Genetic differentiation among Atlantic cod in south and south-east Icelandic waters: synaptophysin (Syp I) and haemoglobin (HbI) variation

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(Received 9 June 1998, Accepted 23 February 1999)

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Significant differences were found at the synaptophysin (Syp I) locus between two groups of Icelandic cod, Gadus morhua; Loftsstaðhraun (spawning ground), Reykjanesgrunn and Eyrabakkaður (feeding grounds) on one hand and Kantur (spawning ground) and Austfjarðadjúp (feeding ground) on the other hand. There was also a considerable genetic heterogeneity within the former group. The results indicate that the cod in south and south-east Icelandic waters do not belong to one panmictic population.

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Key words: Atlantic cod; Gadus morhua; synaptophysin; nuclear RFLP; haemoglobin; population genetics; Iceland.

INTRODUCTION

The Atlantic cod Gadus morhua L. is the major demersal fish resource distributed on the continental shelves and banks on both sides of the North Atlantic ocean (Dahle, 1995; Galvin et al., 1995; Ruzzante et al., 1996). The need for effective management of this species has been demonstrated by the stock reduction to a low level in the western Atlantic (Walters & Maguire, 1996) and in the North Sea (Daan et al., 1994), where over-exploitation has been suggested as one of the main causes. Ever-increasing fishing efforts and over-exploitation has also been pointed out as one reason for the fluctuations in the cod stocks in Icelandic waters (Schopka, 1994), in the waters of the Faroe Islands (Jákupsstovu & Reinert, 1994) and in the Barents Sea (Nakken, 1994).

A variety of stock identification techniques has been used to study the possible subdivision of cod populations in the North Atlantic. Protein variation has indicated heterogeneity of cod stocks in the investigated areas (Cross & Payne, 1978; Mork et al., 1985; Gjøsæter et al., 1992), but not led to a clear-cut conclusion about the population
structure of cod. High variation was found in the haemoglobin (HbI) locus which shows slight spatial variation in allele frequency along the northeast coast of North America (Jamieson, 1975), an apparent cline in frequency along the Norwegian coast (Gjøsæter et al., 1992; Dahle & Jørstad, 1993), and considerable variation in Icelandic waters (Jamieson & Jónsson, 1971). Mitochondrial and nuclear DNA methods have given contradictory results, most mtDNA studies showing limited or no differentiation of populations (e.g. Carr & Marshall, 1991; Árnason & Rand, 1992; Árnason et al., 1992; Pepin & Carr, 1993; Árnason & Pálsson, 1996) whereas one study (Dahle, 1991) revealed large divergence between samples from coastal and Arctic cod in Norway. However, studies based on minisatellite (Galvin et al., 1995), and microsatellite DNA variation (Dahle, 1995; Ruzzante et al., 1996), and restriction fragment length polymorphism (RFLP) of nuclear DNA (Pogson et al., 1995; Fevolden & Pogson, 1997) have displayed considerable population substructuring of cod in the North Atlantic. There is therefore an ongoing debate on the stock structure of Atlantic cod and the enigma of stock identification in this species is far from resolved.

In a study of cod populations in Icelandic waters using transferrin and haemoglobin, Jamieson & Jónsson (1971) described considerable spatial (both loci) and temporal (haemoglobin) variation. Disequilibrium (Hardy-Weinberg tests) were found at some of the sampling sites, indicating that different units of cod are present in Icelandic waters. In contrast, Árnason et al. (1992), using restriction fragment analysis of mitochondrial DNA, found no evidence of population differentiation in Icelandic cod, suggesting that large gene flow hampered establishment of local stocks.

From 38 years of tagging and recapture studies of cod in Iceland, Jónsson (1996) showed that the main migratory routes were related to spawning off the southwest coast. After spawning, the fish migrated northwards along the west coast to the rich feeding grounds off the northwest and north coast. Also prior to spawning, the fish migrated from the north along the west coast to the spawning area off the southwest coast, then continuing to the east along the south coast. Endemic populations were found in some of the fjords of the west and north coast. The lack of recaptured cod tagged at the northeast coast at the spawning grounds in the southwest might be due to a local spawning at the northeast coast. The discrepancy between the studies of Jamieson & Jónsson (1971) and Jónsson (1996), and those of Árnason et al. (1992), shows that knowledge of the structuring of Atlantic cod in Icelandic waters is limited.

The present paper aimed to examine the genetic structure of Atlantic cod from south Icelandic waters using an analysis of nuclear DNA (RFLP) at the synaptophysin (Syp I) locus together with analysis of the haemoglobin locus (HbI), genetic markers which have been used successfully to study the genetic structure of Atlantic cod (Jamieson & Jónsson, 1971; Dahle & Jørstad, 1993; Fevolden & Pogson, 1997). The haemoglobin frequencies were compared to older results (Jamieson & Jónsson, 1971) to test for the temporal stability at this locus.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 344 cod were collected from 6 locations in Icelandic waters (Fig. 1, Table I). Two locations c. 50 nautical miles apart were spawning sites (Jónsson, 1996) namely Loftstaðahraun (L, Fig 1), midpoint 63°43’N, 20°58’W, a submarine rocky mount, depth range 50 - 70 m., and Kantur (K, Fig. 1) midpoint 63°17’N, 19°10’W, a very steep continental slope, with depth ranging from 150 to 450 m. At Kantur, samples taken c. 13
nautical miles apart (called Kantur 1 and Kantur 2) showed no intra-area differences (Fishers exact test, $P > 0.65$), and so were pooled for inter-area comparisons (hereafter called Kantur). The spawning aggregations at Lofstaðahráun and Kantur break into smaller shoals and schools migrating to the feeding grounds, which can be a considerable distance from the spawning grounds. A substantial mixing of shoals and schools takes place on the feeding grounds. Results from tagging experiments (V. Thorsteinsson, Marine Research Institute, Reykjavík, unpublished) were used to find probable feeding grounds for cod migrating from L and K. Cod tagged in the Lofstaðahráun area were recaptured most frequently south-west of Iceland (Reykjanesgrunn (R), and Eyrabakkabugur (E), Fig. 1) during September to January, whereas cod tagged near Kantur were recaptured most frequently at the east coast of Iceland (Austfjarðadjúp (A), Fig. 1) during the same period.

The three SW sample locations (L, R, and E) were called group 1, and the two S and E sample locations (K and A) were called group 2. The data were used to test the null hypothesis that cod in the sampled areas were not distinct. Samples were collected during surveys by the Marine Research Institute, Reykjavík, Iceland, in 1997 (March, April, October) and 1998 (January), using a bottom-trawl (L, A), gill nets (K) and Danish seine (E, R).

**GENETIC ANALYSIS**

Gill arches were dissected from adult fish and stored in 96% ethanol. Approximately 200 mg of gill tissue were used to extract DNA by a modification of the salt-extraction procedure of Miller et al. (1988), and by phenol-extraction (Taggart et al., 1992). The salt-extraction protocol was the same as described in Pogson et al. (1995). The nucleotide sequence data of Fevolden & Pogson (1997) were used to construct primers. These primers amplified a region of the synaptophysin (Syp I) gene by the polymerase chain reaction (PCR) that contained the polymorphic DraI site. The forward primer (Fevolden & Pogson, 1997, 5´-TTGGTCCTCTATCTGGGCTTCG- 3´) was situated in the third exon of the gene. The reverse primer (5´-CGTAGCAGAAGAGTGACACAT- 3´) was located in the 3´flanking region of the gene, beginning 52 bp beyond the termination codon (Fevolden & Pogson, 1997). Amplifications were performed in 17 µl reaction volumes containing 2.5 mM MgCl2, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.1 µM each of the forward and reverse primers, 100 ng of genomic DNA and 1 unit of DyNAzyme™ (Finnzymes Ltd., Espoo, Finland) DNA polymerase. PCR reactions were performed in a Perkin Elmer GeneAmp PCR system 9700, programmed for 25 cycles of denaturation at 94°C (1 min), primer annealing at 55°C (1 min), and primer extension at 72°C (1.67 min). The PCR products were digested with DraI for 50 min at 37°C. Samples were run at 80 mA (100 Volts) for 2 h. Digestion was stopped by adding 1 µl of 0.2 mM EDTA. The digested PCR products were visualized in 2% agarose gels stained with ethidium bromide.

Fresh blood samples were used for the analysis of haemoglobin, either on board the research vessel or at the laboratory. Horizontal agar electrophoresis was used, as described by Sick (1961) with modifications (Jørstad, 1984). Smithies buffer, (45 mM Tris, 25 mM boric acid, 1 mM EDTA, pH 8.6 , Smithies, 1959) was used as an electrode buffer, and diluted 1:1 with distilled water for the gel buffer. A 2% agar concentration (Agar Noble) was used in gels. Electrophoresis was carried out at 25 mA (200 Volts) for 90 min at 3-5°C. The gels were stained with Brilliant Blue G Quick stain in perchloric acid (McFarland, 1977), destained by diffusion and photographed by transmitted light.

**STATISTICAL METHODS**

Allele and genotype frequencies together with pairwise $F_{ST}$ values (Wright, 1978; Reynolds et al., 1983) were calculated using the ARLEQUIN 1.1 computer package
Significance of $F_{ST}$ values was determined using a non-parametric permutation of individuals between the different sampling sites (Schneider et al., 1997). An exact test of population differentiation (Raymond & Rousset, 1995) was performed to compare allele frequencies among the sampling sites. This test is analogous to Fisher’s exact test on a 2x2 contingency table (Schneider et al., 1997) but extended here to a 5 (sampling sites) x 6 (genotypes) contingency table. The genetic structure of the sampling sites was analyzed using an analysis of molecular variance (AMOVA) framework (Weir & Cockerham, 1984). In this test, based on prior empirical data, the sampling sites were divided into two groups: group 1 (L, E and R), and group 2 (K and A). The significance of the variance components associated with the different possible levels of genetic structure (among groups, among populations within groups, among individuals within populations, within individuals) was tested using non-parametric permutation procedures (Excoffier et al., 1992). Population pairwise $F_{ST}$ statistics were calculated for all populations and the significance tested by permuting the individuals between the populations (Reynolds et al., 1983; Schneider et al., 1997). Genotype frequencies were tested for conformity with expected Hardy-Weinberg proportions, and separate tests performed for both loci. Global departure from Hardy-Weinberg equilibrium was tested in the AMOVA test by taking into account the differences between genotypes found within individuals (Schneider et al., 1997). The average heterozygosity was calculated using direct counts, and the estimates based on Hardy-Weinberg expectations. Correlation between the different loci was tested in CSS-STATISTICA (StatSoft, 1994). Sex-specific frequencies of $Syp$ I and $Hbl$ were tested in contingency $\chi^2$ test (Zar, 1996).

Nei’s (1972) genetic distances based on the allele frequencies, were calculated by the GENDIST program in the PHYLIP package (Felsenstein, 1993), and a neighbor-joining dendrogram of the genetic distance matrix was constructed in the NEIGHBOR program in PHYLIP. A Bonferroni correction (Johnson & Field, 1993) of the significance level ($\alpha = 0.05$) was applied when testing for significant differences in allele frequencies and for significant departures from Hardy-Weinberg expectations.

**RESULTS**

THE $SYP$ I POLYMORPHISM

The $Syp$ I genotypes scored using the PCR-based assay appeared as double-banded or triple banded patterns, when visualized on the agarose gel. As previously described by Fevolden & Pogson (1997) individuals homozygous for the absence of the polymorphic $Dra$I site ($Syp$ IA) appeared as 773-bp and 278-bp fragments. Individuals homozygous for the presence of the polymorphic $Dra$I site ($Syp$ IB) appeared as 495-bp and 278-bp fragments, whereas the $Syp$ IAIB heterozygotes appeared as a triple banded pattern (one 773-bp, one 495-bp, and three 278-bp fragments). The allele frequencies for the $Syp$ I alleles differed both between the sampling sites and the sampling groups (group 1, group 2) (Table II). The frequency of the $Syp$ IA allele was highest in the Loftstaðahraun sample (0.874) and lowest in the Kantur sample (mean frequency = 0.299). In general the frequency of the $Syp$ IA allele was higher in the group 1 samples (mean frequency = 0.76) compared to the group 2 samples (mean frequency = 0.38). The frequency of the $Syp$ IB allele was highest in the Loftstaðahraun sample (0.874) and lowest in the Kantur sample (mean frequency = 0.299). In general the frequency of the $Syp$ IB allele was higher in the group 1 samples (mean frequency = 0.76) compared to the group 2 samples (mean frequency = 0.38). When the compiled allele frequencies in the two groups were tested, group 1 and group 2 were highly significantly different at the $Syp$ I locus ($F_{ST} = 0.25$, $P < 0.001$). Although observed frequencies of heterozygotes were in most cases higher than expected Hardy-Weinberg values (Table II), all samples were in Hardy-Weinberg equilibrium ($P > 0.05$).
HAEMOGLOBIN POLYMORPHISM

Three different haemoglobin electrophoretic patterns were described in detail by Sick (1965), Frydenberg et al. (1965), and Dahle & Jørstad (1993). The patterns were interpreted as the two homozygotes and the heterozygote in a two allele (HblI, HblII) system. The frequency of the most common haemoglobin allele, HblI was low, ranging from 0.016 to 0.032 (Table II), with an average of 0.026. The highest value of HblI was at E whereas the lowest was at L (Table II). For all sites the observed and expected heterozygote frequencies at the Hbl locus were similar, and were in Hardy-Weinberg equilibrium (P > 0.75). When the compiled allele frequencies of Hbl in the two groups were tested, group 1 and group 2 did not differ (FST = -0.002, P > 0.55). A very low and non-significant correlation (r = 0.02, P > 0.65) was found between the Syp I and Hbl I loci. No differences were found in frequencies of the Syp I and Hbl loci between sexes (P>0.25).

GENETIC DIFFERENTIATION AMONG POPULATIONS

The total material (all samples compiled) was in Hardy-Weinberg disequilibrium (P < 0.05, Table III) when the individual genetic variation in the analysis of molecular variance (AMOVA, Table III) was included, indicating that the samples were drawn from more than one panmictic population. A significant difference (P < 0.05) was observed between group 1 and group 2 when pooling the material into two groups by the AMOVA test. Also the hierarchical F-statistics (Table III) indicated a population differentiation and 20% of the total allelic variance was due to differences between the two groups. A significant difference between the populations in each group (P < 0.01) was detected, whereas only 6.8% of the allelic variance within groups was observed among the 5 sampling sites (Table III).

The highest level of differentiation was found between the L and the K samples (FST = 0.48, Table IV) both taken during the spawning season, and the lowest (non-significant) between the E and R samples, and the E and A samples (Table IV). Overall, the FST values indicated a strong differentiation among the sampling sites.

Pairwise population differentiation test (analogue to Fishers exact test) revealed highly significant differences (P < 0.005) between all pairs of sampling sites except E vs. R, E vs. A, and K vs. A. Overall (all samples compiled) the pairwise population differentiation test revealed a highly significant dissimilarity (P < 0.0001) of samples.

The neighbor-joining dendrogram constructed using Nei’s (1972) genetic distance (Fig. 2) illustrated the differentiation between the two groups clearly. Further, there was differentiation within group 1 as the L sample differed from the other two (E, R).

DISCUSSION

The present study indicated a genetic differentiation between cod populations in Icelandic waters. Samples from groups 1 and 2 populations differed significantly (Tables III-IV). All the statistical tests employed showed significant genetic differences among the samples. All group 1 populations exhibited high frequencies of the Syp T allele (mean frequency = 0.76), whereas group 2 populations were much lower (mean frequency = 0.38). The distinction in the Syp I allele frequencies between groups 1 and 2 was in line with the findings of Fevolden & Pogson (1997) in Norwegian waters, where coastal cod populations possessed significantly higher frequencies of the Syp I allele (mean frequency = 0.806) than did offshore populations (mean frequency = 0.099). Ruzzante et al. (1996) found a similar distinction between inshore and offshore cod populations off Newfoundland. The findings of Ruzzante et al. (op. cit.) and Fevolden and Pogson (1997) are consistent with morphometric
differences between the cod populations off Newfoundland (Pepin & Carr, 1993) and differences in otolith structure between coastal and Arctic cod in Norwegian waters (Dahle & Jørstad, 1993). Further, significant genetic diversity between coastal and offshore cod populations off Norway (Dahle, 1995), and between northern and southern cod aggregations off Newfoundland (Bentzen et al., 1996) were reported, using DNA microsatellites.

Similar to the microsatellite loci analyzed by Dahle (1995), Bentzen et al. (1996), and Ruzzante et al. (1996), the Syp I polymorphism reveals significant heterogeneity among cod populations at very localized scales (Tables IV, Fig. 2). But unlike the microsatellites and in line with the findings of Fevolden & Pogson (1997), the magnitude of population differentiation revealed by Syp I at small geographic scale (Table IV, Fig. 2) is large. Pogson et al. (1995) and Fevolden & Pogson (1997) discussed the possibility that selection may have been responsible for producing the large differences in Syp I allele frequencies among cod populations. Fevolden & Pogson (1995) documented significant differences in growth rates among Syp I genotypes, lending further support for the selection hypothesis. Fevolden & Pogson (1997) concluded that the Syp I locus is stable enough to be used to identify cod populations based on strong linkage disequilibrium present in the Syp I gene region and the fact that the selective agents responsible for producing allele frequency variation among populations are largely unknown.

Differences in physiological performance have been reported between haemoglobin genotypes in cod (Karpov & Novikov, 1980; Mork et al., 1984a,b; Mork & Sundnes, 1985; Nævdal et al., 1992; Brix et al., 1997), and recently in turbot Scophthalmus maximus, Refinesque (Imsland et al., 1997). Such differences may give rise to high selection pressure on these genes, and reduce their value as population markers. However, no clear indication of directional selection on haemoglobin genotype distribution has been seen when comparing early analyses of cod haemoglobin in Norwegian waters (Frydenberg et al., 1965; Møller, 1966, 1968) with corresponding analyses in recent years (Jørstad & Nævdal, 1989; Gjøsæter et al., 1992; Dahle & Jørstad, 1993; Fyhn et al., 1994). Further, the frequencies of haemoglobin genotypes at the present sites (Table II) are close to those found by Sick (1965) and Jamieson & Jónsson (1971) in similar areas. This does not disprove selection, but it does show that the stability of the gene frequencies is sufficient for them to be used as population markers in cod. Wells (1990) pointed out that one cannot assume automatically that variation in haemoglobin is the result of natural selection. Selection acting on one trait may cause changes in other traits if the other traits are linked genetically to the first.

The Syp I polymorphism (Fevolden & Pogson, 1997) displayed similar distinction between Arctic and coastal cod as did haemoglobin polymorphism (Møller, 1968; Dahle & Jørstad, 1993). In the present study, low individual correlation was found between the Syp I and the haemoglobin loci (r = 0.02), and thus the haemoglobin and the Syp I loci seem to vary independently. As pointed out by Fevolden & Pogson (1997) if selection is occurring at the Hbl locus it should be difficult to find additional loci that distinguish cod populations strongly, both due to the strength of the selection, and because selective gradients may exist at other loci which are not expected to mimic Hbl closely. The fact that two independent loci (Syp I and Hbl) both give similar distinction between cod populations in Norway may challenge the view that the Hbl is not suitable as a population marker.

From these results the hypothesis of genetic homogeneity among cod populations in Icelandic waters is rejected, and these results contrast sharply with those obtained by Árnason et al. (1992), using an RFLP analysis of mitochondrial DNA. Two out of six of their sampling localities were in similar geographic areas to the present study (i.e. Loftstaðahraun, Reykjanesgrunn). The fact that subpopulation structure can be detected with RFLP and haemoglobin variation in Icelandic, Newfoundland (Ruzzante et al., 1996), and Norwegian waters (haemoglobin, Møller, 1968; Dahle & Jørstad, 1993; nuclear DNA
RFLPs, Pogson et al., 1995; Fevolden & Pogson, 1997), but not with analysis of variation of restriction analysis of mitochondrial DNA (Árnason et al., 1992), or mitochondrial cytochrome b DNA sequence variation (Pepin & Carr, 1993; Carr et al., 1995; Árnason & Pálsson, 1996), might imply that the latter technique might not be sufficiently sensitive to detect differences in stock structure, at least at local scales. Ruzzante et al. (1996) pointed out that there may be insufficient variation in the cytochrome b region of mitochondrial DNA to distinguish among local populations. This is supported by the findings of Meyer et al. (1990) on cichlid species in Lake Victoria and Lydeard et al. (1995) on live-bearing poeciliids.

Mitochondrial DNA is inherited maternally and therefore it is important to sample individuals from many locations to avoid overrepresentation of the same mtDNA clone (Dahle, 1991). In the case of the Árnason et al. (1992) study, the majority of samples was collected from only six catches from coastal waters and mainly shallower than 90 m. Thus they were not distributed on a spatial and temporal scale as recommended (Dahle, 1991), and it is possible that this sampling procedure might not be adequate to describe the population structure of cod off Iceland. It has been claimed by some workers (Carvalho & Hauser, 1994; Ward & Grewe, 1994; Ruzzante et al., 1996; Turan et al., 1998) that, if they exist, genetic differences are more likely to be detected by nuclear than by mitochondrial DNA studies. This is supported by the fact that in many cases mtDNA analysis has not led to enhanced resolution of stock issues compared with other molecular methods (Ward & Grewe, 1994 and references therein; Turan et al., 1998). MtDNA is usually treated as a single locus, with the composite genotypes equivalent to alleles, whereas allozyme and nuclear DNA analysis permits the examination of many independent loci (Ward & Grewe, 1994). Further, in many marine fish species common genotypes frequently are distributed widely (Smith et al., 1990; Carvalho & Hauser, 1994), and therefore analysis of frequency distributions demands larger sample sizes than have been employed traditionally in many mtDNA studies (e.g. Dahle, 1991; Árnason et al., 1992; Árnason & Pálsson, 1996).

In conclusion, the present study indicates population differentiation between cod populations off Iceland. However, the evidence for population difference in cod presented here is still preliminary and based on only one locus. More samples, especially from the fishing grounds off northwest Iceland, need to be analyzed to confirm our findings. Also, it remains to be shown whether the population structure detected here remains stable over time.

We thank G. Marteinsdóttir for her valuable contribution; G. Pogson and S. E. Fevolden for help and advice during the analytical work; P. Galvin, J. Coughlan and J. Solvang for their contribution to the analytical work; S. L. Jónsdóttir, H. B. Ingólfsdóttir, E. Ásgeirsson and H. Guðfinnsson for assistance in sampling fish and the crew on board Árni Friðriksson RE-100, Bjarni Sæmundsson RE-30, Friðrik Sigurðsson AR-17 and Valdimar Sveinsson VE-22 for their contribution; and the anonymous referees and Ó. Y. Atladóttir for valuable comments on the manuscript. Financial support was given by the EU FAIR program (FAIR CT95-0282). The work described in this paper will be submitted in partial fulfillment of the requirement for Dr. Scient. Degree, University of Bergen, Norway.

References


### TABLE I. Locations, number of specimens (n), location, date of collection, and sex-ratio of Atlantic cod in Icelandic waters used in this study. Sex could not be determined in all specimens at Austfjarðadjúp.

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>Position</th>
<th>Date</th>
<th>Depth (m)</th>
<th>Sex (male:female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loftstaðahraun (L)</td>
<td>95</td>
<td>63°47 N</td>
<td>20°51 W</td>
<td>3-4 April 1997</td>
<td>82 64:31</td>
</tr>
<tr>
<td>Eyrabakkabugur (E)</td>
<td>31</td>
<td>63°47 N</td>
<td>21°40 W</td>
<td>30 January 1998</td>
<td>54 9:22</td>
</tr>
<tr>
<td>Reykjanesgrunn (R)</td>
<td>49</td>
<td>63°47 N</td>
<td>22°50 W</td>
<td>31 January 1998</td>
<td>110 16:33</td>
</tr>
<tr>
<td>Kantur 1 (Dyrhólaey) (K 1)</td>
<td>43</td>
<td>63°15 N</td>
<td>18°54 W</td>
<td>25-26 March 1997</td>
<td>253 25:18</td>
</tr>
<tr>
<td>Kantur 2 (Dyrhólaey) (K 2)</td>
<td>44</td>
<td>63°19 N</td>
<td>19°24 W</td>
<td>25-26 March 1997</td>
<td>262 22:22</td>
</tr>
<tr>
<td>Austfjarðadjúp (A)</td>
<td>82</td>
<td>64°33 N</td>
<td>12°20 W</td>
<td>26-28 October 1997</td>
<td>355 27:32</td>
</tr>
</tbody>
</table>

### Table II. Frequencies of $Syp^{IA}$, $Syp^{IB}$, $HbI^1$ and $HbI^2$ alleles and heterozygote frequencies of cod samples from five locations in Icelandic waters.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Loftstaðahraun (spaw. area)</th>
<th>Eyrabakkabugur (feeding area)</th>
<th>Reykjanesgrunn (feeding area)</th>
<th>Kantur (spaw. area)</th>
<th>Austfjarðadjúp (feeding area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Syp$ I</td>
<td>$Syp^{IA}$</td>
<td>0.874</td>
<td>0.549</td>
<td>0.673</td>
<td>0.299</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>$Syp^{IB}$</td>
<td>0.126</td>
<td>0.451</td>
<td>0.327</td>
<td>0.701</td>
<td>0.530</td>
</tr>
<tr>
<td></td>
<td>$H$</td>
<td>0.189 (0.220)</td>
<td>0.645 (0.495)</td>
<td>0.571 (0.440)</td>
<td>0.437 (0.419)</td>
<td>0.573 (0.498)</td>
</tr>
<tr>
<td>$HbI$</td>
<td>$HbI^1$</td>
<td>0.016</td>
<td>0.032</td>
<td>0.031</td>
<td>0.028</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>$HbI^2$</td>
<td>0.984</td>
<td>0.968</td>
<td>0.969</td>
<td>0.972</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>$H$</td>
<td>0.032 (0.031)</td>
<td>0.064 (0.062)</td>
<td>0.061 (0.060)</td>
<td>0.057 (0.054)</td>
<td>0.060 (0.058)</td>
</tr>
</tbody>
</table>

n = number of specimens analyzed at each site, $H$ = frequencies of heterozygotes, observed (expected), Spaw. area = spawning area.
TABLE III. Analysis of molecular variance (AMOVA) and hierarchical $F$-statistics (fixation indices) for the cod groups analyzed in the present study. The genetic structure is analyzed at the individual level so that the within individual variance is a test for global departure from Hardy-Weinberg equilibrium. Groups = group 1, group 2. Sampling sites = L, E, R, K, A.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance component</th>
<th>Variance explained by AMOVA model</th>
<th>$F_{XY}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups</td>
<td>1</td>
<td>24.62</td>
<td>0.0616**</td>
<td>20.2</td>
<td>0.201</td>
</tr>
<tr>
<td>Among sampling sites within groups</td>
<td>3</td>
<td>8.45</td>
<td>0.0208**</td>
<td>6.8</td>
<td>0.085</td>
</tr>
<tr>
<td>Among individuals within sampling sites.</td>
<td>339</td>
<td>68.18</td>
<td>-0.0233‡</td>
<td>-7.3‡</td>
<td>-0.099‡</td>
</tr>
<tr>
<td>Within individuals</td>
<td>334</td>
<td>84.50</td>
<td>0.2456*</td>
<td>80.3</td>
<td>0.196</td>
</tr>
<tr>
<td>Total</td>
<td>687</td>
<td>185.79</td>
<td>0.3057</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

$P < 0.05$, **$P < 0.01$, d.f. = degrees of freedom. For calculation of d.f. see Schneider et al. (1997).

‡ Note that the $F$-statistic estimators in the AMOVA model are random variables and can take either positive or negative values (Long, 1986), negative values indicating excess of heterozygotes (Long, 1986; Excoffier et al., 1992). Such negative estimates should be interpreted as zero (Long, 1986) in the AMOVA model i.e. the variance explained by among individuals within sampling sites is zero in the present study.

TABLE IV. Population pairwise $F_{ST}$ estimates among all samples. Permuting the individuals between the sampling sites tested the significance of the pairwise $F_{ST}$ values. 100 permutations were performed for all pair of sampling sites.

<table>
<thead>
<tr>
<th>Kantur</th>
<th>Loftstaðahraun</th>
<th>Reykjanesgrunn</th>
<th>Eyrabakkabugur</th>
<th>Austfjarðadjúp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kantur</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Loftstaðahraun</td>
<td>0.477*</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Reykjanesgrunn</td>
<td>0.219*</td>
<td>0.100*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Eyrabakkabugur</td>
<td>0.101*</td>
<td>0.237*</td>
<td>0.016</td>
<td>—</td>
</tr>
<tr>
<td>Austfjarðadjúp</td>
<td>0.047*</td>
<td>0.289*</td>
<td>0.064*</td>
<td>-0.0001</td>
</tr>
</tbody>
</table>

* $P < 0.005$ (Bonferroni correction for simultaneous tests)
Fig. 1. Atlantic cod. Collection sites in Icelandic waters. Abbreviations: L = Loftstaðahraun, E = Eyrabakkabugur, K = Kantur (Dyrhólaey), R = Reykjanesgrunn, A = Austfjarðadjúp (see Table I for details).

Fig. 2. Neighbour-joining dendrogram of Nei’s (1972) genetic distances among the cod populations in the present study based on both loci investigated.