Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks

Camilla L. Nesbø^{1†}, Eli K. Rueness¹, Svein A. Iversen², Dankert W. Skagen² and Kjetill S. Jakobsen^{1*}

¹Division of General Genetics, Institute of Biology, University of Oslo, PO Box 1031 Blindern, N-0315 Oslo, Norway

²Department of Marine Resources, Institute of Marine Research, N-5024 Bergen, Norway

Despite the resolving power of DNA markers, pelagic and migratory marine fish species generally show very little geographical population structuring. In mackerel (*Scomber scombrus* L.) population differentiation has been detected only at a transatlantic scale. By applying two regions in mitochondrial DNA (mtDNA) (D-loop and cytochrome *b* (*cytb*)) in combination with genealogical and frequency-based statistical approaches, our data suggest population differentiation among eastern Atlantic spawning stocks. In contrast, and indicative of homing behaviour, no genetic structuring was observed among shoals of individuals outside the spawning season. Among spawning stocks, mtDNA D-loop sequences detected differentiation within the eastern Atlantic, while the *cytb* gene detected transatlantic differentiation. The impact of recurrent events (e.g. gene flow restricted by isolation by distance) and historic events (e.g. population range expansions) among spawning stocks was investigated applying a nested cladistic analysis of geographical distribution of *cytb* haplotype lineages. In the eastern Atlantic, historical population range expansion, presumably in connection with recolonization of northern areas after the last glaciation, is suggested to be the main factor determining mtDNA lineage distribution. This was supported by estimates of mtDNA nucleotide diversity, where the highest diversity was observed for the stock spawning in the Bay of Biscay, for which the size estimate is only 15% of the largest stock (Celtic Sea). In addition to revealing population differentiation, our data demonstrate the importance of sampling strategy and the power of applying statistical methods addressing both ongoing and historical population processes.

Keywords: *Scomber scombrus*; mackerel; phylogeography; mitochondrial DNA; D-loop; cytochrome *b*

1. INTRODUCTION

Marine fishes generally show low genetic differentiation among stocks or populations (e.g. Ward *et al.* 1994). This phenomenon is commonly attributed to high level of current gene flow as a result of the high dispersal capabilities in the marine environment (e.g. Ábrnason & Pálsson 1996; Vis *et al.* 1997). However, marine fish populations are usually very large, resulting in low levels of drift and thus increased coalescent time of alleles or haplotypes (e.g. Tajima 1983). Moreover, the distribution and level of divergence of different genetic lineages in highly mobile species reflect both present and historical levels of gene flow (e.g. Camper *et al.* 1993; Magoulas *et al.* 1996; Rosel & Block 1996; Merilä *et al.* 1997). Hence, lack of detectable differentiation at neutral loci could also be due to ancient gene flow, such as colonization events, and a recent (relative to coalescent time) separation of populations. In agreement with this, population differentiation in widely distributed species with large population sizes has in many instances only been detected at a few loci, commonly associated with relatively high mutational turnover (e.g. Wenink *et al.* 1993; Bentzen *et al.* 1996; Bowen & Grant 1997; Ruzzante *et al.* 1998).

Atlantic mackerel (*Scomber scombrus* L.) is found in temperate shelf waters of the North Atlantic Ocean and adjacent seas (Lockwood 1988). In the eastern Atlantic, three stocks (or 'populations') can be defined according to spatial and temporal differences associated with spawning (Iversen 1995; Anonymous 1996); the Southern stock (Bay of Biscay), the Western stock (Celtic Sea) and the North Sea stock. Reproductive isolation of the spawning stocks has been suggested by studies of parasite infestations and otolith morphology, as well as from tagging data (reviewed in Lockwood 1988). Yet, studies of mackerel using protein polymorphisms showed no structuring between sample sites, indicating one panmictic population (reviewed in Jamison & Smith 1987). However, the mackerel stocks are large; at present the Western stock is the largest, about 2.5 million tons, the Southern stock is about 0.4 million tons and the North Sea stock is about 0.1 million tons (Anonymous 1999). Notably, the North Sea stock was probably the largest (about four million tons in 1960; Iversen 1995) prior to the collapse of this stock in the early 1970s (mainly due to overfishing; Hamre 1978). Hence, as suggested above, the lack of detectable differentiation from allozyme markers, might also be due to low mutation rates and/or low levels of drift. The demonstration of transatlantic heterogeneity for mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) haplotype distribution by Scoles *et al.* (1998), may be an indication of this. On the other hand, the mackerel is a highly migratory species, and tagging

* Author for correspondence (k.s.jakobsen@bio.uio.no).

† Present address: Department of Biochemistry and Molecular Biology, Dalhousie University, Canada B3H 4H7.

experiments have demonstrated high dispersal rates; 1200 km in 13 days have been observed (i.e. 93 km a day; Lockwood 1988). Tagging studies have also revealed that fish from all three spawning stocks gather in the Norwegian and North Sea after spawning (e.g. Iversen & Skagen 1989; Uriarte 1995). Hence, these data suggest considerable levels of potential gene flow among populations.

In an effort to investigate population history and population structure in mackerel, we have chosen a phylogeographical approach using genetic markers displaying different levels of polymorphism. We report here the results obtained with the relatively slowly evolving mtDNA cytochrome *b* (*cytb*) gene and the rapidly evolving non-coding mtDNA D-loop.

2. MATERIAL AND METHODS

(a) Sample collection

Information about the samples used is shown in table 1. Samples from the North Sea, Western, Southern and Canadian spawning stocks were individually marked specimens with ripe gonads. The mackerel from the Adriatic Sea were bought from local fishermen in Numana (Ancona, Italy). These samples were juveniles caught in July, by that time this area may have been exposed to migration from other spawning grounds in the Mediterranean Sea. All samples were stored in 96% ethanol.

(b) DNA isolation, amplification and sequencing

DNA was extracted from tail fins or muscle tissue either by a standard phenol–chloroform isolation procedure or using the DNA Direct kit (DynaL AS, Oslo, Norway; Rudi *et al.* 1997). Prior to extraction the tissue was washed in acetone to remove fat. Amplification and sequencing of 272 bp of the 5' control region was carried out using the following primers: Pro, TCC CAC CCC TAA CTC CC; and 12S, CGG TGA CTT GCA TGT GTA AGT TCA (Nesbø *et al.* 1998a); texas-red-GTC CCT CAC CTT CAA TAA CC (Nesbø *et al.* 1998b). Primers for amplifying and sequencing 398 bp of the 3'-end of the *cytb* gene (bp 601–999; accession no. X81564) were CytbL, TTG TTT TCT GCC CAT CC; CytbU, AAA ACA CAC CCA CTG CT; texas-red-ACT TGG CCA ATG ATA ATG. Amplifications were carried out in 50 µl final volume containing 1–5 ng template DNA, 1 × PCR buffer, 2.5 mM MgCl₂, 0.2 mM dNTP, 1 mM of each primer, 0.5–1 U Taq-polymerase (Advanced Biotechnology, Surrey, UK). The reactions were submitted to an initial denaturation at 96 °C for 5 min, and then 30 cycles with denaturation at 96 °C for 1 min, primer annealing at 50 °C for 1 min (mtDNA), and primer extension at 72 °C for 2 min. Cycling was performed on a Perkin Elmer 2400 (PE Biosystems, Foster City, CA, USA). Sequencing of mtDNA PCR products was performed on a Vistra DNA 725 sequencer (Amersham Pharmacia Biotech, Buckinghamshire, UK) using Vistra systems Thermo Sequenase sequencing kit.

(c) Data analysis

A *cytb* haplotype cladogram was estimated according to Templeton *et al.* (1992). This cladogram construction procedure is specifically designed to estimate intraspecific gene trees where most of the nodes (haplotypes) are present in the population under investigation, and are few mutations apart. A set of cladograms that have a high probability (> 95%) of being true is obtained. The resulting cladogram was converted into a nested design as described in Templeton *et al.* (1987). For D-loop

sequences, phylogenetic relationships were estimated using parsimony and neighbour joining (NJ) of Kimura 2P distances (gamma corrected with $\alpha = 0.40$) in PAUP* (Swofford 1998). Inspection of networks constructed between closely related D-loop haplotypes revealed that a very high level of homoplasy was associated with position 84. Hence, this site was excluded in all analyses. Probability of parsimony and θ (expected nucleotide diversity) were estimated using the Mathematica package described in Templeton *et al.* (1992).

For the *cytb* data we used the nested cladistic analysis of geographical distances described in Templeton *et al.* (1995) to test the null hypotheses of no geographical association of haplotypes, and to separate population structure from population history. Four measures of the geographical distance based on the nested cladogram described above are used; $D_c(x)$, $D_n(x)$, $I_c(y)$ and $I_n(y)$, where x and y refer to a specific n -step and $n + 1$ -step clade, respectively. The $D_c(x)$ 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 $D_n(x)$ 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 $I_c(y)$ and $I_n(y)$ are the average D_c or D_n value for all interior clades within the nesting clade y minus the average D_c or D_n value for all terminal clades within nesting clade y . Hence, these indices give a measure of the distribution of old versus young clades. 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).

χ^2 -pseudo-probability tests (Roff & Bentzen 1989) were performed to test for heterogeneity in *cytb* haplotype frequencies between populations using the CHIRXC-program (Zaykin & Pudovkin 1993). Level of between-population differentiation was assessed using pairwise Φ_{ST} calculated in Arlequin v.1.1 (Schneider *et al.* 1997), applying Kimura 2P distances (gamma corrected with $\alpha = 0.40$ for D-loop data).

Analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was used to estimate the amount of genetic structuring at different hierarchical levels by quantifying the inter- and intragroup component of total variance by Φ -statistics in the Arlequin v.1.1 program package (Schneider *et al.* 1997). Estimation of gene and nucleotide diversity, and probability of selective neutrality were done in Arlequin v.1.1. Population UPGMA phenograms from the *cytb* data were constructed in Phylip v.3.572 (Felsenstein 1995). Between-population divergence was estimated as net nucleotide divergence (DA; Nei 1987) and Cavalli-Sforza & Edwards's (1967) chord distance. In calculating chord distances rare haplotypes (from the same one-step clade) were pooled. Confidence of the branches was obtained by bootstrapping haplotypes within populations (100 replicates).

3. RESULTS

(a) High levels of sequence variation in the mtDNA D-loop

In total, 205 mackerel (table 1) were analysed for variation in the 5'-end of the mtDNA D-loop (GenBank

Table 1. *The samples of mackerel assayed in this study*

stock	stock size ^a	sample ^b	cytb (<i>N</i>)	D-loop (<i>N</i>)	date	geographical position
spawning stocks						
Adriatic Sea (AD)	—	AD.a	6	6	23.7.97	43°35' N 13°47' E
		AD.b	10	9	23.7.97	43°35' N 13°47' E
Southern (S)	0.4 × 10 ⁶	S.a	10	7	3.4.97	43°20' N 42°20' W
		S.b	9	3	22.4.97	43°38' N 5°36' W
		S.c	8	5	25.4.97	43°39' N 5°40' W
		S.d	10	1	25.4.97	43°39' N 5°40' W
Western (W)	2.5 × 10 ⁶ t	W.a	10	4	22.5.97	51°25' N 11°15' W
		W.b	7	8	29.5.97	52°55' N 12°30' W
		W.c	10	—	4.6.97	57°45' N 9°25' W
		W.d	10	—	7.6.97	59°15' N 7°20' W
		W.e	11	10	8.6.97	60°25' N 2°55' E
North Sea (NS)	0.1 × 10 ⁶ t	NS.a	10	9	29.6.97	57°00' N 1°45' E
		NS.b	10	—	3.7.97	57°50' N 7°20' E
		NS.c	9	5	11.7.97	59°16' N 4°24' E
		NS.d	10	3	17.7.97	61°23' N 3°40' E
Canada (Can)	5.0 × 10 ⁶ t	Can	27	22	24.6.97	47°59' N 65°05' W
non-spawning stock (North Sea)						
adults		MIX.a ¹	—	7	July 1996	62°32' N 6°08' E
		MIX.b ²	—	8	12.10.96	60°08' N 4°38' E
		MIX.c ³	—	10	16.10.96	61°25' N 3°38' E
		MIX.d ³	—	8	16.10.96	60°24' N 3°04' E
		MIX.e ⁴	—	10	18.10.96	60°25' N 2°55' E
		MIX.f ³	—	9	22.11.96	59°18' N 3°23' E
		MIX.g ⁴	—	9	23.1.97	61°12' N 2°09' E
		FRY.a ⁵	—	7	21.11.96	59°59' N 1°20' E
juveniles		FRY.b ²	—	10	15.10.96	61°14' N 4°34' E
		FRY.c ⁶	—	10	6.12.96	57°00' N 6°10' E
		FRY.d ⁵	—	7	7.12.96	57°49' N 1°28' E
		FRY.e ⁵	—	10	8.12.96	57°21' N 1°12' E
		FRY.f ²	—	5	5.2.97	57°49' N 4°38' E
		FRY.g	—	7	July 1997	58°00' N 8°50' E
		FRY.h	10	—	11.7.97	59°16' N 4°24' E
		FRY.j	10	—	13.7.97	59°37' N 1°21' E
		FRY.i	10	—	14.7.97	60°15' N 4°28' E

^a Absolute stock size (in tons) was obtained from Anonymous (1999).

^b Numbers attached to the non-spawning samples analysed for D-loop variation refers to the group assigned to in AMOVA grouping samples according to geographical proximity. Several other grouping schemes (geographical and temporal) were also tried; however, no significant geographical structuring was observed.

accession no. AJ132395). In a 272-bp region 106 variable sites were observed, defining 124 haplotypes. Two haplotypes Dcom1 and Dcom2 (separated by a single mutation in position 84) were most abundant, occurring in 14 and 16 specimens, respectively. Very high levels of sequence diversity were observed; including all sequences, mean corrected divergence was 2.9% and maximum divergence was 8.9%. Among spawning individuals mean divergence was 2.6%.

A considerable level of homoplasy was indicated by a high probability of non-parsimonious relationships between sequences ($p=0.10$). An NJ tree based on D-loop haplotypes observed in spawning individuals is presented in figure 1. A consensus tree calculated from the first 2700 trees obtained from parsimony analyses, revealed the same overall topology with the divergence of clade I and II supported by all trees. Mean corrected divergence was 4.7% between clade I and II, 3.7% within clade I and 2.1% within clade II. There was no clear relationship between spawning site and phylogeny (figure 1). However,

no individuals from the Western stock were observed in clade I, and low sequence diversity of the Western and North Sea stocks compared with the others was evident (see figure 1).

(b) *Pattern of molecular variation, phylogeny and haplotype frequencies of the cytb gene*

Among the 197 cytb sequences obtained (see table 1), 27 substitutions were observed, defining 29 haplotypes (figure 2). Mean sequence divergence among the haplotypes was 0.6%. An Ewens–Watterson test (Ewens 1972; Watterson 1978) suggested that the cytb data were in agreement with the expectation of selective neutrality ($p=1.0$). All mutations, but two, were at third position silent sites; the 874 T→C and 700 G→A transitions were in first codon position. The latter results in an amino-acid replacement; V→M in haplotype A12 (amino-acid position 233).

A haplotype cladogram (figure 2) was constructed according to Templeton *et al.* (1992). No haplotypes were

Figure 1. Neighbour-joining tree of the D-loop haplotypes observed among spawning individuals. Nomenclature of haplotypes corresponds to their respective sample (see table 1); AD, Adriatic Sea; S, Southern stock; W, Western stock; NS, North Sea; Can, Canada. Individuals possessing the Dcom1, Dcom2 and Dcom3 haplotypes are given below the tree. An alignment of the D-loop haplotypes is available at our internet site (<http://biologi.uio.no/fellesavdelinger/dna-lab/d-loop.html>). Individuals with *cytb* haplotypes not belonging to the 1–1A clade are indicated (see figures 2 and 3). Individuals with D-loop Dcom1 or Dcom2 haplotypes possessed A, B or C *cytb* haplotypes, and individuals with the D-loop Dcom3 haplotype possessed A and C haplotypes. Individuals with unique D-loop haplotypes, from which *cytb* sequences were not obtained are S.a.10, Can15 and Can10. Dcom1: AD.b.1, S.a.7, W.a.1, W.a.4, W.e.4, NS.a.3, Can1; Dcom2: AD.b.2, AD.b.4, AD.b.8, S.c.2, W.b.1, W.e.4, NS.c.6, NS.c.8, Can22; Dcom3: W.b.8, W.b.12, W.e.2.

separated by more than five mutations. The confidence of the cladogram, calculated as probability of parsimony among haplotypes, was estimated to be 98% (Templeton *et al.* 1992). However, some ambiguities were evident. In site 909, T is observed in both A2 and B7. This can be explained either by independent C–T transitions at site 909 or by a G–A reversal at site 903 in A2. Due to the high frequency of haplotype A (see figure 2) the transition in site 909 was

favoured. D1 shares the substitution 874 T–C with D and D2, and 780 T–C with E. For this haplotype we preferred the first-position codon change in site 874.

The distribution of haplotypes within each stock is presented in figures 2 and 3. The A haplotype was the most frequent in all stocks with a mean of 0.46, ranging from 0.63 in the Adriatic Sea to 0.33 in the Southern stock (figure 3). None of the common haplotypes was

Figure 2. Nested minimum-spanning network (Templeton *et al.* 1987) derived from the 29 *cytb* haplotypes found in mackerel. Each line represents one mutation, except for haplotype D2 (two mutations). The mutated site is indicated at each branch, and the base at each variable site for haplotype A is given as follows: 618 t; 648 c; 660 c; 663 t; 666 g; 700 g; 726 a; 780 t; 783 a; 789 t; 792 c; 870 a; 874 t; 879 t; 885 g; 897 g; 903 g; 906 a; 909 c; 930 t; 939 a; 945 g; 963 a; 966 a; 984 t. Mutations that have occurred more than once are indicated by *. Positions refer to the complete mackerel *cytb* sequence (accession no. x81564; Cantatore *et al.* 1994). The number of individuals displaying the haplotype, and the populations where the haplotypes occur are indicated; AD, Adriatic Sea; S, Southern; W, Western; NS, North Sea; Can, Canada; m, samples from the mixed winter and autumn population in the North Sea.

unique to a particular population. Haplotype C was, however, not present in the Canadian sample and E was absent from the Adriatic sample. A consensus UPGMA tree based on between-stock distances (DA) calculated by bootstrapping haplotypes within stocks, is shown in figure 4. Support was obtained only for the divergence of the Canadian stock (60%). Using Cavalli-Sforza & Edwards's (1967) chord distances gave the same topology. However, here only the clustering of the North Sea and Western stock showed strong bootstrap support (87% support; figure 4).

(c) Phylogeny of composite mtDNA haplotypes

How do the *cytb* haplotypes relate to the D-loop haplotypes? This has been addressed in the D-loop NJ tree in figure 1, where the *cytb* haplotype (when $\neq$ A) of 85 individuals from which both segments were sequenced is superimposed on the tree (indicated by B, C, D or E). D-loop clade I individuals possessed the *cytb* A haplotype, except Can13 which possessed the D2 *cytb* haplotype. D-loop haplotypes associated with *cytb* E haplotypes form a monophyletic group in the D-loop tree, while the *cytb* C and B haplotypes occur in several D-loop clusters. An NJ tree of the two sequences combined showed the same overall topology as the tree in figure 1, i.e. a division into two major clades. However,

the composite haplotypes formed monophyletic groups according to *cytb* one-step clades, except for the composite haplotypes with D-loop Dcom3 haplotypes, with *cytb* clade 1-4D haplotypes (AD.b.6 and Can13), and with *cytb* clade 1-1A haplotypes (i.e. indicating the A haplotype as the root of the *cytb* cladogram in figure 2). The lack of D-loop groups consistent with the *cytb* clades is therefore probably mainly due to high level of homoplasy in the D-loop. However, the inconsistency associated with the D-loop Dcom3 and *cytb* D haplotypes could also result from homoplasies in the *cytb* gene.

(d) The Southern stock shows the highest level of mtDNA diversity among eastern Atlantic stocks

Diversity indices calculated for the different spawning stocks and for non-spawning mackerel in the North Sea during winter and autumn are presented in table 2. For the D-loop overall gene diversity was high (0.99; s.d. = 0.003), and did not vary between stocks. However, variation in nucleotide diversity was observed. The level of diversity within the *cytb* gene was considerably lower (about ten times lower nucleotide diversity) compared with the D-loop, and both gene and nucleotide diversity varied between stocks. Among eastern Atlantic spawning stocks, within-stock diversity measures were highest in the sample from the Southern stock for both sequences

Figure 3. The distribution of *cytb* haplotypes in spawning stocks. The haplotype composition of juveniles (FRY samples) caught in the North Sea in July is also given. The names of tip haplotypes are not shown (except the E haplotype), but are displayed in figure 2. The number of individuals analysed (*N*) from each stock and the frequencies of the most common haplotype (*A*) were AD: *N* = 16, *A* = 0.63; S: *N* = 37, *A* = 0.32; W: *N* = 48, *A* = 0.56; NS: *N* = 39, *A* = 0.44; Can: *N* = 27, *A* = 0.33; FRY: *N* = 30, *A* = 0.30. AD, Adriatic Sea; S, Southern; W, Western; NS, North Sea; Can, Canada.

analysed. For the Canadian sample, high diversity indices were observed for both sequences, while the Adriatic specimens showed low genetic diversity for the *cytb* gene and high diversity for the D-loop. Pairwise *t*-tests revealed significant differences in diversity (log arcsine transformed) between the following stocks: Southern–Western (*p* = 0.01), Southern–North Sea (*p* = 0.01), Western–North Sea (*p* = 0.04), Western–Canadian (*p* = 0.01), and Adriatic–Canadian (*p* = 0.03). The non-spawning individuals caught in the North Sea revealed high levels of diversity. Notably, the nucleotide diversity for North Sea spawning individuals is about half of what is observed for juveniles caught in the same area (table 2).

(e) **Low levels of between-spawning stock differentiation**

AMOVA was performed in order to assess how much of the genetic variation occurred between and within stocks. There was no evidence of within-stock structuring, and the highest Φ estimates were obtained by pooling indi-

viduals from different samples from the same spawning stock. However, low levels of between-stock structuring were observed, as most of the variation is between individuals within stocks (> 97%). Results from AMOVA both with and without a hierarchical structure imposed are presented in table 3. When the Canadian sample was compared with other stocks; 6% of the *cytb*, and 3% of the D-loop variation was due to transatlantic differentiation. For the two mtDNA sequences combined 4% of the variation was explained by this structure. Comparing the Adriatic sample with the eastern Atlantic stocks revealed low differentiation (*cytb*: 1.00% *p* = 0.49, D-loop 1.04% *p* = 0.25; data not shown).

Pairwise Φ_{ST} estimates based on D-loop sequences revealed differentiation of the Western stock compared with all other stocks (table 4). For the *cytb* gene, χ^2 -analyses detected significant differences in overall haplotype composition (χ^2 = 133, *p* < 0.001). Pairwise Φ_{ST} estimates revealed significant differentiation in comparisons involving the Canadian stock, while pairwise χ^2 -analyses

Figure 4. Consensus UPGMA phenogram obtained by bootstrapping *cytb* haplotypes within stocks 100 times. Distance measures used were net nucleotide divergence and chord distances. Bootstrap support for the branches obtained from DA distances is indicated in bold and from *cytb* chord distances in italic. Observed nucleotide divergence (%) between the Canadian and the other stocks was 0.0176, between Adriatic Sea and eastern Atlantic stocks 0.0043, between Southern and Western–North Sea 0.0004, and between Western and North Sea 0.0019.

detected heterogeneity between the Southern and Western stock as well (table 4).

(f) No differentiation among shoals of non-spawning individuals

AMOVA was also performed on the D-loop data set obtained from non-spawning individuals collected in the North Sea during autumn and winter (table 1). Grouping samples according to whether they consisted of juvenile or adult individuals, revealed no differences in genetic composition (table 3), suggesting temporal stability. Moreover, no geographical structure was detected when grouping the samples according to proximity of sampling sites (table 3). Similarly, pairwise Φ_{ST} values obtained from the juveniles analysed for *cytb* sequence variation were negative (data not shown).

(g) Population history inferred from the *cytb* and D-loop

A nested cladistic analysis of geographical distances was used to test the null hypothesis of no geographical association of *cytb* haplotypes, and to separate the influences of recurrent events (such as restricted gene flow due to isolation by distances) and different historical events (such as fragmentation, population range expansion, long-distance colonization). This analysis uses the temporal information in the haplotype tree combined with the geographical range of the haplotype lineages to make inferences about how haplotype lineages have spread geographically through time (Templeton *et al.* 1995; Templeton 1998, 1999).

Since only single observations were made of each of the clade 1–4D haplotypes (figure 2), the entire clade was excluded from the analysis. Two analyses were performed

Table 2. Diversity indices within stocks of Atlantic mackerel obtained from the mtDNA data set

marker/stock	<i>N</i>	gene diversity	nucleotide diversity ^a
D-loop			
spawning stock			
Adriatic Sea	15	0.971	0.026
Southern	17	1.000	0.022
Western	22	0.948	0.016
North Sea	17	0.993	0.016
Canada	18	1.000	0.028
non-spawning			
adults	61	0.992	0.030
juveniles	55	0.980	0.028
total	205	0.988	0.029
<i>cytb</i>			
spawning stock			
Adriatic Sea	16	0.625	0.0019
Southern	37	0.853	0.0037
Western	48	0.666	0.0020
North Sea	39	0.746	0.0026
Canada	27	0.775	0.0031
non-spawning			
juveniles	37	0.869	0.0037
total	197	0.766	0.0028

^a Kimura 2P correction (gamma corrected with $\alpha = 0.40$ for the D-loop) was used when calculating nucleotide diversity.

including (i) all populations and (ii) eastern Atlantic populations (figure 5). From the inferences reached, it is obvious that both population history and population structure have played important roles in determining the geographical distribution of mackerel *cytb* haplotypes. Analysing all stocks, contiguous range expansion (i.e. population range expansion achieved through expanding population fronts) was suggested for the 1–1A and 1–3C clade (figure 5a), while recurrent gene flow restricted by distance was suggested for all clades combined. *Cytb* haplotype distribution within the eastern Atlantic samples suggested contiguous range expansion for clade 1–1A and all one-step clades combined, and isolation by distance for the 1–2C clade (figure 5b).

4. DISCUSSION

(a) Pattern of genetic variation in Atlantic mackerel

The amount and pattern of genetic variation in both mtDNA segments analysed is similar to that of other marine fishes (e.g. Árnason & Pálsson 1996; Rosel & Block 1996). The level of *cytb* between-haplotype diversity, within-stock gene diversity (Western and Canadian) and transatlantic divergence, were in concordance with what was observed from mtDNA RFLP analysis of mackerel samples from England and the USA (Scoles *et al.* 1998). Mean divergence in the D-loop was at least 4.3 (2.6/0.6) times higher than in the *cytb* gene, which is comparable to estimates obtained for other species of the suborder Scombroidei (e.g. Alvarado Bremer *et al.* 1995).

Distinctiveness of the Canadian stock was demonstrated by the *cytb* data, while the D-loop data revealed differentiation within the eastern Atlantic. A plausible

Table 3. *Results from AMOVA*^a

structure tested ^b /marker	Φ_{CT}	ρ	Φ_{SC}	ρ	Φ_{ST}	ρ
spawning stocks						
(Can, AD, S, W, NS)						
cytb	—	—	—	—	0.02	0.02
D-loop	—	—	—	—	0.02	0.01
mtDNA ^c	—	—	—	—	0.02	0.02
(AD, S, W, NS)						
cytb	—	—	—	—	0.00	0.39
D-loop	—	—	—	—	0.02	0.05
mtDNA ^c	—	—	—	—	0.01	0.24
(Can)(AD, S, W, NS)						
cytb	0.06	0.20	0.00	0.33	0.06	0.02
D-loop	0.03	0.21	0.01	0.06	0.04	0.01
mtDNA ^c	0.04	0.20	0.00	0.21	0.04	0.02
non-spawning individuals						
juveniles–adults ^d						
D-loop	−0.001	0.52	−0.002	0.46	−0.003	0.58
sampling sites ^d						
D-loop	−0.02	0.96	0.01	0.62	−0.005	0.56

^a Φ_{ST} is the correlation of random haplotypes within populations relative to that of random pairs of haplotypes drawn from 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 from the complete data set analysed, and 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 Φ estimates is obtained by permutation of the original data. For further statistical methodology see for example Schneider *et al.* (1997).

^b Structure tested refers to either the geographical hierarchy (or no hierarchy) given below, or for the non-spawning stock to juveniles–adults or geographical location (sampling site). AD, Adriatic Sea; S, Southern stock; W, Western stock; NS, North Sea stock; Can, Canadian stock.

^c mtDNA refers to the cytb and D-loop combined.

^d See table 1 for group assignment.

Table 4. *Divergence statistics for mackerel spawning stocks*^a *obtained from mtDNA*

marker		statistic	AD	S	W	NS
S	D-loop	Φ_{ST} (p) ^b	−0.028 (0.90)	—	—	—
	cytb	Φ_{ST} (p)	0.016 (0.21)	—	—	—
		ρ hom. (χ^2) ^c	0.324 (16.8)	—	—	—
W	D-loop	Φ_{ST}	0.053 (0.01)	0.029 (0.07)	—	—
	cytb	Φ_{ST}	0.003 (0.37)	0.009 (0.21)	—	—
		ρ hom. (χ^2)	0.067 (16.1)	0.030 (21.2)	—	—
NS	D-loop	Φ_{ST}	0.023 (0.08)	−0.008 (0.60)	0.038 (0.03)	—
	cytb	Φ_{ST}	0.012 (0.26)	−0.009 (0.67)	−0.008 (0.59)	—
		ρ hom. (χ^2)	0.080 (15.9)	0.231 (18.4)	0.839 (6.6)	—
Can	D-loop	Φ_{ST}	0.020 (0.16)	0.009 (0.29)	0.062 (0.01)	0.026 (0.08)
	cytb	Φ_{ST}	0.052 (0.06)	0.068 (0.01)	0.053 (0.02)	0.048 (0.03)
		ρ hom. (χ^2)	0.006 (19.0)	0.003 (26.1)	0.008 (18.1)	0.014 (17.5)

^a AD, Adriatic Sea; S, Southern stock; W, Western stock; NS, North Sea stock; Can, Canadian stock.

^b The significance of the pairwise Φ_{ST} estimates was obtained by performing 1000 permutations. In calculating the pairwise Φ_{ST} we applied Kimura 2P distances between haplotypes. The D-loop distances were gamma corrected (gamma distribution $\alpha = 0.40$). For the cytb gene no gamma correction was applied.

^c Probabilities of homogeneity (ρ hom.) of cytb was obtained by 1000 randomizations of haplotype frequencies. The pairwise χ^2 -values are given in parentheses.

explanation for this discrepancy is that nucleotide saturation in the D-loop obscures the informativeness in this segment for distantly related sequences. Sequence characters equal in state are then not necessarily identical by descent (e.g. Smouse 1998). The high level of polymorphism in the D-loop segment, however, results in a higher genetic resolution, and thus reveals differences undetectable by the cytb gene (i.e. differentiation of the North Sea and Western stocks). Similar relationships,

where divergence in the D-loop level off at high cytb divergence, have been demonstrated for rainbow fishes (Melanotaeniidae; Zhu *et al.* 1994; see also Grant *et al.* 1998). Zhu *et al.* (1994) found that the most probable cause of the discrepancy is rapid saturation of transitions in the D-loop, presumably as a result of a more biased base composition (i.e. higher A + T content) compared with the cytb. Notably, the differences in A + T content between the sequences analysed are very similar to what

(a)

haplotypes			1-step clades		
no.	<i>D_c</i>	<i>D_n</i>	no.	<i>D_c</i>	<i>D_n</i>
B	1834	1835			
b1	782	1770			
b2	0 nc	1343			
b3	0 nc	1146			
b4	0 nc	1146			
b6	0 nc	1151			
b7	0 nc	3472			
It	1704	163	1-2-B	1804	1685
A	1390	1481			
a1	0 nc	1022			
a2	0 nc	2114			
a3	0 nc	1051			
a4	0 nc	3744L			
a5	0s	1022			
a8	0 nc	127s			
a9	432	1133			
a10	0 nc	1022			
a11	0 nc	64s			
a12	0 nc	2114			
E	2119L	1989L			
It	1158	82			
contiguous range expansion (1-2-11-12-NO)			1-1-A	1560	1548
C	901s	918s	1-3-C	921s	1086 s
c1	0	981	It	197	162
c2	0 nc	948	isolation by distance (1-2-3-4-NO)		
c3	0 nc	844			
It	901	-7			
contiguous range expansion (1-2-11-12-NO)					

(b)

haplotypes			1-step clades		
no.	<i>D_c</i>	<i>D_n</i>	no.	<i>D_c</i>	<i>D_n</i>
B	797	840			
b1	0 nc	1034			
b2	0 nc	548			
b3	0 nc	1034			
b4	0 nc	1034			
b6	0 nc	1035			
It	797	-97	1-2-B	860	853
A	751s	784s			
a1	0 nc	1172L			
a3	0 nc	452			
a5	0s	1172L			
a8	0 nc	624			
a9	432	706			
a10	0 nc	1172L			
a11	0 nc	612			
E	775	791			
It	600	-53			
contiguous range expansion (1-2-11-12-NO)			1-1-A	800s*	818
C	862	883	1-3-C	895L	888
c1	0	1001	It	-77s	-53
c2	0 nc	981	contiguous range expansion (1-2-11-12-NO)		
c3	0 nc	839			
It	862L	-58			
isolation by distance (1-2-3-4-NO)					

observed by Zhu *et al.* (1994); 52% in *cytb* and 68% in the D-loop. Moreover, when plotting observed percentage of transitions and transversions, transitions appear to plateau at 5–7% (not shown), in agreement with what was observed in rainbow fishes.

(b) *Phylogeography and population history of Atlantic mackerel*

The tree topology inferred from the D-loop haplotypes, displaying a split into two major clades (figure 1), is also seen for other pelagic scombroids (e.g. Graves & McDowell 1994; Alvarado Bremer *et al.* 1995). Alvarado Bremer *et al.* (1995) suggested the division into two clades in swordfish (*Xiphias gladius*) to be caused by isolation of the Mediterranean Sea from the Atlantic Ocean due to drop in sea level during one of the Pleistocene glaciations. However, since a division into two clades also is consistent with the expectations in a large population under neutral coalescent theory (see for example, Donnelly & Tavaré 1995), allopatric evolution need not be invoked to explain the topology of the tree.

Considering all stocks, the distribution of the *cytb* haplotypes matches the V pattern (phylogenetic continuity, partial spatial separation) of Avise *et al.* (1987). In agreement with this, hierarchical organization of genetic variation between the spawning populations was suggested from AMOVA and population UPGMA clustering (see table 3 and figure 4). Restricted gene flow between the eastern and western Atlantic is also supported by the fact that no transatlantic migrations have been detected from the numerous fish tagged in the last 20 years (S. A. Iversen and D. W. Skagen, unpublished data).

The geographical analysis of the haplotype tree reinforced these conclusions. When including the Canadian and the Adriatic sample, recurrent gene flow restricted by isolation by distance was suggested for the highest order nesting clade (figure 5a). This indicates that as far back as can be traced with mtDNA there has been gene flow restricted by distance between mackerel populations inhabiting the eastern and western Atlantic and the Mediterranean Sea. Since the recurrent gene flow is relative to the time-scale defined by the coalescence rate of

Figure 5. (Opposite) Nested geographical analysis of the mackerel *cytb* haplotypes according to the nomenclature of Templeton (1998). The nested design is given in figure 2. Interior clades are shaded. Following the name of all clades are the clade *D_c* and *D_n* values given in kilometres. *D_c* (x) is the average distance of individuals displaying clade x haplotypes to the geographical centre of clade x. *D_n* (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 denote that the distance measure was significantly small or large at the 5% level (if not otherwise noted) (**p* = 0.075); nc, not calculated due to only one observation of the haplotype. At the bottom of each box representing a clade with significantly small or large distances, the biological inference is given. The numbers in parentheses refer to the chain of inference when the key given in Templeton *et al.* (1995, pp. 781–782) is applied to the statistical results. (a) Analysis including all samples, (b) analysis excluding the samples from Canada and the Adriatic Sea.

the *cytb* gene, it is likely to have been sporadic events over a time-span of several thousand years (see Templeton 1999). Figure 5a also indicate two significant population range expansions; one across the Atlantic Ocean (clade 1-1A) and one involving populations in the eastern Atlantic and Mediterranean Sea (clade 1-3C). These range expansions do probably not reflect colonization events into previously unoccupied areas, since recurrent gene flow was detected for the 'older' haplotype level (see for example, Hammer *et al.* 1998; Templeton 1999).

The nested cladistic analysis of eastern Atlantic samples suggested an initial population range expansion (detected by both clade 1-1A and all one-step clades combined; figure 5b), followed by recurrent restricted gene flow due to isolation by distance (detected by the 1-3C clade; figure 5b). A southern contraction of eastern Atlantic stocks during the last glaciation with a northern range expansion following the retreat of the glacier is highly plausible, at least into the North Sea area which was land or covered by ice in glacial time (Andersen & Borns 1994). Hence, the population range expansions (figure 5b) suggested within the eastern Atlantic could correspond to the recolonization of uninhabited northern areas after the last glaciation. Difference in relative age of the stocks was also indicated by the observation of no clear relationship between present population size and level of diversity. The highest levels of diversity were associated with the Southern stock, which is estimated to be small in absolute numbers (15% of the Western stock; Anonymous 1999). Hence, this could reflect an older age of this population.

The patterns of differentiation observed with mtDNA are also supported by results from five microsatellites (data available from <http://biologi.uio.no/fellesavdelinger/dna-lab/micro.html>). These loci indicate allelic heterogeneity among spawning stocks, both within the eastern Atlantic and across the Atlantic Ocean, supporting a similar differentiation pattern as observed from the mtDNA.

(c) *Patterns of population differentiation in the eastern Atlantic*

Taken together, the data obtained in this study suggest that the eastern Atlantic spawning stocks, or at least the Western stock, can be considered reproductively isolated, and therefore support the data obtained from studies of parasite infestations and otolith measures (reviewed in Lockwood 1988). 'Overspill' of Western spawners into the North Sea has been suggested from tagging (e.g. Eltink *et al.* 1986). However, the D-loop data suggested that the current level of actual gene flow is insufficient to overcome the effect of high mutation rate and mechanisms (see Avise *et al.* 1987; Palumbi 1994), such as homing, leading to population structuring in mackerel. The spawning stocks, thus, confirm to the short-term management units defined in Moritz (1994). This means that the eastern Atlantic mackerel should be managed as composed of at least three stocks, not as a single stock as done presently (Anonymous 1996).

The stock in the North Sea during winter and autumn revealed no genetic structuring. This finding, together with the high diversity estimates obtained (table 2), support the notion of a mixed stock outside the spawning

season observed from tagging. Moreover, the negative Φ_{ST} s obtained suggest that both juvenile and adult mackerel from all three stocks shoal 'randomly' (relative to spawning area) in the feeding season. Earlier studies using protein polymorphism did not detect any structuring among Atlantic mackerel (reviewed in Jamison & Smith 1987). This is probably a result of low levels of resolution of the markers used, and the fact that sampling in these studies did not concentrate on spawning individuals (i.e. similar to the analysis of non-spawning individuals).

(d) *A genealogical approach reveals heretofore undetected structuring of Atlantic mackerel*

In sum, our data suggest that the low level of differentiation among present mackerel populations is mainly due to shared ancestral polymorphism, and a low differentiation rate (low level of drift, large coalescence time) caused by large effective sizes. Hence, this study clearly demonstrates the usefulness of a genealogical approach in understanding the present population structure of a highly migratory species. Similar patterns with highly skewed *cytb* haplotype frequencies and relatively low levels of differentiation over large geographical distances (e.g. across the northern Atlantic Ocean) are commonly observed in marine species (e.g. Camper *et al.* 1993; Alvarado Bremer *et al.* 1995; Carr *et al.* 1995; Stanley *et al.* 1996; Vis *et al.* 1997). Lack of population differentiation detected from frequency and F_{ST} -based analyses in some of these species, could also here reflect a recent establishment of population (relative to coalescent time of the markers used), and may not be due to panmictic populations (see, for example, Bentzen *et al.* 1996). Moreover, few earlier studies of marine fishes have concentrated on spawning individuals. The observation of 'random' shoaling of mackerel in the feeding season underscores the importance of sampling strategy to obtain meaningful genetic information when studying highly migratory species.

The authors are very grateful for the mackerel samples provided by A. Uriarte (Southern stock), F. Grégoire (Canadian stock), and E. Nesbø and T. E. Nesbø (the Mix.a sample). We thank A. R. Templeton for providing the program for calculating the significance of the minimum spanning network estimation of θ . We are grateful to T. E. Nesbø and K. Østbye for comments on earlier drafts of this work. This work was supported by grant no. 107622/420 from the Norwegian Research Council (NFR) to K.S.J.

REFERENCES

- Alvarado Bremer, J. R., Baker, A. J. & Mejuto, J. 1995 Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) population in the Atlantic Ocean. *Can. J. Fish. Aquat. Sci.* **52**, 1720–1732.
- Andersen, B. G. & Borns Jr, H. W. 1994 *The Ice Age world*. Oslo: Scandinavian University Press.
- Anonymous 1996 Report of the working group on the assessment of mackerel, horse mackerel, sardine and anchovy. *ICES C.M.* 1996/Assess: 7.
- Anonymous 1999 Report of the working group on the assessment of mackerel, horse mackerel, sardine and anchovy. *ICES C.M.* 1999/ACFM: 6.
- Árnason, E. & Pálsson, S. 1996 Mitochondrial cytochrome b DNA sequence variation of Atlantic cod *Gadus morhua*, from Norway. *Mol. Ecol.* **5**, 715–724.

- Awise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. & Saunders, N. C. 1987 Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *A. Rev. Eco. Syst.* **18**, 489–522.
- Bentzen, P., Taggart, C. T., Ruzzante, D. E. & Cook, D. 1996 Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Can. J. Fish. Aquat. Sci.* **53**, 2706–2721.
- Bowen, B. W. & Grant, W. S. 1997 Phylogeography of the sardines (*Sardinops* spp): assessing biogeographic models and population histories in temperate upwelling zones. *Evolution* **51**, 1601–1610.
- Camper, J. D., Barber, R. C., Richardson, L. R. & Gold, J. R. 1993 Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. *Mol. Mar. Biol. Biotechnol.* **2**, 154–161.
- Cantatore, P., Roberti, M., Pescole, G., Ludovico, A., Milella, F., Gadatela, M. N. & Saccone, C. 1994 Evolutionary analysis of cytochrome *b* sequences in some Perciformes: evidence for a slower rate of evolution than in mammals. *J. Mol. Evol.* **39**, 589–597.
- Carr, S. M., Snellen, A. J., Howse, K. A. & Wroblewski, J. S. 1995 Mitochondrial DNA sequence variation and genetic stock structure of Atlantic cod (*Gadus morhua*) from bay and offshore locations on the Newfoundland continental shelf. *Mol. Ecol.* **4**, 79–88.
- Cavalli-Sforza, L. L. & Edwards, A. W. 1967 Phylogenetic analysis. Models and estimation procedures. *Am. J. Hum. Gen.* **19**, 233–257.
- Donnelly, P. & Tavaré, S. 1995 Coalescents and genealogical structure under neutrality. *A. Rev. Genet.* **29**, 401–421.
- Eltink, A., Wamerdam, M. & Heine, A. 1986 Origin, migration and spawning of southern North Sea mackerel with respect to the overspill of the Western mackerel to the North Sea stock. *ICES, C.M.1986/H:49*.
- Ewens, W. J. 1972 The sampling theory of selectively neutral alleles. *Theor. Pop. Biol.* **3**, 87–112.
- Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.
- Felsenstein, J. 1995 *PHYLIP (phylogeny inference package)*, v. 3.572. Native-mode Executables for PowerMac.
- Grant, S. W., Clark, A. M. & Bowen, B. W. 1998 Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cytochrome *b* sequences. *Can. J. Fish. Aquat. Sci.* **55**, 2539–2547.
- Graves, J. E. & McDowell, J. R. 1994 Inter-ocean genetic divergence of istiophorid billfishes. *Mar. Biol.* **122**, 193–203.
- Hammer, M. F., Karafet, T., Rasanayagam, A., Wood, E. T., Altheide, T. K., Jenkins, T., Griffiths, R. C., Templeton, A. R. & Zegura, S. L. 1998 Out of Africa and back again: nested cladistic analysis of human Y chromosome variation. *Mol. Biol. Evol.* **15**, 427–441.
- Hamre, J. 1978 The effect of recent changes in the North Sea mackerel fishery on stock and yield. *Rap. P.-v. Cons. Int. Explor. Mer.* **172**, 197–210.
- Iversen, S. A. 1995 Makrell. In *Fiskeribiologi* (ed. N. Torsvik, S. Mortense & K. H. Nedraas), pp 95–101. Oslo: Landbruksforlaget. (In Norwegian)
- Iversen, S. A. & Skagen, D. W. 1989 Migration of Western mackerel to the North Sea 1973–1988. *ICES, C.M. 1989/H: 20*.
- Jamison, A. & Smith, P. J. 1987 Atlantic mackerel (*Scomber scombrus* L.) stocks and their genes: a review. *Rep. Proc. Verb.* **44**, 66–72.
- Lockwood, S. J. 1988 *The mackerel, its biology, assessment and the management of a fishery*. Farnham, UK: Fishing News Books.
- Magoulas, A., Tsimenides, N. & Zouros, E. 1996 Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: the case of the European anchovy (*Engraulis encrasicolus*). *Mol. Biol. Evol.* **13**, 178–190.
- Merilä, J., Björklund, M. & Baker, A. J. 1997 Historical demography and present day population structure of the greenfinch, *Carduelis chloris*—an analysis of mtDNA control region sequences. *Evolution* **51**, 946–956.
- Moritz, C. 1994 Applications of mitochondrial DNA analysis in conservation: a critical review. *Mol. Ecol.* **3**, 401–411.
- Nei, M. 1987 *Molecular evolutionary genetics*. Columbia University Press.
- Nesbø, C. L., Arab, M. O. & Jakobsen, K. S. 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, C. L., Magnhagen, C. & Jakobsen, K. S. 1998b Genetic differentiation among stationary and anadromous perch (*Perca fluviatilis*) in the Baltic sea. *Hereditas* **129**, 241–249.
- Palumbi, S. R. 1994 Genetic divergence, reproductive isolation, and marine speciation. *A. Rev. Eco. Syst.* **25**, 547–572.
- Roff, D. A. & Bentzen, P. 1989 The statistical analysis of mitochondrial DNA polymorphism: χ^2 and the problem of small samples. *Mol. Biol. Evol.* **6**, 539–545.
- Rosel, P. E. & Block, B. A. 1996 Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. *Mar. Biol.* **125**, 11–22.
- Rudi, K., Kroken, M., Dahlberg, O. J., Deggerdal, A., Jakobsen, K. S. & Larsen, F. 1997 A rapid, universal method to isolate PCR-ready DNA using magnetic beads. *BioTechniques* **22**, 506–511.
- Ruzzante, D. E., Taggart, C. T. & Cook, D. 1998 A nuclear DNA basis for shelf- and bank-scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. *Mol. Ecol.* **7**, 1663–1680.
- Schneider, S., Kueffer, J.-M., Roessli, D. & Excoffier, L. 1997 Arlequin v. 1.1: a software for population genetic data analysis. Geneva: Genetics and Biometry Laboratory, University of Geneva, Switzerland. Available from <http://anthropologie.unige.ch/arlequin>.
- Scoles, D. R., Collette, B. B. & Graves, J. E. 1998 Global phylogeography of mackerels of the genus *Scomber*. *Fish. Bull.* **96**, 823–842.
- Smouse, P. E. 1998 To tree or not to tree. *Mol. Ecol.* **7**, 399–412.
- Stanley, H. F., Casey, S., Carnahan, J. M., Goodman, S., Harwood, J. & Wayne, R. K. 1996 Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). *Mol. Biol. Evol.* **13**, 368–382.
- Swofford, D. L. 1998 *PAUP* (phylogenetic analysis using parsimony): preliminary test version*. Sunderland, MA: Sinauer.
- Tajima, F. 1983 Evolutionary relationship of DNA sequences in finite populations. *Genetics* **105**, 437–460.
- Templeton, A. R. 1998 Nested clade analysis of phylogeographical data: testing hypotheses about gene flow and population history. *Mol. Ecol.* **7**, 381–397.
- Templeton, A. R. 1999 Human races: a genetic and evolutionary perspective. *Am. Anthr.* **100**, 632–650.
- Templeton, A. R., Boerwinkle, E. & Sing, C. F. 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, A. R., Crandall, K. A. & Sing, C. F. 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, A. R., Routman, E. & Phillips, C. A. 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.
- Uriarte, A. 1995 Preliminary results of a tagging survey of the mackerel in the Bay of Biscay in 1994. *ICES, C.M.* 1995/H:24.
- Vis, M. L., Carr, S. M., Bowering, W. R. & Davidson, W. S. 1997 Greenland halibut (*Reinhardtius hippoglossides*) in the North Atlantic are genetically homogeneous. *Can. J. Fish. Aquat. Sci.* **54**, 1813–1821.
- Ward, R. D., Woodwark, M. & Skibinski, D. O. F. 1994 A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *J. Fish Biol.* **44**, 213–232.
- Watterson, G. 1978 The homozygosity test of neutrality. *Genetics* **88**, 405–417.
- Wenink, P. W., Baker, A. J. & Tilanus, G. J. 1993 Hypervariable control-region sequences reveal global population structuring in a long-distance migrant shorebird, the dunlin (*Calidris alpina*). *Proc. Natl Acad. Sci. USA* **90**, 94–98.
- Zaykin, D. & Pudovkin, A. 1993 Two programs to estimate significance of χ^2 -values using pseudo-probability tests. *J. Hered.* **84**, 152.
- Zhu, D., Jamison, B. G. M., Hugall, A. & Moritz, C. 1994 Sequence evolution and phylogenetic signal in control region and cytochrome b sequences of rainbow fish (Melanotaeniidae). *Mol. Biol. Evol.* **11**, 672–683.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.