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Haemoglobin variation in cod: a description of new variants and their geographical distribution

Åse Husebø*, Albert Kjartansson Imsland* & Gunnar Nævdal

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Blood from 1209 individual cod was sampled from nine localities in the Northeast Atlantic, and analysed using agar gel electrophoresis (AGE) and isoelectric focusing (IEF) to reveal individual and population variation in haemoglobin genotypes. The second aim of the study was to compare our data with data collected some 30 years ago in the same area to investigate possible directional selection. A new haemoglobin polymorphism of Atlantic cod was documented in samples from Danish waters using IEF electrophoresis (pH 5.5–8.5) and, in addition to the five genotypes seen in earlier studies, 11 subtypes are described. Significantly different genotype distributions were found between the samples from northern Norway on the one hand and southern Norway and Danish waters on the other, and the frequency of the *Hb-I(1)* allele increased from north to south. With the possible exception of two samples, the frequency of the main haemoglobin genotypes was found to be very similar to corresponding frequencies described in these areas three decades ago. The frequencies of the *Hb-I* subtypes off Norway varied among the sampling sites and were only found in Norwegian coastal cod with increasing frequency from north to south. In the Danish samples, 11 novel *Hb-I* subtypes were found and are described.

Åse Husebø & Gunnar Nævdal, Department of Biology, University of Bergen, P.O. Box 7800, N-5020 Bergen, Norway.
Albert Kjartansson Imsland, Akvaplan-niva, Iceland Office, Akralind 4, 201 Kópavogur, Iceland.
E-mail: albert.imsland@akvaplan.niva.no
**Equal authorship.*

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INTRODUCTION

A considerable number of studies on the genetic structure of cod, *Gadus morhua*, in Norwegian waters have been performed, revealing findings that appear to be conflicting. The earliest studies (Frydenberg & al. 1965; Møller 1966, 1968, 1969) focused on haemoglobin, serum transferrin and blood types. Based on these markers, Møller (1969) concluded that two populations of cod inhabit Norwegian waters, i.e. Arcto-Norwegian (AN) and Norwegian coastal (NC) cod. On the basis of his observations, Møller (1969) hypothesized that the AN and the NC forms of cod represent sibling species. This has been described as the "historical" hypothesis (Árnason & Pálsson 1996). Furthermore, Frydenberg & al. (1965) reported considerable subdivision of cod into several local populations, based on haemoglobin. Williams (1975) argued that the differences described by Møller (1969) were the result of strong natural selection (i.e. the "selection" hypothesis). Later studies have all shown substantial variation in frequency distributions of cod haemoglobin genotypes in Norwegian waters (Gjøsæter & al. 1992; Dahle & Jørstad 1993; Nordeide & Pettersen 1998) in line with the historical hypothesis.

Sick (1965) demonstrated differences in haemoglobin frequency between the Baltic and the Danish Belt Sea, an area characterized by a steep salinity gradient from near oceanic salinities in the North Sea to the brackish Baltic Sea. His main conclusion was that there were two well-defined cod populations (Belt Sea and Baltic Sea) with a narrow zone of pure mechanical mixing. These findings were challenged by Moth-Poulsen (1982), as allozyme studies indicated the presence of four distinct populations in this area and a potential intraspecific hybrid zone. Recently, this was supported (Nielsen & al. 2003) in a study based on DNA microsatellites that indicated a hybrid zone in the transition area between the North Sea and the Baltic proper. In the present study, we investigated the haemoglobin frequencies in cod in the transient area found in these previous studies.

Fyhn & al. (1994) documented a new Hb-I polymorphism using isoelectric focusing (IEF) electrophoresis, and described new phenotypes. They distinguished the AN cod from NC cod by an absence of double bands for both *Hb-I(1/2)* and *Hb-I(2/2)* genotypes. The

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Table 1. Summary of the Atlantic cod samples.

Sample number	Location	n	Sample position	Date	Length (cm)	Length interval (minimum–maximum, cm)	Age (years)	Age interval (minimum–maximum)	Otolith classification (%NC)	Females (%)
1	Balsfjorden	46	69°17′N, 19°54′E	14 March 1994	NA	NA	7.4	6–12	66.7	NA
2	Eidsfjorden	23	68°50′N, 15°05′E	16 March 1994	68.9	53–87	6.4	4–11	100	NA
3	Henningsværstraumen 1	71	68°10′N, 14°05′E	19 March 1994	71.5	52–115	6.9	5–12	48.1	NA
4	Henningsværstraumen 2	85	68°10′N, 14°05′E	21 March 1994	81.6	57–108	7.9	5–12	28.3	NA
5	Henningsværstraumen 3	95	68°10′N, 14°05′E	22 March 1994	79.3	53–115	7.8	4–12	31.0	NA
6	Henningsværstraumen 4	96	68°10′N, 14°05′E	24 March 1994	81.3	56–119	7.8	4–12	21.9	NA
7	Henningsværstraumen 5	96	68°10′N, 14°05′E	25 March 1994	85.7	62–122	8.6	5–12	9.6	NA
8	Storegga	20	63°17′N, 5°54′E	20 April 1994	NA	NA	5.0	2–14	89.5	NA
9	Buagrunnen	102	63°07′N, 6°51′E	21 April 1994	NA	NA	4.3	3–8	98.7	NA
10	Risør 1	101	58°45′N, 9°10′E	4 May 1994	42.3	27–92	2.6	1–8	NA	53
11	Risør 2	90	58°45′N, 9°18′E	4 September 1994	37.8	13–86	2.0	1–5	NA	36
12	Øresund	66	55°54′N, 12°44′E	12 October 1994	34.2	21–71	1.9	1–5	NA	56
13	Lillebelt	129	55°15′N, 10°05′E	13 October 1994	32.9	21–65	2.1	1–3	NA	57
14	Øygarden 1	97	60°30′N, 4°44′E	25 March 1994	29.7	19–48	1.6	1–4	NA	NA
15	Øygarden 2	92	60°30′N, 4°44′E	7 April 1994	64.2	41–91	NA	NA	NA	25

NA – Not available; NC – Norwegian Coastal cod.

existence of such subtypes in other parts of the cod distribution areas is imperfectly known, but could offer additional markers for use in population studies. These phenotypes might also be of some value in breeding programmes in the emerging aquaculture of this species, as a haemoglobin genotype-dependent growth rate has been indicated (Mork & al. 1984a, b; Nævdal & al. 1992), where the genotype with the highest growth (*Hb-I(2/2)*) also has the highest oxygen affinity (Brix & al. 1998) and the highest competitive performance (Salvanes & Hart 2000).

In the present paper, observations on genotype frequency distributions in cod from the Norwegian coast and from the Danish Belt Sea are presented and compared with those described three decades ago. In addition, subtype variations in haemoglobin are described and preliminary observations on subtype frequencies are given. The results are discussed in relation to application in studies of natural cod populations and to possible use in aquaculture.

MATERIAL AND METHODS

Sampling

Blood samples from cod (n = 1209, Table 1, Fig. 1) were collected along the Norwegian coast (seven sampling sites) and from the Danish Belt Sea (two sampling sites) in the period March–October 1994. All samples were kept on ice until analysed within 96 h after sampling. When possible, biological information (length and sex) was collected and otoliths were sampled for age determination. For the mid- and north Norway samples (sample numbers 1–9), visual inspection of the otoliths was used to classify each individual as NC or AN cod (Rollefsen 1934). Most samples were collected close to the estimated spawning time in each area. To follow the mixing of AN and NC cod at known spawning grounds, samples from Henningsværstraumen were gathered during a 7 day consecutive period in the spawning season (Table 1) and these samples were kept separate during further analysis. Samples from Risør (south Norway) were sampled at a different time of the year and were kept separate during the analysis. Samples from Øygarden (west Norway) were not pooled, as these represent two independent samples collected during a 2 week period.

Agar gel electrophoresis (AGE)

All samples were analysed by AGE and the method described by Sick (1961) was applied with modifications (Jørstad 1984). Smithies buffer, pH 8.6 was used as an electrode buffer, and diluted 1:1 with distilled

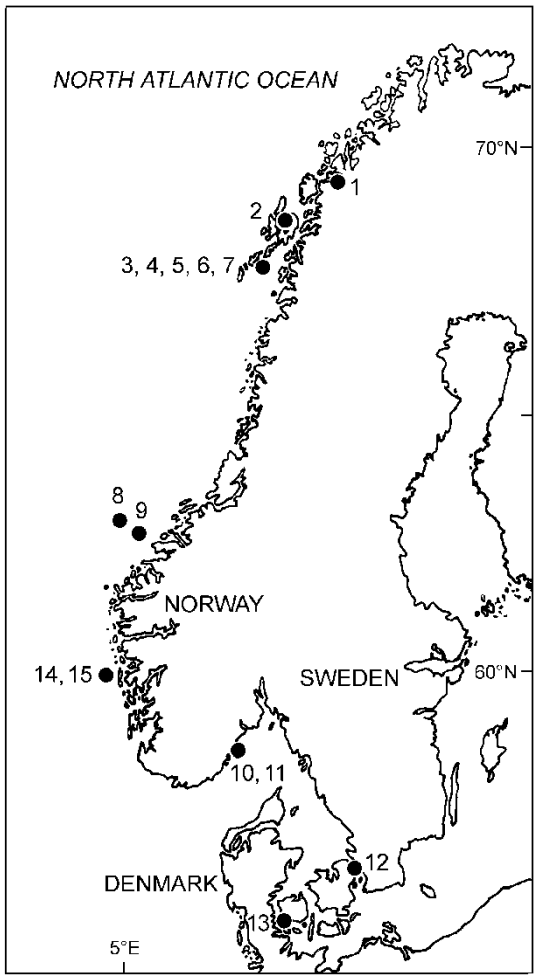

Fig. 1. Sampling locations of Atlantic cod in Norwegian waters and in the Danish Belt Sea (see Table 1 for details).

water for the gel buffer. A 2% agar concentration was used in the gels (Agar Noble, Difco Laboratories). The samples were run at 200 V (20 mA) for 90 min. During the run, the gels were cooled with ice water circulating in a cooling plate beneath the agar gel. The gels were stained with Brilliant Blue G Quick stain in perchloric acid and then destained by diffusion (14% acetic acid, 7% methanol) overnight.

IEF

IEF was carried out on all samples in accordance with the instruction manuals using pre-cast slab gels (Ampholine PAG plates, Pharmacia, pH 5.5–8.5) with modifications described by Fyhn & al. (1994). The gels were pre-focused for 30 min. After pre-focusing, filter papers containing haemolysate were applied on the anodic side of the gel. The filter papers were removed after 10 min and the gel was run for 110 min or until the haemoglobin bands were clearly visible. The voltage used was 1600 V. At the anode side, a 0.4 mol l^{-1} HEPES buffer (28 ml of distilled water and 2.8 g HEPES) was used, and at the cathode side a 0.1 mol l^{-1} NaOH buffer (0.4 g NaOH dissolved in distilled water up to 100 ml). The same staining procedure was used as for AGE and the haemoglobin components were identified manually using a transmitted light. In AGE a constant pH is applied, but in IEG a pH gradient is applied so that each protein component can be studied at its isoelectric point (i.e. where the protein is neutral). These two methods were used in combination when studying both main and subcomponents of proteins in the present study.

Statistical methods

The calculations and statistics were performed using BIOSYS-1 (release 1.9, Swofford & Selander 1981). A chi-squared goodness of fit test (Zar 1984) was performed on each sample to test the accordance between observed and expected Hardy–Weinberg equilibrium. Levene's formula for small samples was used as the chi-squared test is sensitive to low expected genotype frequency. This test was also used to test for heterogeneity within and between sampling sites. To describe the genetic stability within each sample site, Selander's D test statistic for heterozygote deficiency or excess was calculated for each sample site.

The range of genetic distance was calculated by pairwise comparisons of the sample units (Swofford & Selander 1981) using Nei's (1972, 1987) genetic distance measure. A hierarchical cluster analysis (Swofford & Selander 1981) was performed using the unweighted pair-group method with arithmetic averaging (UPGMA). Bonferroni corrections (Johnson & Field 1993) of the significance level ($\alpha = 0.05$) were applied when testing for significant departures from Hardy–Weinberg expectations.

Results

AGE

By AGE, the three different electrophoretic patterns described by Sick (1961) were found: *Hb-I(1/1)*, *Hb-I(1/2)* and *Hb-I(2/2)* (most anodic), and interpreted as the two homozygotes and the heterozygote in a two allele system (Fig. 2A). The chi-squared goodness of fit test did not reveal any significant difference between

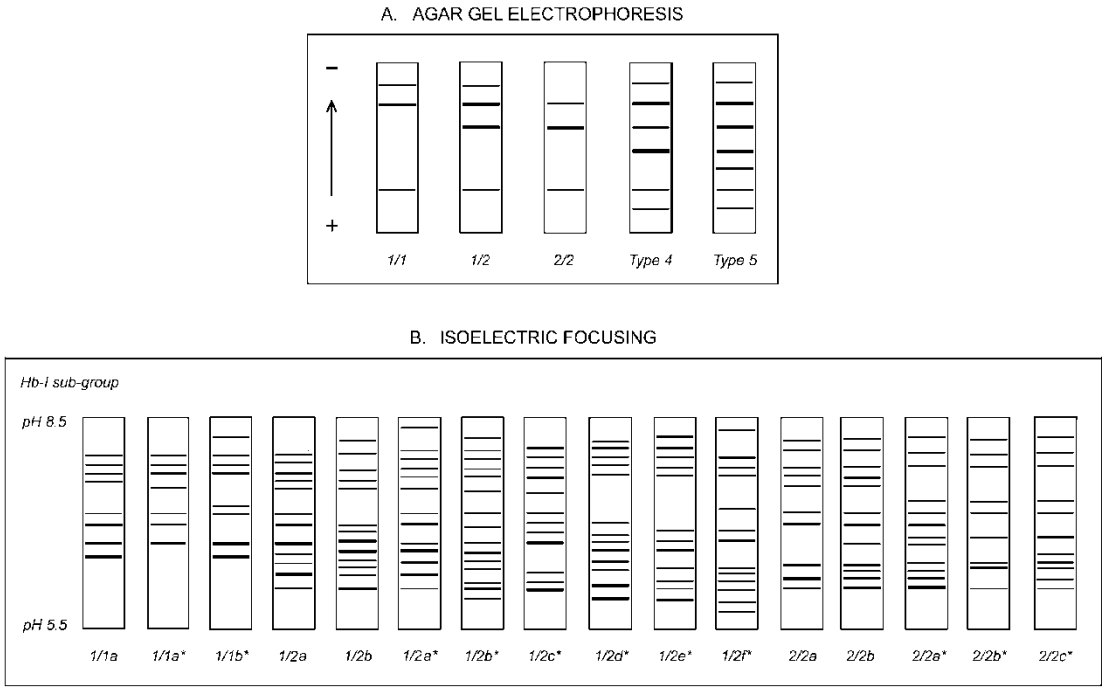

Fig. 2. A schematic representation of the patterns of components from agar gel electrophoresis and isoelectric focusing of haemoglobins of cod. (A) For agar gel electrophoresis, the origin is at the anode (+) and the proteins migrate towards the cathode (–), as indicated by the arrow. 1/1, 1/2 and 2/2 show the genotypes *Hb-I(1/1)*, *Hb-I(1/2)* and *Hb-I(2/2)*, respectively. Types 4 and 5 show the pattern of the "rare" haemoglobin types found in the samples from west and north Norway. (B) For isoelectric focusing, the origin is approximately 2 cm from the anode side (i.e. at pH 5.5) and the proteins migrate towards their isoelectric point on the pH gradient. Subtypes marked with an asterisk are only found in Danish waters.

Table 2. Observed and expected (in parentheses) phenotypic distributions and allelic frequencies of the *Hb-I* locus analysed with agar gel electrophoresis in different cod populations.

Sample number	n	Genotype					Allele frequency		χ^2	p
		Hb-I(1/1)	*Hb-I(1/2)*	*Hb-I(2/2)*	*4*	*5*	*Hb-I(1)*	*Hb-I(2)*		
1	46	6 (6.2)	22 (21.7)	18 (18.2)			0.37	0.63	0.01	0.92
2	23	1 (1.5)	10 (9.1)	12 (12.5)			0.26	0.74	0.26	0.61
3	71	6 (5.1)	26 (27.7)	37 (36.1)	1	1	0.27	0.73	0.28	0.60
4	85	3 (3.4)	28 (27.2)	52 (52.4)	1	1	0.20	0.80	0.07	0.79
5	95	4 (3.6)	29 (29.9)	61 (60.6)		1	0.20	0.80	0.08	0.77
6	96	1 (2.4)	29 (26.1)	66 (67.4)			0.16	0.84	1.19	0.28
7	96	1 (1.1)	19 (18.8)	74 (74.1)	2		0.11	0.89	0.02	0.90
8	20	3 (3.7)	11 (9.7)	5 (5.7)	1		0.45	0.55	0.39	0.53
9	102	11 (10.7)	44 (44.7)	46 (45.7)		1	0.33	0.67	0.02	0.88
10	101	38 (36.7)	46 (48.6)	17 (15.7)			0.60	0.40	0.28	0.60
11	90	26 (30.5)	53 (44.0)	11 (15.5)			0.58	0.42	3.82	0.06
12	66	30 (29.9)	29 (29.2)	7 (6.9)			0.67	0.33	0.01	0.95
13	129	43 (44.1)	65 (62.9)	21 (22.1)			0.59	0.41	0.15	0.70
14	97	37 (39.5)	50 (45.0)	10 (12.5)			0.64	0.36	1.23	0.27
15	92	27 (28.5)	48 (45.1)	16 (17.5)	1		0.56	0.44	0.39	0.54

n – Sample size.
χ^2_1-statistic for deviations from expected Hardy–Weinberg phenotypic distributions and p values for the χ^2_1-statistic. Rare main groups (4 and 5) are listed in this table but not included in the calculations.

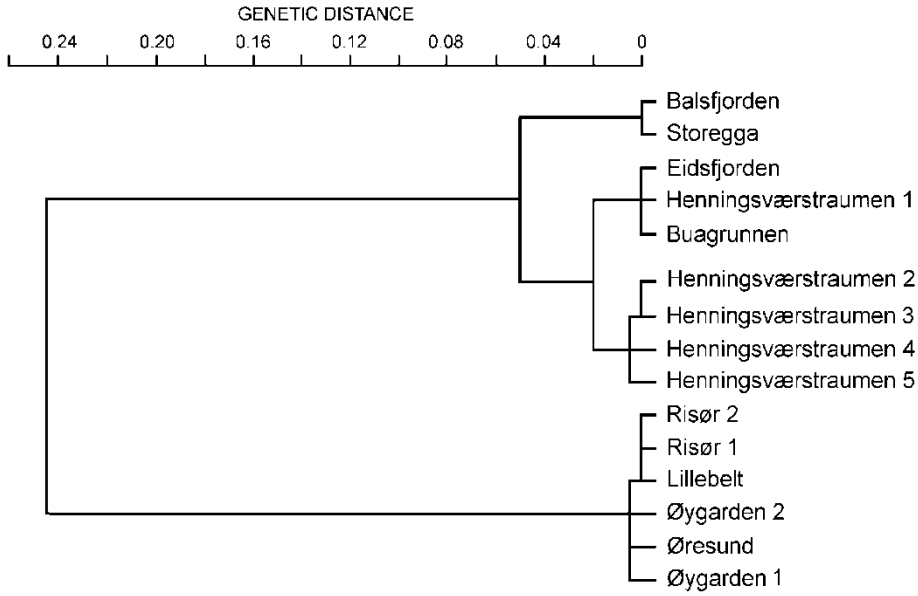

Fig. 3. An unweighted pair-group method with arithmetic averaging (UPGMA) dendrogram of Nei's (1972) genetic distance matrix among the 15 sampling sites analysed with agar gel electrophoresis in the present study.

observed and expected Hardy–Weinberg distributions in any sample (Table 2). One borderline case was found (Risør 2, $p = 0.06$), but when corrected for multiple tests ($\alpha = 0.05/15 = 0.003$) the deviation was no longer significant. Likewise, a comparison of observed and expected heterozygosities and an exact probability test revealed no significant heterozygote deficiencies in the samples.

In addition to the three common types, two rare genotypes (types 4 and 5 in Fig. 2A) were found at low frequencies in samples from west and north Norway (Table 2). These genotypes have additional bands on the anodic side of the usual Hb-I bands, and they have been described as the result of variation at a locus that controls another polypeptide chain in the haemoglobin molecule (Manwell & Baker 1970). They are not treated further here.

The *Hb-I** allele frequencies were significantly different ($\chi^2_{14} = 387.4$, $p < 0.001$, Table 2) among the samples, showing that different populations were sampled. The frequency of *Hb-I(1)* was low in the northern samples and, with the exception of two Henningsværstraumen samples, an apparent cline with increasing frequency of *Hb-I(1)* from north to south was observed (Table 2), in line with previous studies and first described by Frydenberg & al. (1965). The highest frequency of *Hb-I(1)* was observed in one of the samples from the Danish Belt Sea (Table 2). The samples from Henningsværstraumen were collected in the spawning season in one of the main spawning areas of AN and NC cod. A homogeneity test of these five successive samples showed that the frequency of the *Hb-I** allele differed among these samples ($\chi^2_4 = 15.5$, $p < 0.01$, Table 2). The dendrogram constructed from the matrix of pairwise genetic distance values (UPGMA) revealed two clearly distinct groups, as samples from north and mid-Norway represented one group, whereas west and south Norway and the Danish Belt Sea samples represented another (Fig. 3).

No age-, size- or sex-dependent frequencies were observed ($p > 0.15$).

IEF

On IEF gels, haemoglobin of the genotypes *Hb-I(1/2)* and *Hb-I(2/2)* from Norway displayed between 10 and 13 bands (Fig. 2B), whereas the *Hb-I(1/1)* genotype displayed eight bands (Fig. 2B). Furthermore, in *Hb-I(1/2)* and *Hb-I(2/2)*, the position and strength of the band components on the IEF gels varied highly. No variation was found in the *Hb-I(1/1)* genotype in samples from Norway. Accordingly, the *Hb-I(1/1)*, *Hb-I(1/2)* and *Hb-I(2/2)* genotypes could be divided by the position and strength of the IEF band patterns into five subtypes: *Hb-I(1/1)a*, *Hb-I(1/2)a*, *Hb-I(1/2)b*, *Hb-I(2/2)a*, and *Hb-I(2/2)b* (Fig. 2B). Here, "a" is used

Table 3. Observed numbers of haemoglobin subtypes in all 15 sample units. See text for details on classification.

Sample number	*Hb-I(1/1)*			*Hb-I(1/2)*								*Hb-I(2/2)*				
	1/1a	*1/1a**	*1/1b**	*1/2a*	*1/2b*	*1/2a**	*1/2b**	*1/2c**	*1/2d**	*1/2e**	*1/2f**	*2/2a*	*2/2b*	*2/2a**	*2/2b**	*2/2c**
1	6			21	1							16	2			
2	1			10								11	1			
3	6			25	1							36	1			
4	3			28	1							49	3			
5	4			29								61				
6	1			29								85	1			
7	1			19								74				
8	3			11								5				
9	11			42	2							41	5			
10	38			42	4							16	1			
11	26			49	4							8	3			
12		30				6	5	5	5	3	4			3	1	
13		42	1			34	11	8	6	5				10	5	4
14	37			46	4							8	2			
15	27			38	10							14	2			
Total	164	72	1	389	27	40	16	13	11	8	4	404	21	13	6	4

for the most common subtype and "b" for the second most common (Table 3).

The samples from the Danish Belt Sea displayed different band patterns compared with the Norwegian samples. Here, six *Hb-I(1/2)* and three *Hb-I(2/2)* subtypes were found (Fig. 2B). In addition, a rare *Hb-I(1/1)* subtype was found in one individual from Lillebelt [*Hb-I(1/1)b** in Fig. 2B]. These subtypes are named: *Hb-I(1/1)a**, *Hb-I(1/1)b**, *Hb-I(1/2)a**, *Hb-I(1/2)b**, *Hb-I(1/2)c**, *Hb-I(1/2)d**, *Hb-I(1/2)e**, *Hb-I(1/2)f**, *Hb-I(2/2)a**, *Hb-I(2/2)b**, and *Hb-I(2/2)c**. Here also, "a" is used for the most common subtype, "b" for the second most common, etc. The observed frequency distributions of these subtypes are shown in Table 3. The haemoglobin subtypes were represented in all sample units apart from samples 5, 7 and 8 (Table 3). The subtypes were not found in individuals classified by otoliths as AN. Accordingly, all AN individuals (n = 604) were removed when testing for significant differences in the frequencies of the subtypes among the Norwegian sampling units. The remaining individuals (n = 605) were classified as belonging to north, mid-, west or south Norway in accordance with the results seen in the main band polymorphism at the *Hb-I* locus. Using this classification, a significantly different subtype frequency was found ($\chi^2_6 = 15.3$, $p < 0.05$, Table 4) among the samples of NC cod.

Table 4. Observed frequencies of *Hb-I* subtypes analysed with isoelectric focusing in different cod populations in Norwegian waters, after removing all Arcto-Norwegian cod based on otolith examination. The samples were grouped into four categories (north, mid-, west or south Norway) based on geographical location and agar gel electrophoresis classification of the samples.

Group	*Hb/I(1/2)a*	*Hb/I(1/2)b*	*Hb/I(2/2)a*	*Hb/I(2/2)b*
A	0.40	0.01	0.56	0.03
B	0.50	0.02	0.43	0.05
C	0.68	0.11	0.18	0.03
D	0.72	0.06	0.19	0.03

A – North Norway (sample units 1–7); B – Mid-Norway (sample units 8 and 9); C – West Norway (sample units 14 and 15); D – South Norway (sample units 10 and 11).

DISCUSSION

The present data are in line with many other studies that have indicated a subdivision of Atlantic cod in Norwegian waters into AN and NC (Frydenberg & al. 1965; Møller 1966, 1968, 1969; Jørstad 1984; Dahle 1991; Fyhn & al. 1994; Fevolden & Pogson 1995, 1997). Pogson & Fevolden (2003) used the pantophysin (*Pan* I = *Syp* I) locus of Atlantic cod to examine the role of contemporary selection in maintaining significant allele frequency differences. Their results failed to support either the historical (Møller 1969) or the selection (Williams 1975) hypotheses, but were consistent with a recent separation of coastal and Arctic populations rendered more visible by the action of diversifying selection in the two environments. Based on their findings, Pogson & Fevolden (2003) suggested that AN and NC populations may be more independent than indicated by previous studies.

But what evolutionary mechanism could be acting on these genes causing the divergence between genotypes

and populations? Several mechanisms can be postulated. First, this might be an example of balanced polymorphism. Genotype-dependent differences in physiological performance seen in earlier studies on Atlantic cod (Karpov & Novikov 1980; Nævdal & al. 1992) may indicate differential selection pressure on the controlling genes. However, such selection pressure may be balanced by other factors. Imsland (1999) investigated age at first maturation among the haemoglobin genotypes of turbot. For both sexes, fewer fish of the *Hb-I(1/1)* and *Hb-I(1/2)* genotypes matured at 2 years of age compared with the *Hb-I(2/2)* genotype. These findings were in accordance with genotypic growth properties (Imsland & al. 1997, 2000), and differences in oxygen affinity (Imsland & al. 1997; Samuelsen & al. 1999). Taken together these findings may imply a balanced polymorphism of the haemoglobin in turbot. Whether there exists a similar balancing system in cod is still unresolved. However, Salvanes & Hart (2000) compared the competitive performance of cod of the three main Hb-I genotypes. Randomly chosen 1-year-old cod were tested for individual responses to prey offered sequentially and found that the most successful fish were usually among the first to feed and tended to possess haemoglobin genotype *Hb-I(2/2)*. Their findings indicate that there exists a link between genotypic growth and feeding behaviour. Taken together, the differences in growth, oxygen affinity and competitive performance in the cod haemoglobin genotypes could be viewed as an example of balanced polymorphism.

Second, differences in haemoglobin frequencies could be an adaptation to variable environmental conditions. Atlantic cod is the major demersal fish resource distributed on the continental shelves and banks on both sides of the North Atlantic Ocean (Imsland & Jónsdóttir 2003), distributed in a variety of temperature conditions (e.g. Brander 1995). Brix & al. (1998) found the highest oxygen affinity for *Hb-I(2/2b)*, suggesting that this type is a more efficient oxygen binder at high temperatures, supporting the results of Fyhn & al. (1994) that this type is more restricted to coastal and warmer water and thus a better marker of the coastal cod population. Our data are in line with these findings, as we found this type more restricted to coastal and warmer water. This might suggest that the sub-band polymorphism could be used as a population marker in cod and that the different subgroups represent adaptation to specific environmental conditions (here temperature). In the present study, the haemoglobin subtype system varied between sampling groups in Norwegian waters (Table 4). Furthermore, the totally different subtype system found in the Danish Belt Sea (Fig. 2B, Table 3) might suggest that the sub-band polymorphism could be used as a population marker in cod and that the different subtypes represent adaptation to specific environmental conditions (possibly temperature). In turbot, six haemoglobin subtypes were recently described (Imsland & al. 2003) and indicated that differences in frequencies of these subtypes varied among the sampling sites in line with different temperature ranges. Furthermore, the sub-band polymorphism in cod could also represent adaptation to other environmental conditions, e.g. oxygen availability, as differences are found in oxygen affinity between the main groups and one subtype in Atlantic cod (Brix & al. 1998) and between the three main haemoglobin genotypes in turbot (Imsland & al. 1997; Samuelsen & al. 1999). However, as long as the inheritance mode of this system is unresolved, utilization of the subtypes as population markers should be done with caution.

Similar sub-bands as reported in the present study have been documented by Fyhn (1991) and Fyhn & al. (1994) for cod, and suggested as a marker for NC cod versus AN cod populations, as sub-bands were not found in cod from the Barents Sea or the Faeroe Bank. In their study, Fyhn & al. (1994) documented five subtypes, of which two were characterized by sub-bands. In the present study, we report a high variation in the sub-bands of cod haemoglobin from the Danish Belt Sea and suggest 11 new subtypes that are only represented in this area. Furthermore, a cline in the frequencies of the subtypes, as higher frequencies in west and south Norway compared with Lofoten and north Norway, is indicated. Brix & al. (1998) investigated the oxygen affinity of four of the haemoglobin genotypes found in cod [*Hb-I(1/1)*, *Hb-I(1/2)*, *Hb-I(2/2)*, and *Hb-I(2/2b)*]. They found the highest oxygen affinity for *Hb-I(2/2b)*, suggesting that this type is a more efficient oxygen binder at high temperatures, supporting the results of Fyhn & al. (1994) that this type is more restricted to coastal and warmer water and, thus, a good marker for the coastal cod population.

The fact that there seems to be a haemoglobin genotype-dependent variation in physiological traits (Nævdal & al. 1992; Brix & al. 1998) and haemoglobin polymorphism in cod might be utilized in aquaculture production. Similar results have been found for turbot, and Imsland & al. (2000) suggested that a genotype-dependent growth rate in turbot variation between the haemoglobin genotypes might reflect differences in metabolic capacity rather than metabolic efficiency. This is supported by the results of Imsland & al. (1997) and Samuelsen & al. (1999), who showed that *Hb-I(2/2)* binds dissolved oxygen in the water more efficiently, thus increasing the metabolic capacity

of the fish. This suggests that the co-variation between haemoglobin genotypes and growth may be of some value in future breeding programmes of turbot. Imsland (1999) and Imsland & al. (2000) suggested that the haemoglobin variations may represent quantitative trait loci in turbot. Much the same is true for Atlantic cod and the possible use of haemoglobin variation could be included in future breeding programmes of Atlantic cod.

Differences in physiological and behavioural properties of the cod haemoglobin genotypes (Karpov & Novikov 1980; Nævdal & al. 1992; Salvanes & Hart 2000) and differences in age at maturity and within-season spawning time (Mork & Sundnes 1985 and references therein) are probably best explained by natural selection (Mork & Giæver 1999). 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 & al. 1965; Møller 1968) with corresponding analyses in recent years (Jørstad & Nævdal 1989; Gjøsæter & al. 1992; Dahle & Jørstad 1993; Fyhn & al. 1994; present study). This does not disprove the action of selection forces, but it does show that the stability of the gene frequencies is high enough to use these frequencies as genetic population markers in cod (Jørstad & Nævdal 1989). However, it would be very interesting to monitor these gene frequencies in the near future, especially with concerns about global climatic change and its effect on cod stocks (Pörtner & al. 2001). As the distribution of these genotypes seems to mirror sea temperatures (Petersen & Steffensen 2003), changes in sea temperatures should be reflected in changes in the geographical cline (i.e. present study) of *Hb-I* frequencies. The results from the study of Petersen & Steffensen (2003) indicate that increasing water temperatures will possibly increase the frequency of *Hb-I(1/1)*, as the preferred sea temperature for this genotype was found to be 15.4 ± 1.1 °C compared with 8.4 ± 1.5 °C for *Hb-I(2/2)*. Other factors may also contribute to changes in gene frequencies, e.g. directed fisheries. Such fishery policy would tend to remove the "fast growing" *Hb-I* genotype (i.e. *Hb-I(2/2)*; Nævdal & al. 1992), as these will reach minimum fishery size before the two other genotypes. We recommend that possible temporal changes in *Hb-I* frequencies be studied, with special focus on these aspects.

The distribution of the subtypes reported here should also be studied on samples from other parts of the species distribution. Preferentially detailed studies should be carried out to reveal the genetic background and to determine whether this system can be used as a correlated trait to select for an improved growth rate of cod. A fruitful way to continue this research might be to conduct controlled experiments where performance (growth, feeding behaviour, etc.) and environmental factors (e.g. temperature, oxygen, photoperiod, predation risk and food availability) are studied simultaneously for the haemoglobin genotypes.

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