ICES Journal of Marine Science, 58: 114–122. 2001
doi:10.1006/jmsc.2000.0995, available online at http://www.idealibrary.com on IDEAL®

Genetic differentiation among Atlantic cod (*Gadus morhua* L.) in Icelandic waters: temporal stability

Ó. D. B. Jónsdóttir, A. K. Daníelsdóttir, and G. Nævdal

Jónsdóttir, Ó. D. B., Daníelsdóttir, A. K., and Nævdal, G. 2001. Genetic differentiation among Atlantic cod (*Gadus morhua* L.) in Icelandic waters: temporal stability. – ICES Journal of Marine Science, 58: 114–122.

Temporal variability in genetic structure of Atlantic cod was investigated at two locations (Loftstaðahraun and Kantur) on the main cod spawning grounds off south Iceland using synaptophysin (*Syp* I) and haemoglobin as genetic markers. During the spawning season (late March and early April) cod were sampled at each sampling location and then at the same locations two to four weeks later, during two consecutive years (1997 and 1998). Significant genetic differences were found at the *Syp* I locus between the two samples (Loftstaðahraun and Kantur) and the difference was temporally stable. Very low variation was found in the haemoglobin and no temporal changes were found for this marker. The results strongly indicates that cod in south Icelandic waters do not belong to one panmictic population and that this genetic difference is stable from year-to-year.

Key words: *Gadus morhua*, Iceland, genetic differentiation, temporal stability.

Received 4 November 1999; accepted 4 July 2000.

Ó. D. B. Jónsdóttir: Marine Research Institute, Division of Population Genetics; A. K. Daníelsdóttir: Marine Research Institute, Division of Population Genetics, Keldnaholti, 112 Reykjavík, Iceland; G. Nævdal: Institute for Fisheries and Marine Biology, High Technology Center, N-5020 Bergen, Norway. Correspondence to A. K. Daníelsdóttir; tel: +354 570 7200; fax: +354 570 7111; email: andán@iti.is

Introduction

Examination of temporal variability in gene frequencies is essential for the proper understanding of population genetic structure (Allendorf and Utter, 1979; Utter *et al.*, 1980). Inferences about underlying population structure deduced from particular patterns of spatial variation may be inaccurate if such patterns vary markedly over time (Kornfield *et al.*, 1982). Significant temporal genetic differentiation might indicate the existence of stochastic effects due to small effective population size, or perhaps the occurrence of previously cryptic genetic subpopulations of stocks (Gold *et al.*, 1993). A proper understanding of population structure is of primary importance for the conservation of genetic resources in exploited marine fisheries. This includes determining whether or not the genetic structure of a stock complex remains stable over time. A temporally stable genetic structure implies that existing, separate breeding components persist over time and are thus likely to experience independent population dynamics (Gold *et al.*, 1993; Brown *et al.*, 1996; Ruzzante *et al.*, 1997).

Atlantic cod (*Gadus morhua*) is a significant resource in the North Atlantic ocean. Considerable effort has been made in the past to elucidate the possible subdivision of the Atlantic cod population complex (Cross and Payne, 1978; Carr and Marshall, 1991; Galvin *et al.*, 1995; Pogson *et al.*, 1995; Ruzzante *et al.*, 1996; Fevolden and Pogson, 1997). However, these studies have not led to a clear-cut conclusion about population structure of cod and some results are contradictory (e.g. Jamieson and Jónsson, 1971; Árnason *et al.*, 1992; Pepin and Carr, 1993; Ruzzante *et al.*, 1996). Therefore, uncertainty on the stock structure of Atlantic cod prevails. Studies of cod populations in Icelandic waters have also led to contradictory results. The results of Jamieson and Jónsson (1971), Jónsson (1996) and Jónsdóttir *et al.* (1999) indicate the presence of different units of cod in Icelandic waters, whereas Árnason *et al.* (1992) found no evidence of population differentiation of cod in the same area. The discrepancy between these studies shows that our knowledge of the structuring of Atlantic cod in Icelandic waters is incomplete.

1054–3139/01/010114+09 $35.00/0

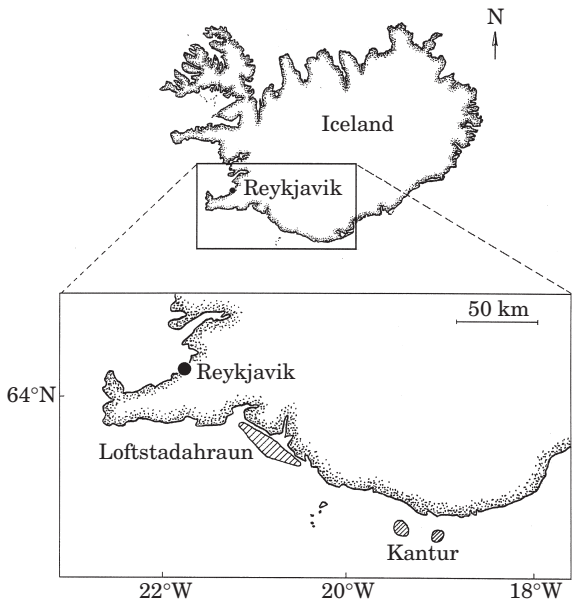

Figure 1. Sampling areas of Atlantic cod off south Iceland (see Table 1 for details).

Jónsdóttir *et al.* (1999) described the population structure within the Northern cod complex in south and southeast Icelandic waters using a nuclear gene locus, synaptophysin (*Syp* I), and the haemoglobin loci (*HbI*). The purpose of the present study was to examine the temporal stability at the *Syp* I and the *HbI* loci, and to study the frequency distributions of the variants within these loci.

Materials and methods

Sample collection

A total of 749 cod was collected from two locations in south Icelandic waters (Figure 1, Table 1) which is the main spawning ground of cod (Jónsson, 1982; Jónsson, 1996). The samples consisted of 79–99 specimens (Table 1). The sampling locations, which are approximately 50 nautical miles apart, were Loftstaðahraun (Figure 1), a submarine rocky mount, depth range 50–70 m, and Kantur (Figure 1) a very steep continental slope, with a depth range of 150–450 m. Samples were collected during surveys by the Marine Research Institute, Reykjavík, Iceland, during spawning seasons in March and April 1997 and 1998. Each location was sampled twice, at intervals of two to four weeks (Table 1). The fish were caught using bottom trawls (L-Apr1-97, L-Apr2-97, L-Mar-98, see below) and gill-nets (Kantur sampling locations and L-Apr-98). The length and sex of each fish were recorded. The two spawning grounds appear to be geographically isolated from each other as Loftstaðahraun is located in shallow water within the area referred to as the main spawning grounds of cod on the south coast (Jónsson, 1982), whilst Kantur is located further to the west in much deeper water on the continental edge. Results from tagging experiments have indicated that spawners from these two locations may display different patterns of migration when leaving the spawning areas (Jónsson, 1996). Previous investigations (Jónsdóttir *et al.*, 1999) have revealed considerable genetic differences between cod captured at these locations. Two of the samples in this study (L-Apr1-97, K-Mar-97) were also included in the study of Jónsdóttir *et al.* (1999) but are included here to allow comparison of genetic structure within and between years at the same spawning location.

Genetic analysis

Gill arches were dissected from every fish and stored in 96% (v/v) ethanol. Approximately 200 mg of gill tissue were then used to extract DNA by the phenol-extraction method (Taggart *et al.*, 1992). The nucleotide sequence data of Fevolden and Pogson (1997) were used to construct primers that amplify, by the polymerase chain reaction (PCR), a region of the synaptophysin (*Syp* I) gene that contains the polymorphic *Dra*I site. All samples were screened for variation at the *Syp* I locus using the reaction conditions described by Jónsdóttir *et al.* (1999) modified from Fevolden and Pogson (1997). Digested PCR products were visualized in 2% (w/v) agarose gels stained with ethidium bromide.

Fresh blood samples were used for the analysis of haemoglobin. Horizontal agar electrophoresis was used, as described by Sick (1967) 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% (w/v) agar concentration (Agar Noble) was used in the gels. Electrophoresis was carried out at 25 mA (8 V cm^{-1}) 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, pairwise F_{ST} values (Wright, 1978; Reynolds *et al.*, 1983) and an exact test of population differentiation (analogous to Fishers exact test) were calculated using the ARLEQUIN 1.1 computer package (Schneider *et al.*, 1997). The genetic structure of the sampled populations were analysed using an analysis of molecular variance (AMOVA) framework (Weir and Cockerham, 1984). The eight samples were grouped according to the two sampling locations: Loftstaðahraun (L-Apr1-97, L-Apr2-97, L-Mar-98, and L-Apr-98) and Kantur (K-Mar-97, K-Apr-97,

Table 1. Summary statistics of cod samples collected in March and April 1997 and 1998, used for testing the temporal genetic stability of spawning populations at Loftstaðfahraun and Kantur. Differences in mean length of specimens between sampling locations were tested with one-way ANOVA. Different letters indicate significant differences ($p<0.05$) in mean length between sampling locations, with [a] indicating significantly higher mean length compared to [b] etc.

Collection date	Sampling locations	Sample identifier	n	Latitude (°N)	Longitude (°W)	Mean depth (m)	Mean length (s.d.) (cm)	Proportion females (%)
3–4 April 1997	Loftstaðahraun	*L-Apr1-97**	95	63°46	20°50	44	109 (14)[a]	33.6
18–20 April 1997	Loftstaðahraun	L-Apr2-97	79	63°44	20°53	80	103 (14)[b]	12.6
25–26 March 1997	Kantur	K-Mar-97	87	63°17	19°13	285	85 (6)[d]	46.0
15–16 April 1997	Kantur	*K-Apr-97**	96	63°17	19°12	288	83 (7)[d]	64.6
28–30 March 1998	Loftstaðahraun	L-Mar-98	99	63°47	21°00	45	98 (14)[c]	39.4
26 April 1998	Loftstaðahraun	L-Apr-98	96	63°50	21°10	52	95 (12)[c]	36.5
25–26 March 1998	Kantur	K-Mar-98	99	63°17	19°12	286	84 (7)[d]	41.4
16–17 April 1998	Kantur	K-Apr-98	98	63°17	19°12	288	78 (7)[e]	49.0

*These samples were also included in the study of Jónsdóttir *et al.* (1999) but are included here to allow comparison of genetic structure within and between years at the same spawning location.

K-Mar-98 and K-Apr-98), and the significance of the variance components was tested using non-parametric permutation procedures (Excoffier *et al.*, 1992). One-way ANOVA (Zar, 1996) was applied to test for significant differences in length distribution of the specimens. Significant results from the ANOVA were followed by a Student-Newman-Keuls multiple comparison test to locate differences among the eight samples (Zar, 1996). Population pairwise F_{ST} statistics were calculated for all samples and the significance tested by permuting (1000 permutations) the individuals between the samples (Reynolds *et al.*, 1983; Schneider *et al.*, 1997). Global departure from Hardy-Weinberg equilibrium was tested in the AMOVA test by taking into account genotypic differences between individuals (Schneider *et al.*, 1997). The Selander's D-statistics for the excess or deficiency of heterozygotes (Selander, 1970) and the average heterozygosity were calculated using direct counts, and the estimates based on Hardy-Weinberg expectations. Sex-specific frequencies of *Syp* I and *HbI* were tested with a contingency χ^2-test (Zar, 1996). Departures from 1:1 sex ratios at each sampling location were tested with χ^2-test.

Reynolds genetic distances (Reynolds *et al.*, 1983) were calculated by the GENDIST program in the PHYLIP package (Felsenstein, 1993), and a UPGMA dendrogram of Reynolds genetic distance matrix was constructed in the NEIGHBOR program in PHYLIP. A Bonferroni correction (Johnson and Field, 1993) of the significance level ($\alpha=0.05$) was applied when testing for significant differences in allele frequencies and for significant departures of genotypes proportions from Hardy-Weinberg expectations.

Results

Syp I and *HbI* polymorphisms

The allele frequencies for the *Syp* I alleles differed both between the sampling locations (Loftstaðahraun and Kantur) and between samples within sampling location (Loftstaðahraun, Table 2). The frequency of the *Syp* I^A allele was significantly higher in the Loftstaðahraun samples (mean frequency = 0.766) compared to the Kantur samples (mean frequency = 0.270). Although observed frequencies of heterozygotes were in most cases higher than the expected Hardy-Weinberg values (Table 2), no significant deviations from Hardy-Weinberg equilibrium were detected in any sample when using the Bonferroni correction for simultaneous tests ($p>0.15$) and Selander's D-statistics values were non-significant for all samples ($\chi^2_1<2.4$, $p>0.10$).

The frequency of the most cathodic haemoglobin allele, HbI^1 was low, ranging from 0.005 (K-Apr-98) to 0.032 (L-Apr2-97, Table 2), with an average of 0.021. When the compiled allele frequencies of *HbI* in the two sampling locations were tested, Loftstaðahraun and Kantur did not differ significantly ($F_{ST}<0.015$, $p>0.5$). For all samples the observed and expected heterozygote frequencies at the *HbI* locus were similar and were in Hardy-Weinberg equilibrium ($p>0.9$). The Selander's D-statistics values were non-significant for all samples ($\chi^2_1<1.0$). No differences were found in frequencies of the *Syp* I ($p>0.09$) and *HbI* loci ($p>0.7$) between sexes within sampling locations.

Genetic differentiation within sampling locations

Kantur

At Kantur, samples taken approximately 13 nautical miles apart (Kantur 1 and Kantur 2) showed no differences in allelic frequencies (Fisher's exact text, $p>0.65$), and were therefore pooled for inter-area comparisons. No significant differences (F_{ST} and population pairwise differentiation test, $p>0.2$) were observed between samples from Kantur ($n=380$). Further, the differences between Kantur 1997 and 1998 samples accounted only

Table 2. Frequencies of *Syp* I^A, *Syp* I^B, *HbI*^{1} and *HbI*^{2} alleles, heterozygosity and Selander's D-statistics of cod samples from two successively sampled locations in south Icelandic waters in 1997 and 1998.

Locus	Allele	L-Apr1-97	L-Apr2-97	L-Mar-98	L-Apr-98	K-Mar-97	K-Apr-97	K-Mar-98	K-Apr-98
	n	95	79	99	96	87	96	99	98
Syp I	*Syp* I^A	0.874	0.791	0.732	0.667	0.299	0.287	0.247	0.250
	Syp I^B	0.126	0.209	0.268	0.333	0.701	0.713	0.753	0.750
	H	0.189	0.291	0.454	0.500	0.436	0.510	0.434	0.397
		(0.220)	(0.330)	(0.392)	(0.444)	(0.419)	(0.409)	(0.372)	(0.375)
	D	− 0.141	− 0.118	0.159	0.126	0.042	0.249	0.167	0.057
HbI	*HbI*^{1}	0.016	0.032	0.010	0.031	0.029	0.016	0.030	0.005
	HbI^2	0.984	0.968	0.989	0.969	0.971	0.984	0.970	0.995
	H	0.032	0.063	0.020	0.062	0.057	0.031	0.061	0.010
		(0.031)	(0.061)	(0.020)	(0.060)	(0.056)	(0.031)	(0.059)	(0.010)
	D	0.019	0.034	0.010	0.042	0.032	0.018	0.032	0.020

n=number of specimens analyzed, *H*=heterozygosity, observed (expected), *D*=Selander's (1970) D-statistic for the excess of deficiency of heterozygotes.

Table 3. Population pairwise F_{ST} estimates at *Syp* I locus among all samples. Permuting the individuals between the samples tested the significance of the pairwise F_{ST} values. 1000 permutations were performed for all pairs of samples.

	L-Apr1-97	L-Apr2-97	K-Mar-97	K-Apr-97	L-Mar-98	L-Apr-98	K-Mar-98	K-Apr-98
L-Apr1-97	—	—	—	—	—	—	—	—
L-Apr2-97	0.021	—	—	—	—	—	—	—
K-Mar-97	*0.478***	*0.347***	—	—	—	—	—	—
K-Apr-97	*0.500***	*0.372***	− 0.004	—	—	—	—	—
L-Mar-98	*0.050***	0.001	*0.294***	*0.318***	—	—	—	—
L-Apr-98	*0.107***	0.028	*0.203***	*0.226***	0.008	—	—	—
K-Mar-98	*0.537***	*0.413***	0.001	− 0.002	*0.360***	*0.266***	—	—
K-Apr-98	*0.547***	*0.421***	0.002	− 0.002	*0.367***	*0.271***	− 0.004	—

*p<0.0018, **p<0.00036 (Bonferroni correction for simultaneous tests).

for 0.4% of total genetic variation when tested with AMOVA.

Loftstaðahraun

The Loftstaðahraun samples (n=369) did, however, display some genetic heterogeneity as L-Apr1-97 differed significantly from L-Apr-98 (Table 3). L-Apr1-97 differed from L-Mar-98 when tested with pairwise F_{ST} test (Table 3), whereas the population pairwise differentiation test did not indicate any differences between these samples (p>0.10). An AMOVA test indicated that only 3.8% of the variation within Loftstaðahraun samples was due to differences between years.

Genetic differentiation between sampling locations

The total material (all samples for both loci compiled) was not in Hardy-Weinberg equilibrium (p<0.01, Table 4) when the individual genetic variation in the analysis of molecular variance (AMOVA, Table 4) was tested, strongly indicating that the samples were drawn from more than one panmictic population. Significant difference was observed at the *Syp* I locus (F_{ST}>0.234, p<0.001) when testing the compiled allele frequencies in the samples from the two sampling locations. The population pairwise F_{ST} test and the population pairwise differentiation test revealed highly significant differences (p<0.001) between samples from Loftstaðahraun vs. Kantur (Table 3). When pooling the material into two groups (Loftstaðahraun and Kantur) the AMOVA test indicated a significant difference (p<0.05) between the two sampling locations (Table 4). Also, hierarchical F-statistics indicated a clear population differentiation and 36.4% of the total allelic variance was due to differences between the two sampling locations (Table 4). A significant difference was detected between the samples within each sampling location (p<0.01), whereas only 1.1% of the allelic variance within sampling locations was observed among the eight samples (Table 4).

The UPGMA dendrogram constructed using Reynolds (Reynolds *et al.*, 1983) genetic distance (Figure 2) illustrated the differentiation between the two

Table 4. 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. Sampling location=Loftstaðfahraun (L-Apr1-97, L-Apr2-97, L-Mar-98, L-Apr-98), Kantur (K-Mar-97, K-Apr-97, K-Mar-98, K-Apr-98).

Source of variation	d.f.	Sum of squares	Variance component	Variance explained by AMOVA model	F_{XY}
Among sampling locations	1	87.861	0.1162*	36.45	0.364
Among samples within sampling location	6	5.128	0.0036**	1.13	0.018
Among individuals within samples	741	136.553	−0.0147‡	−4.60‡	−0.073‡
Within individuals	749	160.000	0.2136**	67.02	0.330
Total	1497	389.543	0.3187	100	

*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 locations is zero in the present study.

sampling locations. The genetic distance between Loftstaðahraun and Kantur samples was eight-fold higher than the genetic distance within these two groups. Further, there was differentiation within the Loftstaðahraun sampling location as the L-Apr1-97 sample differed significantly from L-Apr-98 (Table 3, Figure 2). It should be noted that the dendrogram is based on just two loci and should be interpreted with caution.

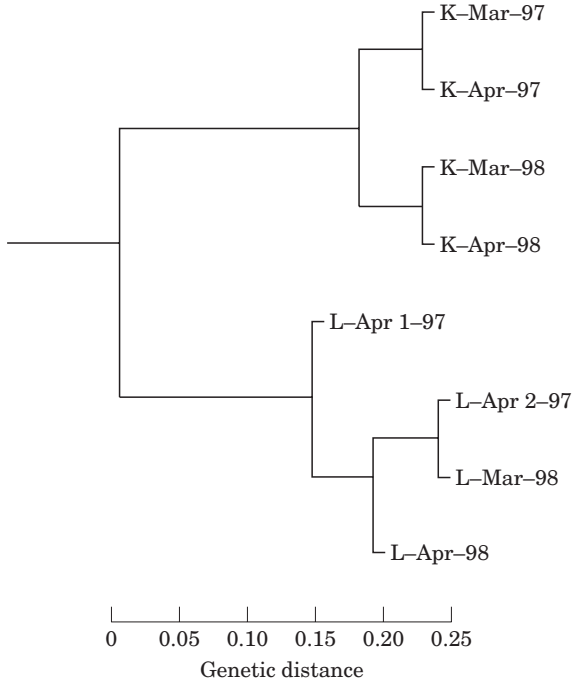

Figure 2. UPGMA dendrogram of the eight samples based on Reynolds genetic distance. See Table 1 for details of sampling locations. It should be noted that the dendrogram is based on just two loci and should be interpreted with caution.

Sex ratio

Percentage females at each sampling location varied from 13–65% (Table 1). The sex ratio was significantly different from a 1:1 distribution at all Loftstaðahraun samplings in both years (χ^2_1>11.5, p<0.01) and at Kantur in April 1997 and March 1998 (χ^2_1=7.2, p<0.01). At Kantur the proportion of females increased from March to April in both years whereas at Loftstaðahraun the female proportion decreased between the subsequent samplings in both years.

Discussion

Genetic diversity at Loftstaðahraun and Kantur

In accordance with our previous study (Jónsdóttir *et al.*, 1999), the present study showed a genetic differentiation among spawning units of cod populations in Icelandic waters. Differences in the *Syp* I allele frequencies were highly significant for Loftstaðahraun vs. Kantur in both years (1997 and 1998, Table 3, Figure 2), and 36.4% of the total allelic variance detected was due to differences between the two sampling locations (Table 4). The distinction in the *Syp* I allele frequencies between the two groups (Loftstaðahraun and Kantur) in the present paper was in line with the findings of Fevolden and Pogson (1997) in Norwegian waters, where coastal cod populations possessed significantly higher frequencies of the *Syp* I^A allele (mean frequency=0.81) than did off-shore populations (mean frequency=0.10). Ruzzante *et al.* (1996) found a similar distinction between inshore and offshore cod populations off Newfoundland. The

findings of Ruzzante *et al.* (1996) and Fevolden and Pogson (1997) are consistent with morphometric differences between the cod populations off Newfoundland (Pepin and Carr, 1993) and differences in otolith structure between coastal and Arctic cod in Norwegian waters (Dahle and 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) was reported, using DNA microsatellites. Ruzzante *et al.* (1998) analysed variation in five microsatellite DNA loci in 14 cod populations spanning the range of the species in the Northwest Atlantic. Differences were revealed among populations at continental shelf scales (NE Newfoundland, Grand Banks, Flemish Cap, Scotian Shelf, Georges Bank) where regions are separated by submarine saddles, channels and trenches. They also found evidence of genetic structure at spawning-bank scales consistent with variation in oceanographic features and in the spatiotemporal distribution of spawning which may represent barriers to gene flow among geographically contiguous populations inhabiting a highly advective environment. Overall, these findings indicate genetic heterogeneity among cod populations at very localized scales contrasting with earlier findings which have indicated minor sub-structuring (Cross and Payne, 1978; Mork *et al.*, 1985) or have indicated panmixia (Árnason *et al.*, 1992; Pepin and Carr, 1993; Árnason *et al.*, 2000).

The frequency of haemoglobin genotypes did not display any significant differences in the present study, neither between sampling locations, samples or years (Table 2), in line with previous studies (Jónsdóttir *et al.*, 1999). It has been argued whether the haemoglobin locus in cod is a suitable population marker for genetic studies since it has been linked to differences in physiological performance leading to selection (Karpov and Novikov, 1980; Mork *et al.*, 1984a,b; Mork and Sundnes, 1985; Nævdal *et al.*, 1992; Brix *et al.*, 1997). The fact that the haemoglobin frequencies are close to those found by Sick (1965) and Jamieson and Jónsson (1971) in similar areas show that the stability of the gene frequency is sufficient to be used as population marker in cod at least in the area investigated here.

Temporal stability in genetic structure of cod at Loftstaðahraun and Kantur

This study revealed a significant temporal stability for most samples, at least at the scale of one year. The allelic frequencies of the *HbI* were stable between years as well as the differences in *Syp* I allelic frequencies between Loftstaðahraun and Kantur (Table 2, Figure 2). The allelic frequency of *Syp* I^A from Kantur samples was stable, ranging from 0.247–0.299 (K-Mar-98 and K-Mar-97, respectively). However, the allelic frequencies of *Syp* I^A from Loftstaðahraun samples ranged from 0.667–0.874 between years (L-Apr-98 and L-Apr1-97, respectively, Table 2). The observed stability in the differences between Loftstaðahraun and Kantur is consistent with very similar results reported by Jónsdóttir *et al.* (1999; unpublished results) and therefore provides evidence of temporal stability in genetic differences between the two sampling locations at the scale of one year. Temporal stability in allelic frequency of Atlantic cod has also been reported by Ruzzante *et al.* (1997), where the magnitude of the genetic differences between inshore and offshore cod populations off Newfoundland remained unchanged during the period 1992–1995.

The issue of temporal stability in genetic structure has previously been considered for a number of marine species using a variety of genetic techniques. Gold *et al.* (1993) demonstrated temporal stability in both nuclear gene allele frequencies and mtDNA haplotype frequencies of a red drum (*Sciaenops ocellatus*) population among four year-classes (1984–1987). Temporal stability has also been detected by King *et al.* (1987) throughout three developmental stages and eight year-classes of herring (*Clupea harengus*) using enzyme loci. However, former studies by Kornfield *et al.* (1982) showed significant temporal heterogeneity of Atlantic herring allozymes. Brown *et al.* (1996) used mitochondrial DNA haplotype frequencies to demonstrate a relatively small temporal variation in the allele frequency of American shad (*Alosa sapidissima*), compared with the existing geographically based variation among populations from different river drainages spanning the distribution ranges of the species. Gyllensten and Ryman (1988) observed pronounced allozymic variation through time for sculpin (*Myoxocephalus quadricornis*) from several Baltic Sea spawning aggregations, where the highest temporal allele frequency shifts were at highly polluted localities. They suggested that the reproductive units of sculpin might not be geographically stationary. Also, Chapman (1987, 1989) observed temporal genetic changes in Chesapeake Bay striped bass (*Morone saxatilis*) and suggested a cohort-related distribution of mtDNA size polymorphisms. Finally, significant temporal genetic variation has been found among populations of queen conch (*Stombus gigas*) in the Florida Keys (Campton *et al.*, 1992).

The temporally stable genetic structure observed for Atlantic cod in Icelandic waters implies that existing, separate breeding components persist over time. Nordeide (1998) investigated the intermingling of Norwegian coastal (NC) and Arcto-Norwegian (AN) cod at their spawning grounds at Lofoten (northern Norway) using haemoglobin alleles and biological data. He found that NC and AN cod did not mingle randomly, although specimens from both populations may stay simultaneously at the same local spawning ground within an area of less than 0.012 km^2. Our data are in

line with the findings of Nordeide (1998). It may thus be speculated whether the spawning behavior of cod at Loftstaðahraun and Kantur may also be different.

At Loftstaðahraun the sex ratio in the samples was skewed towards males, whereas the overall proportions of males and females were more equal at Kantur (Table 1). Skewed sex ratios in the spawning aggregations of Atlantic cod in the wild have been reported earlier (McKenzie, 1940; Chrzan, 1950; Templeman and May, 1965; Jónsson, 1982; Nordeide, 1998). The bias towards males at Loftstaðahraun is in accordance with Jónsson's research (Jónsson, 1982) in the same area. His studies revealed unbalanced sex ratios in the cod stock during the spawning season; females predominated in the samples from January to mid-March, but males predominated from the end of March until the end of the season. In the spawning areas of Newfoundland, Morgan and Trippel (1996) observed shoals with unequal sex ratios during the spawning season (March–June), depending on sampling location and depth. Male-biased shoals were detected at the spawning grounds prior to the arrival of females, which is in accordance with the findings of Jónsson (1982) considering the time of the sample collections. If differences in sex ratios among samples can be explained by differences in sampling depth (Morgan and Trippel, 1996), the equality of the sexes observed at Kantur might be explained by the sampling methods where gillnets were set at depths ranging from approximately 130–430 m. Applying the hypothesis of Morgan and Trippel (1996) to our data, it might be speculated that the genetic difference between cod in Loftstaðahraun and Kantur is maintained by e.g. different timing of spawning and/or different behavioural mechanisms (Brawn, 1961a,b; Hawkins and Rasmussen, 1978; Hawkins, 1986). Hutchings *et al.* (1999) present results from cod spawning in tanks and argue that cod has a lek mating system and is not a promiscuous group spawner, as is the general view. Also, Nordeide and Folstad (2001) reviewed the literature on cod spawning behaviour and conclude that a lek mating system with several options for female choice best describes the spawning behaviour of cod. It is argued that different courtship display, combined with different mate preferences, may be important pre-mating mechanisms reducing or preventing interbreeding between groups of cod. Although our data can not verify or falsify the hypothesis of Hutchings *et al.* (1999) and Nordeide and Folstad (2001), the relationship between spawning behaviour and reduced interbreeding are in line with our findings. Alternatively, the observed sex-biased shoals of cod could simply be an artifact of the sampling gear used in the surveys (Rollefsen, 1953; Jónsson, 1982). Also, the sex-biased shoals might be related to the bottom type as there are indications that it may be more difficult to catch females on rough lava bottom (Loftstaðahraun) during spawning as females tend to swim less than males on the spawning ground (Hutchings *et al.*, 1999; Nordeide and Folstad, 2000).

Conclusion

The results of the present study strongly indicate that Atlantic cod collected at the spawning grounds south of Iceland do not belong to one panmictic population and the observed genetic difference was stable between years. Significant genetic difference was found at the *Syp* I locus between samples collected at two localities, Loftstaðahraun and Kantur. The observed genetic difference was stable from 1997–1998, indicating temporal stability, at least at the scale of one year. Significant genetic heterogeneity was observed within the Loftstaðahraun samples, but not within the Kantur samples.

Acknowledgements

We thank A. K. Imsland for valuable comments and assistance; V. Thorsteinsson, G. Marteinsdóttir for helpful advice; G. Pogson and S. E. Fevolden for advice during the analytical work; J. T. Nordeide and a anonymous referee for comments on the manuscript; S. L. Jónsdóttir, H. B. Ingólfsdóttir, Ó. Ý. Atladóttir, E. Ásgeirsson, H. Guðfinnsson and K. Kristinsson for assistance in sampling fish and the crew on board Valdimar Sveinsson VE-22, Árni Friðriksson RE-100, Dröfn RE-35, and Eyrún EX-1315 for their contribution. Financial support was given by the EU FAIR program (FAIR CT95-0282).

References

Allendorf, F. W., and Utter, F. M. 1979. Population genetics of fish. *In* Fish Physiology, Vol. 8, pp. 407–454. Ed. by W. S. Hoar, D. S. Randall, and J. R. Brett. Academic Press, New York, NY.

Árnason, E., Pálsson, S., and Arason, A. 1992. Gene flow and lack of population differentiation in Atlantic cod, *Gadus morhua* L., from Iceland, and comparison of cod from Norway and Newfoundland. Journal of Fish Biology, 40: 751–770.

Árnason, E., Petersen, P. H., Kristinsson, K., Sigurgíslason, H., and Pálsson, S. 2000. Mitochondrial cytochrome *b* DNA sequence variation of Atlantic cod from Iceland and Greenland. Journal of Fish Biology, 56: 409–430.

Bentzen, P., Taggart, C. T., Ruzzante, E., and Cook, D. 1996. Microsatellite polymorphism and population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences, 53: 2706–2721.

Brawn, V. M. 1961a. Reproductive behaviour of the cod (*Gadus callarias* L.). Behaviour, 18: 177–198.

Brawn, V. M. 1961b. Sound production by the cod (*Gadus callarias* L.). Behaviour, 18: 239–245.

Brix, O., Forås, E., and Strand, I. 1997. Genetic variation and functional properties of Atlantic cod haemoglobin: Introducing a modified tonometric method for studying fragile haemoglobin. Comparative Biochemistry and Physiology, 116A: 575–583.

Brown, B. L., Epifanio, J. M., Smouse, P. E., and Kobak, C. J. 1996. Temporal stability of mtDNA haplotype frequencies in American shad stocks: to pool or not to pool across years? Canadian Journal of Fisheries and Aquatic Sciences, 53: 2274–2283.

Campton, D. E., Berg, C. J. Jr, Ropison, L. M., and Glazer, R. A. 1992. Genetic patchiness among populations of queen conch *Stombus gigas* in the Florida Kays and Bimini. Fishery Bulletin U.S., 90: 250–259.

Carr, S. M., and Marshall, H. D. 1991. Detection of intraspecific DNA sequence variation in the mitochondrial cytochrome b gene of Atlantic cod (*Gadus morhua*) by the polymerase chain reaction. Canadian Journal of Fisheries and Aquatic Sciences, 48: 48–52.

Chapman, R. W. 1987. Changes in the population structure of male striped bass, *Morone saxatilis*, spawning in the three areas of Chesapeake Bay from 1984 to 1986. Fishery Bulletin, 85: 167–170.

Chapman, R. W. 1989. Spatial and temporal variation of mitochondrial DNA haplotype frequencies in the striped bass (*Morone saxatilis*) 1982 year class. Copeia, 1989: 344–348.

Chrzan, F. 1950. Investigations on the Baltic cod. Journal du Conseil International pour l'Exploration de la Mer, 16: 192–207.

Cross, T. F., and Payne, R. H. 1978. Geographic variation in Atlantic cod, *Gadus morhua*, off eastern North America: a biochemical systematic approach. Canadian Journal of Fisheries and Aquatic Sciences, 35: 117–123.

Dahle, G. 1995. Genetic structure of the North-East Atlantic cod (*Gadus morhua* L.), an appraisal of different molecular techniques. Ph.D. thesis. University of Bergen, Norway.

Dahle, G., and Jørstad, K. E. 1993. Haemoglobin variation in cod – a reliable marker for Arctic cod (*Gadus morhua* L.). Fisheries Research, 16: 301–311.

Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred form metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics, 131: 479–491.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) Version 3.5c. University of Washington, Seattle.

Fevolden, S. E., and Pogson, G. H. 1997. Genetic divergence at the synaptophysin (*Syp* I) locus among Norwegian coastal and north-east Arctic populations of Atlantic cod. Journal of Fish Biology, 51: 895–908.

Galvin, P., Sadusky, T., McGregor, D., and Cross, T. 1995. Population genetics of Atlantic cod using amplified single locus minisatellite VNTR analysis. Journal of Fish Biology, 47: 200–208.

Gold, J. R., Richardson, L. R., King, T. L., and Matlock, G. C. 1993. Temporal stability of nuclear gene (allozyme) and mitochondrial DNA genotypes among red drums from the Gulf of Mexico. Transactions of the American Fisheries Society, 122: 659–668.

Gyllensten, U., and Ryman, N. 1988. Biochemical genetic variation and population structure of fourhorn sculpin *Myoxocephalus quadricornis cottidae* in Scandinavia. Heriditas (Lund), 108: 179–186.

Hawkins, A. D. 1986. Underwater sound and fish behaviour. *In* The Behaviour of Teleost Fishes, pp. 114–151. Ed. by T. J. Pither. Croom Helm, London.

Hawkins, A. D., and Rasmussen, K. J. 1978. The calls of gadoid fish. Journal of the Marine Biological Association, UK, 58: 891–911.

Hutchings, J. A., Bishop, T. D., and McGregor-Shaw, C. R. 1999. Spawning behaviour of Atlantic cod, *Gadus morhua*: evidence of mate choice in a broadcast spawner. Canadian Journal of Fisheries and Aquatic Sciences, 56: 97–104.

Jamieson, A., and Jónsson, J. 1971. The Greenland component of spawning cod at Iceland. Journal du Conseil International Pour l'Exploration de la Mer, 161: 65–72.

Johnson, C. R., and Field, C. R. 1993. Using fixed-effects model multivariate analysis of variance in marine biology and ecology. Marine Biology Annual Review, 31: 177–221.

Jónsdóttir, Ó. D. B., Imsland, A. K., Daníelsdóttir, A. K., Thorsteinsson, V., and Nævdal, G. 1999. Genetic differentiation among Atlantic cod in south and south-east Icelandic waters: synaptophysin (*Syp* I) and haemoglobin variation. Journal of Fish Biology, 54: 1259–1274.

Jónsson, E. 1982. A survey of spawning and reproduction of the Icelandic cod. Rit Fiskideildar (Journal of the Marine Research Institute, Reykjavik), 6: 1–45.

Jónsson, J. 1996. Tagging of cod (*Gadus morhua*) in Icelandic waters 1948–1986. Rit Fiskideildar (Journal of the Marine Research Institute, Reykjavik), 14: 7–82.

Jørstad, K. E. 1984. Genetic analyses of cod in northern Norway. *In* The Propagation of Cod *Gadus morhua* L., pp. 745–760. Ed. by E. Dahl, D. S. Danielsen, E. Moksness, and P. Solemdal. Flødevigen rapportserie, Vol. 1.

Karpov, L. K., and Novikov, G. G. 1980. The haemoglobin alloforms in cod (*Gadus morhua* L.), their functional characteristics and distribution in the populations. Journal of Ichthyology, 20: 45–50.

King, D. P., Ferguson, A., and Moffett, I. J. 1987. Aspect of the population genetics of herring *Glupea harengus* around the British Isles UK and in the Baltic Sea. Fisheries Research (Amst.), 6: 35–52.

Kornfeld, I., Sidell, B. D., and Gagnon, P. S. 1982. Stock definition in Atlantic herring (*Clupea harengus harengus*): genetic evidence for discrete fall and spring spawning populations. Canadian Journal of Fisheries and Aquatic Sciences, 39: 1610–1621.

Long, J. C. 1986. The allelic correlation structure of gainj and kalam-speaking people. I. The estimation and interpretation of Wright's *F*-statistics. Genetics, 112: 629–647.

McFarland, G. 1977. The staining of starch gels with Coomassie Brilliant Blue G 250 perchloric acid solution. Experentia, 33: 16–68.

McKenzie, R. A. 1940. Nova Scotian autumn cod spawning. Journal of the Fisheries Research Board of Canada, 5: 105–120.

Morgan, M. J., and Trippel, E. A. 1996. Skewed sex ratio in spawning shoals of Atlantic cod (*Gadus morhua*). ICES Journal of Marine Science, 53: 820–826.

Mork, J., and Sundnes, G. 1985. 0-group cod (*Gadus morhua*) in captivity: differential survival of certain genotypes. Helgoländer Meeresuntersuchungen, 39: 63–70.

Mork, J., Giskeødegård, R., and Sundnes, G. 1984a. Population genetic studies in cod (*Gadus morhua* L.) by means of the haemoglobin polymorphism; observations in a Norwegian coastal population. Fiskeridirektoratets skrifter, Serie Havundersøkelser, 17: 449–471.

Mork, J., Giskeødegård, R., and Sundnes, G. 1984b. The haemoglobin polymorphism in Atlantic cod (*Gadus morhua* L.): Genotype differences in somatic growth and in maturing age in natural population. *In* The Propagation of Cod *Gadus morhua* L., pp. 721–732. Ed. by E. Dahl, D. S. Danielsen, E. Moksness, and P. Solemdal. Flødevigen rapportserie, Vol. 1.

Mork, J., Ryman, N., Ståhl, G., Utter, F., and Sundnes, G. 1985. Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. Canadian Journal of Fisheries and Aquatic Sciences, 42: 1580–1587.

Nordeide, J. T. 1998. Coastal cod and northeast Arctic cod – do they mingle at the spawning grounds in Lofoten? Sarsia, 83: 373–379.

Nordeide, J. T., and Folstad, I. 2001. Is cod a lekking or a promiscuous group spawner? Fish and Fisheries (in press).

Nævdal, G., Folkvord, A., Otterlei, E., and Thorkildsen, S. 1992. Growth rate related to genotype of 0-group cod at three environmental temperatures. Sarsia, 77: 71–73.

Pepin, P., and Carr, S. M. 1993. Morphological, meristic, and genetic analysis of stock structure in juvenile Atlantic cod (*Gadus morhua*) from the Newfoundland shelf. Canadian Journal of Fisheries and Aquatic Sciences, 50: 1924–1933.

Pogson, G. H., Mesa, K. A., and Boutilier, R. G. 1995. Genetics population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics, 139: 375–385.

Reynolds, J., Weir, B. S., and Cockerham, C. C. 1983. Estimation for the coancestry coefficient: basis for a short-term genetic distance. Genetics, 105: 769–779.

Rollefsen, G. 1953. The selectivity of different fishing gear used in Lofoten. Journal du Conseil International pour l'Exploration de la Mer, 19: 191–194.

Ruzzante, D. E., Taggart, C. T., Cook, D., and Goddard, S. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Canadian Journal of Fisheries and Aquatic Sciences, 53: 634–645.

Ruzzante, D. E., Taggart, C. T., Cook, D., and Goddard, S. 1997. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: a test and evidence of temporal stability. Canadian Journal of Fisheries and Aquatic Sciences, 54: 2700–2708.

Ruzzante, D. E., Taggart, C. T., and Cook, D. 1998. A nuclear DNA basis for shelf and bank-scale population structure in northwest Atlantic cod (*Gadus morhua*): Laborador to Georges Bank. Molecular Ecology, 7: 1663–1680.

Schneider, S., Kueffer, J. M., Roessli, D., and Excoffier, L. 1997. Arlequin ver. 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Selander, R. K. 1970. Behaviour and genetic variation in natural populations. American Zoologist, 10: 53–66.

Sick, K. 1961. Haemoglobin polymorphism in fishes. Nature, 192: 894–896.

Sick, K. 1965. Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean. Hereditas, 54: 49–69.

Smithies, O. 1959. An improved procedure for starch-gel electrophoresis: Further variants in the serum proteins of normal individuals. Biochemical Journal, 71: 585.

Taggart, J. B., Hynes, R. A., Prodöhl, P. A., and Ferguson, A. 1992. A simplified protocol for routine total DNA isolation form salmonid fishes. Journal of Fish Biology, 40: 963–965.

Templeman, W., and May, A. W. 1965. Research vessel catches of cod in the Hamilton inlet bank area in relation to depth and temperature. ICNAF Special publication, 6: 149–165.

Utter, F. M., Campton, D., Grant, S., Milner, G., Seeb, J., and Wishard, L. 1980. Population structure of indigenous salmonid species of the Pacific Northwest. *In* Proceedings of symposium Salmonid ecosystems of the North Pacific, pp. 285–304. Ed. by W. J. McNeil, and D. C. Himsworth. Oregon St Univ. Press, Corvallis, OR.

Weir, B. S., and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358–1370.

Wright, S. 1978. Evolution and the genetics of populations: variability within and among natural populations. University of Chicago Press, Chicago, IL.

Zar, J. H. 1996. Biostatistical analysis. 3rd ed. Prentice Hall, New Jersey, USA. 662 pp.