot of log ($N_f m_f$) vs. log (geographical distance) indicated an isolation-by-distance effect with a regression line of $y = -0.33x + 1.02$ (Fig. 4a), although the coefficient of determination was low ($r^2 = 0.12$). It appeared from the tests for homogeneity of haplotype frequencies between populations (Table 4), the AMOVA analyses (Table 3) and the neighbour-joining dendrogram (Fig. 3), that populations within river systems/regions tended to be more closely related to each other than to populations from other river systems/regions. Consequently, samples within river systems/regions were pooled, and gene flow was estimated between pooled populations. Levels of gene flow corresponded well to the geographical locations of the populations, with minimal gene flow among Karup River and Bornholm trout (the two regions separated by the largest geographical distances) and high levels of gene flow among Gudenaa River and Odder River trout (the river systems separated by the smallest geographical distances). A plot of log ($N_f m_f$) vs. log (geographical distance) (Fig. 4b) indicated a strong isolation-by-distance effect ($y = -1.94x + 5.19$, $r^2 = 0.44$), although it should be considered that this plot involved only six data points.

The tests for relationships between the phylogeny of haplotypes and their geographical distribution were based on permuting haplotypes among clades I, II and III, as defined previously. A test involving all four regions yielded a nonsignificant result ($P = 0.16$). Tests between all pairs of regions yielded

individually significant results for the Karup River vs. Bornholm ($P = 0.014$) and the Odder River vs. Bornholm ($P = 0.016$), although the results were not significant at a table-wide level, i.e. after application of the sequential Bonferroni procedure (data not shown).

Table 2 Composite genotypes (haplotypes), haplotype frequencies, and unbiased nucleon diversity of the sampled populations of *Salmo trutta*. Composite genotypes are denoted by capital letters in the following order: ND-1: *Ava*II, *Hin*fI, *Alu*I, *Hae*III, *Hpa*II, *Dde*I. ND-5/6: *Ava*II, *Hin*fI, *Hae*III, *Taq*I, and *Xba*I

Haplotype genotype	Composite	Sample localities										
		KA1	KA2	KA3	KA4	KA5	KA6	OD1	OD2	OD3	OD4	OD5
Type 1	AAAAAAAAAAB	0	0.04	0	0	0	0	0	0	0	0	0
Type 2	BAACBACABAA	0	0.04	0.07	0	0	0	0.31	0.26	0.33	0.41	0.10
Type 3	AACCAABCABA	0	0	0	0	0.33	0.03	0	0	0	0	0
Type 4	AABCAABCABA	0.39	0.56	0.39	0.58	0.67	0.52	0.07	0.02	0.03	0	0.10
Type 5	AAABAABCABA	0.32	0.32	0.14	0.22	0	0.24	0.07	0.05	0.10	0.08	0.05
Type 6	AABCAAACABB	0	0	0	0	0	0	0	0	0	0	0
Type 7	AAAAAABAAAA	0	0	0	0.02	0	0	0.36	0.21	0.38	0.15	0.28
Type 8	AAACAABCABA	0.29	0.04	0.39	0.18	0	0.15	0.10	0.24	0	0.05	0.23
Type 9	AAAAAACABAA	0	0	0	0	0	0	0	0	0	0	0
Type 10	ABABBABCABA	0	0	0	0	0	0	0.10	0.21	0.15	0.31	0.25
Type 11	AABCAABAABA	0	0	0	0	0	0	0	0	0	0	0
Type 12	BAACBACCBAA	0	0	0	0	0	0	0	0	0	0	0
Type 13	AAAAABCAAAA	0	0	0	0	0	0	0	0	0	0	0
Type 14	AAAAAABBCAA	0	0	0	0	0	0.06	0	0	0	0	0
N		28	25	28	50	21	79	42	42	39	39	40
Nucleotide diversity		0.0016	0.0034	0.0030	0.0018	0.0008	0.0019	0.0071	0.0076	0.0072	0.0071	0.0062
Nucleon diversity		0.69	0.60	0.58	0.59	0.47	0.65	0.77	0.80	0.72	0.72	0.81

Haplotype	Sample localities														
	OD6	OD7	GU1a 89	GU1a 94	GU1b	GU2 94	GU2 95	GU3	GU4	GU5	GU6a	GU6b	BO1	BO2	BO3
Type 1	0	0	0.04	0	0	0.03	0.10	0	0	0.02	0	0	0.18	0.30	0.13
Type 2	0.33	0.13	0.16	0.22	0.65	0.30	0.38	0.17	0.62	0.82	0.03	0.15	0.64	0.70	0.30
Type 3	0	0	0	0.02	0	0.10	0	0.03	0.19	0	0.71	0.18	0	0	0
Type 4	0.17	0.08	0.40	0.06	0.12	0.20	0.14	0.07	0.10	0.16	0.11	0.03	0.14	0	0.17
Type 5	0	0.48	0.16	0.16	0.02	0.23	0.07	0.53	0.04	0	0.09	0.23	0	0	0.09
Type 6	0	0	0.08	0.04	0.16	0.03	0.09	0.09	0	0	0	0.28	0	0	0
Type 7	0.31	0.13	0.12	0.16	0.02	0	0.10	0	0	0	0.03	0.05	0.05	0	0.04
Type 8	0.05	0.03	0.04	0.06	0	0	0.02	0.10	0.02	0	0	0	0	0	0
Type 9	0	0	0	0.02	0	0.03	0	0	0	0	0	0	0	0	0
Type 10	0.14	0.18	0	0.24	0	0.07	0.10	0	0.02	0	0.03	0	0	0	0
Type 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.13
Type 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.09
Type 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04
Type 14	0	0	0	0.04	0.02	0	0	0	0.02	0	0	0.08	0	0	0
N	42	40	25	51	49	30	58	58	52	44	35	39	22	23	23
Nucleotide diversity	0.0055	0.0077	0.0070	0.0077	0.0077	0.0081	0.0091	0.0054	0.0069	0.0044	0.0022	0.0074	0.0071	0.0056	0.0087
Nucleon diversity	0.76	0.72	0.80	0.85	0.54	0.83	0.81	0.67	0.58	0.31	0.48	0.82	0.59	0.47	0.87

Discussion

Genetic variation and differentiation

The reduced variability in Karup River trout is likely to be the result of a population bottleneck, as the Karup River was heavily polluted during the 1960s and 1970s. Probably, the only original populations are those at the KA3 and KA4 localities. The populations at the other localities are assumed to have been re-established by a combination of natural recolonization and stocking activity, involving offspring of local trout (Hansen *et al*., 1995).

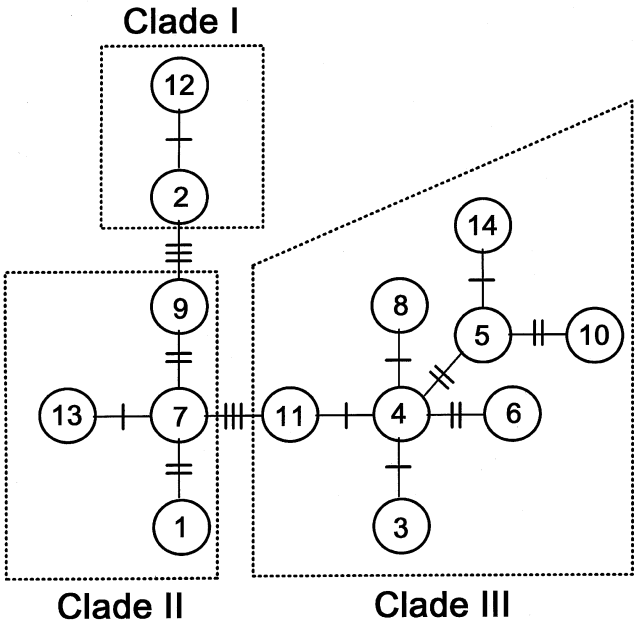

Fig. 2 Maximum parsimony tree showing the relationships among the observed haplotypes of *Salmo trutta*. Bars across lines denote the number of mutational steps separating pairs of haplotypes. Clades I, II and III denote clades of haplotypes separated from each other by three mutation steps.

The neighbour-joining dendrogram, based on sequence divergence between populations (Fig. 3) and the tests for homogeneity of haplotype frequencies between samples (Table 4), generally demonstrated close relationships between populations from the same river system/region. There were, however, some notable exceptions to the general pattern. The two dammed populations, KA5 and GU1b, diverged considerably from other populations from the same river systems and exhibited reduced variability, so restricted gene flow and exclusion of the anadromous portion of the populations had major effects on genetic diversity at the mtDNA level. Founder effects may be of importance for the divergence of some of the other populations. In particular, the recently recolonized population OD7 diverged significantly from other populations from the Odder River system (Table 4). In other recolonized populations, however, it was not obvious that founder effects had resulted in increased divergence (OD1 and KA1, 2, 3, 6). Finally, the GU6a sample diverged considerably from another sample from the same tributary (GU6b). This was analysed in more detail by Hansen *et al*. (1997b). By a combined mtDNA and microsatellite approach it was shown that nearly all 0+trout belonged to just three families, and that this had probably resulted in biased estimates of haplotype frequencies.

The outcome of the hierarchical analyses of molecular variance, showing that a large part of the total variance was distributed among river systems/regions (Table 3), contrasts with results from several studies of mainly resident or landlocked trout, based on allozyme electrophoresis (e.g. Ryman, 1983; Riffel *et al*., 1995). As an example, Riffel *et al*. (1995) studied trout populations from the Rhine and Danube drainages in Germany, and found that only 1% of the total variance was distributed among drainages, whereas 19% was distributed among subpopulations within drainages.

Table 3 Analysis of molecular variance (AMOVA) as described by Excoffier *et al*. (1992) among populations of *Salmo trutta*

Variance components			Φ-statistics		
Among regions	Among populations within region(s)	Within populations	Φ_{CT}	Φ_{SC}	Φ_{ST}
0.0010 (17.1%)	0.0008 (13.3%)	0.0040 (69.6%)	0.17***	0.16***	0.30***

Φ_{CT} is the correlation of haplotypes drawn from populations within regions relative to the total; Φ_{SC} is the correlation of haplotypes drawn from populations relative to the region; and Φ_{ST} is the correlation of haplotypes drawn from populations relative to the total. The significance of the Φ-statistics was tested by permutations.
***$P<0.001$.

Table 4 Pairwise tests for homogeneity of haplotype frequencies between samples of *Salmo trutta*. The significances of χ^2-statistics were estimated by the procedure described by Roff & Bentzen (1989)

	KA 1	KA 2	KA 3	KA 4	KA 5	KA 6	OD 1	OD 2	OD 3	OD 4	OD 5	OD 6	OD 7	GU 1a—94	GU 16	GU 2—95	GU 3	GU 4	GU 5	GU 6a	GU 6b	BO 1	BO 2
KA2	—																						
KA3	—	—																					
KA4	—	—	—																				
KA5	***	**	***	**																			
KA6	—	—	—	—	***																		
OD1	***	***	***	***	***	***																	
OD2	***	***	***	***	***	***	—																
OD3	***	***	***	***	***	***	—	—															
OD4	***	***	***	***	***	***	—	—	—														
OD5	***	***	**	***	***	***	—	—	—	—													
OD6	***	***	***	***	***	***	—	—	—	—	—												
OD7	***	***	***	***	***	***	**	***	*	*	*	***											
GU1a—94	***	***	***	***	***	***	—	—	—	—	—	—	—										
GU1b	***	***	***	***	***	***	***	***	***	***	***	***	***	***									
GU2—95	***	***	***	***	***	***	—	*	—	—	***	—	**	—	—								
GU3	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***	***							
GU4	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***						
GU5	***	***	***	***	***	***	***	***	***	***	***	***	***	***	—	***	***	—					
GU6a	***	***	***	***	*	***	***	***	***	***	***	***	***	***	***	***	***	***	***				
GU6b	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***	***	***			
BO1	***	***	***	***	***	***	*	***	***	***	***	*	***	***	—	—	***	—	—	***	***		
BO2	***	***	***	***	***	***	***	***	***	***	***	***	***	***	**	—	***	***	*	***	***	—	
BO3	***	***	***	***	***	***	**	***	***	***	***	***	***	**	**	—	***	***	***	***	***	—	*

*$P<0.05$, **$P<0.01$, ***$P<0.001$, — NS.

Relationship between geographical distance and gene flow

The Mantel tests and the plots of log ($N_f m_f$) vs. log (geographical distance) (Fig. 4a,b) showed that there was indeed an isolation-by-distance effect, but that this was weak when all populations were considered individually (Fig. 4a). It was found that Mantel tests involving only populations from the same river systems all yielded nonsignificant results, and that much stronger isolation-by-distance patterns were observed when samples from the same river systems/regions were pooled. This suggests that geographical distances between river systems are important, whereas geographical distance between populations within river systems is of limited importance to the genetic structure of populations. It could be argued that the differences in isolation-by-distance patterns for populations within river systems, as opposed to populations from different river systems, could be a result of the relatively small geographical distances within river systems, whereas distances between river systems were much larger. However, Morán *et al.* (1995) studied relationships between geographical and genetic distance in Spanish trout populations, based on allozyme electrophoresis, and reached a

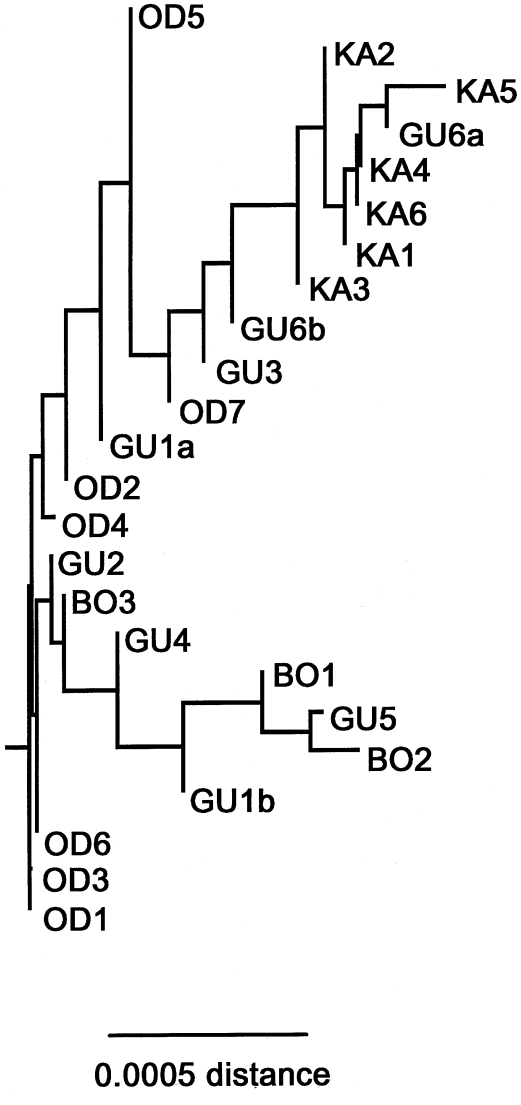

Fig. 3 Neighbour-joining dendrogram (Saitou & Nei, 1987), based on sequence divergence between populations (Nei & Miller, 1990), summarizing the genetic relationships among populations of *Salmo trutta*.

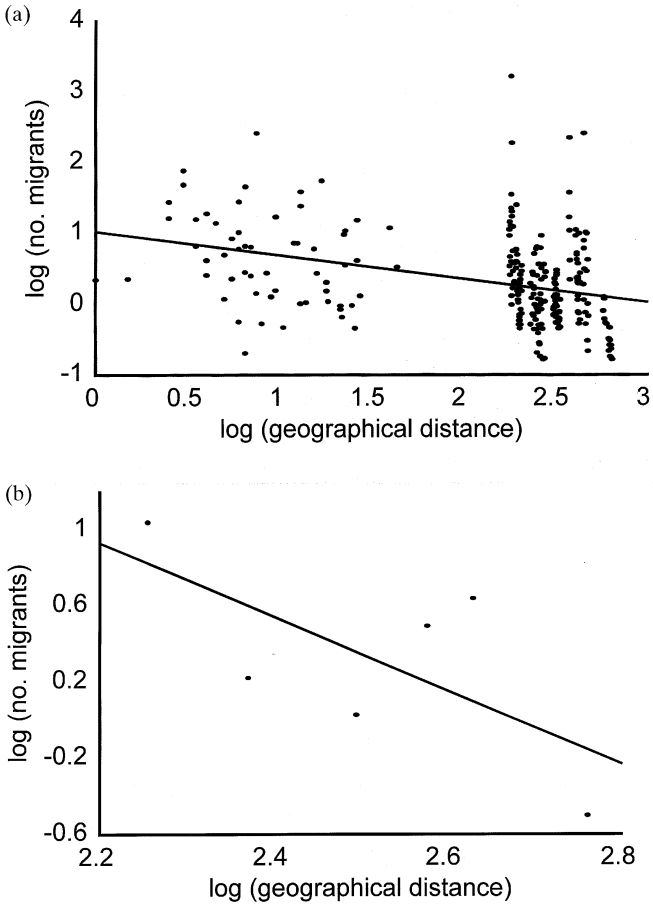

Fig. 4 Plots of log (estimated number of migrants between pairs of populations) vs. log (geographical distance between pairs of populations) for analysing isolation-by-distance patterns in *Salmo trutta*, as described by Slatkin (1993). (a) Plots involving all sampled populations. (b) Plots involving only the four main regions/river systems sampled, i.e. samples within each of the Karup, Gudenaa and Odder River systems and Bornholm Island were pooled. Regression lines are (a) $y = -0.33x + 1.02$, $r^2 = 0.12$ and (b) $y = -1.94x + 5.19$, $r^2 = 0.44$.

similar conclusion. This study operated on a much smaller geographical scale than our study (maximum distances between populations were ≈ 45 km), so distances within and between river systems were of the same order of magnitude.

The differences in patterns of gene flow within and between river systems may result from several factors. It is possible that gene flow takes place in a hierarchical manner, where gene flow within river systems is mainly in accordance with an island model (Wright, 1951), whereas gene flow among river systems follows a stepping-stone model (Kimura & Weiss, 1964). Alternatively, the lack of correspondence between gene flow and geographical distances between populations within river systems could be the result of small effective population sizes of tributary populations resulting in strong drift, which might obscure patterns of gene flow. The lack of temporal stability of haplotype frequencies observed in the GU1a and GU2 populations supports this explanation, and in general there are several examples of temporal shifts of mtDNA haplotype frequencies in natural populations resulting from the small effective population number of mtDNA relative to nuclear genes (e.g. Hansen & Loeschcke, 1996b). It remains to be seen, however, at what time scale significant temporal changes in nuclear allele frequencies are likely to take place. Analysis of microsatellite DNA from scale samples taken over many years would be an obvious way of addressing this problem (Nielsen *et al*., 1997).

Finally, there is the possibility that nonrepresentative sampling from tributary populations, in particular sampling offspring from just a few families, has taken place (Allendorf & Phelps, 1981). As mentioned previously, this was shown to be the case in one sample of juvenile trout (Hansen *et al*., 1997b) and we cannot rule out that this has happened in other samples as well. Although this could result in biased estimates of allele or (in this case) haplotype frequencies in individual tributary populations, the pooling of all samples from each river system could lead to a more representative estimate of haplotype frequencies for trout of the river system as a whole. This might explain why a much stronger isolation-by-distance pattern was observed when populations within river systems were pooled than when all populations were considered individually (Fig. 4b vs. 4a).

Phylogeographical patterns

Distinct phylogeographical patterns have been observed in brown trout populations over large parts of the species' range of distribution (e.g. Bernatchez *et al*., 1992). On a smaller northern European scale, Hamilton *et al*. (1989) suggested that the distribution of alleles at the *LDH-5** locus in trout populations reflected at least two postglacial recolonization events that had taken place in this region from different refugia. However, in a later study Hynes *et al*. (1996) did not find an obvious correspondence between geographical distribution of trout mtDNA haplotypes in northern Europe and their phylogenetic relationships, and there was no apparent correlation between *LDH-5** alleles and specific phylogenetic groups of haplotypes.

In the present study, the tests for nonrandom geographical distribution of phylogenetic groups of haplotypes did not yield any significant results, and drift and gene flow are probably the most important factors involved in the geographical distribution of haplotypes. The pairwise tests between the Karup River and Bornholm regions and the Odder River and Bornholm regions did, however, approach significance. Also, there were some indications of clines in the distribution of clades of haplotypes (as defined in Fig. 2). Going from the Karup River in Northern Jutland over the Gudenaa and Odder Rivers and into the Baltic Sea area, i.e. Bornholm Island, the frequencies of clade I haplotypes were 0.01, 0.37, 0.27 and 0.58, the frequencies of clade II haplotypes were 0.01, 0.07, 0.26 and 0.25, and the frequencies of clade III haplotypes were 0.98, 0.56, 0.47 and 0.18, respectively. It is important, however, to keep in mind that Karup River trout may have passed through a recent population bottleneck, and this may have lead to significant shifts of haplotype frequencies including loss of some haplotypes. Clearly, screening of many more trout populations would be necessary to verify the presence of clines in the distribution of trout haplotypes in the Kattegat and Baltic Sea area, but this might be worth the effort. It has previously been shown that Atlantic salmon from the Baltic Sea area are genetically distinct from populations from the Atlantic area (Ståhl, 1987). In general, the Scandinavian peninsula and the Baltic region appears to be a secondary contact hot-spot zone (e.g. Jaarola & Tegelström, 1995).

Conclusions

In conclusion, our results showed that even though there may be significant genetic differentiation among tributary populations within river systems, sea trout populations from one river system were generally more closely related to each other than to

populations from other river systems. Also, there did appear to be a correlation between geographical distance and estimated gene flow between populations, but mainly at the level of gene flow from one river system/region to another. Drift and gene flow were most likely to be the main forces affecting the geographical distribution of haplotypes, but there were some indications that phylogeographical factors, i.e. secondary contact between trout exhibiting different phylogenetic groups of haplotypes, could also play a role.

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