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Genetic variation in Atlantic cod (*Gadus morhua* L.): A quantitative estimate from a Norwegian coastal population*

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Samples of blood, eye, heart, liver and skeletal muscle from 80 specimens of Atlantic cod (*Gadus morhua* L.) collected from a restricted area of Trondheimsfjorden, Norway, were examined electrophoretically. Analyses of 20 proteins resolved 38 loci, 30 of which were considered potentially useful for population genetic surveys. We describe the electrophoretic expression and tissue predominance of these loci. The proportion of polymorphic loci (0.99 criterion) was 0.30 and average heterozygosity 0.082±0.029.

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The Atlantic cod (*Gadus morhua* L.) is the economically most important species in the north-east Atlantic fisheries. The applicability of electrophoretic techniques as a valuable tool for stock identification and management was early recognized for this species (SICK 1961, 1965a, b; FRYDENBERG et al. 1965; MØLLER 1966). Those and subsequent studies (e.g., JAMIESON 1975; CROSS and PAYNE 1978; and references therein) indicated a complex population structure in the cod and stressed the need for detailed studies on the amount and distribution of genetic variation among different subunits of the species. As pointed out by several authors (e.g., NEI and ROYCHOUDHURY 1972; NEI 1973, 1975; LEWONTIN 1974) such studies should be based on large numbers of loci to provide reliable estimates of evolutionary and genetic relationships among subunits as well as of fundamental population genetics parameters such as average heterozygosity and the frequency of polymorphic loci.

Although there have been several studies on the cod describing the distribution of allelic variants at one or a few selected polymorphic loci, there has been no attempt to analyze in detail the amount and distribution of genetic variability in this species using a large number of loci, polymorphic as well as monomorphic. It was the purpose of the present study to screen for a large number of electrophoretically detectable loci that could be used on a routine basis for population genetic surveys in the cod. We present the results of such an analysis of a Norwegian coastal cod population; the loci described in the present paper will be used in future reports on the population structure of the Atlantic cod.

Material and methods

Eighty cod of various size and age (30–50 cm, 2–5 years old as estimated from otolith reading (ROLLEFSEN 1933)) were captured with shrimp trawl in January 1979 in Borgenfjorden, a shallow part of Trondheimsfjorden, Norway (Fig. 1). Blood samples were drawn from the heart with a heparinized syringe and centrifuged, whereafter the serum fraction was removed and frozen. Tissue samples of eye, heart, liver and skeletal muscle were immediately cut•out and kept frozen at −35°C.

Sample preparation and horizontal starch gel electrophoresis were conducted as described by UTTER et al. (1974). The fat extraction was removed from the liver supernatant before sample applications on the gel. Electrophoretic conditions and specific enzyme staining procedures followed

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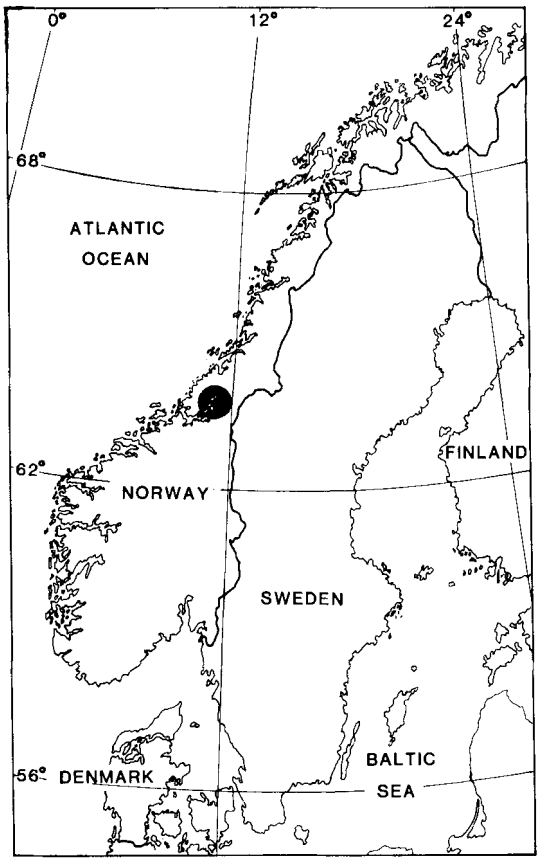

Fig. 1. Map of the Scandinavia with sampling area indicated.

ALLENDORF et al. (1977). A total of 19 enzymes were stained for (Table 1), and two buffer system were used:

A. (RIDGWAY et al. 1970):
Gel: 0.03 M Tris—0.005 M citric acid, pH 8.5.
Electrode: 0.06 M Lithium hydroxide—0.3 M boric acid, pH 8.1.
Gels contained 99 % gel buffer and 1 % electrode buffer.

B. (CLAYTON and TRETIAK 1972):
Gel: 0.002 M citric acid, pH 6.0.
Electrode: 0.04 M citric acid, pH 6.1.
Both buffers are pH adjusted with N-(3Aminopropyl)-morpholine.

In addition to the starch gel electrophoresis, the haemoglobins were analyzed on agar gels as described by SICK (1961).

In the preliminary analyses all the 19 enzymes were tested on all combinations of tissue (eye, liver, heart and muscle) and buffer system for a minimum of 30 specimens; all fish were analyzed for all loci permitting reliable scoring using the best tissue—buffer combination

When interpreting the electrophoretic banding patterns genetically we follow the prinicples which have been previously outlined with special reference to brown trout (*Salmo trutta* L.) population data (ALLENDORF et al. 1977). Notations about enzyme subunit structure are based on DARNALL and KLOTZ (1975). We use the system of nomenclature proposed by ALLENDORF and UTTER (1979): An abbreviation is chosen for each protein; these same abbreviations in italics represent the loci coding for these proteins. If multiple forms of an enzyme are found, a hyphenated numeral is included; the form with the least anodal migration is designated one, the next two and so on. Allelic variants are designated according to their relative electrophoretic mobility. One allele (generally the most common one) is arbitrarily designated *100*. Other alleles are then assigned a numerical value representing the electrophoretic mobility of their gene products relative to the *100*-allele. Thus, an allele of the least anodal LDH locus coding for an enzyme migrating one-half as far as the common allele product would be designated *LDH-l(50)*.

Results and discussion

Interpretation of zymograms

The main results of the electrophoretic analyses are compiled in Table 1. A minimum of 35 enzyme coding loci were identified and 27 of them were considered usable in routine population surveys. In all cases our estimates of the number of monomorphic loci are conservative and reflect the minimum number of loci controlling the expression of each particular enzyme (cf. ALLENDORF et al. 1977). For many of the enzymes the products from specific loci predominated in different tissues. However, all the polymorphic enzyme loci listed may be satisfactorily scored from either liver or skeletal muscle extracts.

Generally, freezing and thawing did not reveal isozyme patterns different from those obtained from fresh extracts. Repeated analyses after storage of tissues for approximately 20 months at −35°C were performed for all the polymorphic loci. Except for SDH, which could not be reliably scored after long storage due to loss of activity, the zymograms from the reanalyses were consis-

Table 1. Enzymes stained for, with abbreviations, locus designations and Enzyme Commission (E. C.) number. Conditions for best electrophoretic results are also given. E = eye, H = heart, L = liver, M = skeletal muscle, R = red cells and S = serum. Comments: C = cathodal mobility, N.A. = no activity and U. R. = unsufficient resolution.

Protein	E.C.No.	Abbreviation	Locus designation (if multiple)	Best buffer system	Ranking of tissues with respect to activity/resolution	Polymorphic	Variant alleles	Comments
Aspartate aminotransferase	2.6.1.1	AAT	*1*	B	M L E	No		C.
			2	B	L E	No		
			3	B	L	No		
			4	B	M			U.R.
Alcohol dehydrogenase	1.1.1.1	ADH						N.A.
α-glycerophosphate dehydrogenase	1.1.1.8	α-GPDH						N.A.
Esterase		EST	*1*	A	S			U.R.
			2	A	M E	No		
			3	A	M L E	No		
			4	A	L			U.R.
			5	A	M L	Yes	*93*	
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH	*1*	B	L	No		C
			2	B	M H	No		C.
			3	B	E			U.R.
Glycerol dehydrogenase	1.1.1.6	GDH		B	L	No		C.
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH						U.R.
Haemoglobin		HB	*1*	*	R	No		
			2	*	R	Yes	*83*	
			3	*	R	No		
Isocitrate dehydrogenase	1.1.1.42	IDH	*1*	B	L H	Yes	*140*	
			2	B	M	No		
Lactate dehydrogenase	1.1.1.27	LDH	*1*	B	L	No		
			2	B	M H E L	No		
			3	B	H E L M	Yes	*70*	
Malate dehydrogenase	1.1.1.37	MDH	*1*	B	L			C. U.R.
			2	B	M E	Yes	*280*	
			3	B	L M E H	Yes	*60*	
Malic enzyme	1.1.140	ME		B	M	No		
Peptidase	3.4. .	PEP	*1*	A	M H	No		
			2	A	L H	No		
			3	A	M L E H	No		
Phosphoglucoisomerase	5.3.1.9	PGI	*1*	A	M	Yes	*135, 65*	
			2	A	M L E H	Yes	*90*	
Phosphoglucomutase	2.7.5.1	PGM		A	M L H	Yes	*80, 70*	
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGDH		B	L M	No		
Phosphomannose isomerase	5.3.1.8	PMI		B	M			U.R.
Sorbitol dehydrogenase (Polyol dehydrogenase)	1.1.1. .	SDH	*1*	B	L	No		C.
			2	A	L	Yes	*80*	
Superoxide dismutase	1.15.1.1	SOD		A	M	No		
Xanthine dehydrogenase	1.2.1.37	XDH		A	M			U.R.

* Following SICK (1961)

tent with those obtained initially. The *IDH-1* banding patterns also became weaker and it appears that liver tissue preferably should be stored at ultra-low temperatures.

Our interpretation of a genetic basis for the polymorphisms were always supported (except for SDH, which is only expressed in liver) by consistency of banding patterns in at least two tissues (cf. ALLENDORF and UTTER 1979). Further, electrophoretic banding patterns at polymorphic loci were always consistent with those expected from the quaternary structure of each particular enzyme (except for esterase, the quaternary structure of which is not known in the cod). No activity was obtained when staining for ADH and α-GPDH and no conclusion concerning their genetic control could therefore be drawn.

GDH, 6PGDH, ME, SOD. — Each of these enzymes were represented by a single invariant band that in each case was assumed to reflect the expression of a single locus. Additional zones, potentially reflecting SOD activity, appeared after prolonged staining; however, as these zones could not be reliably scored we have chosen to indicate SOD as being coded for by a single locus.

AAT. — A total of four zones of activity with different tissue predominance were observed when staining for this enzyme; they were interpreted as representing the expression of four loci. The most anodal zone of activity (*AAT-4*) showed some variability potentially reflecting genetic variation; however, the resolution was too poor to permit reliable scoring.

EST. — Five zones of activity were observed when staining for this rather unspecific group of enzymes; they were assumed to reflect the expression of five loci (*EST-1, 2, 3, 4* and *5*). Two of the zones (*EST-1* and *4*) were too poorly resolved to permit reliable scoring. NYMAN (1965) reported variability of the electrophoretic expression of cod serum esterases and JAMIESON (1975) reported a serum esterase locus segregating for three alleles among north-western Atlantic cod. In our sample variation was observed only at *EST-5* exhibiting single- and double-banded phenotypes consistently expressed in muscle and liver tissue and apparently reflecting allelic segregation at a locus coding for a monomeric enzyme. Several authors have pointed out the difficulties associated with the interpretation of esterase zymograms (e.g., UTTER et al. 1974; ALLENDORF et al. 1975; ALLENDORF and UTTER 1979), as the expression of these enzymes is frequently influenced by environmental and ontogenetic conditions. Our electrophoretic patterns were perfectly reproducible after at least 20 months of storage at −35°C, and the electrophoretic expression was consistent in liver and muscle tissue in all individuals.

GAPDH, PEP. — Each of these enzymes was represented by three invariant zones. The difference between the zones with regard to tissue predominance indicated that each enzyme was coded by three loci, which all appeared monomorphic in the present material.

G6PDH, PMI. — Staining for these enzymes resulted in fairly good activity but very poor resolution. Therefore, noting can be concluded about the number of loci involved or possible polymorphisms.

HB. — The electropherograms obtained when analyzing this protein appeared identical to those previously described by SICK (1961). According to SICK (1965a) and MANWELL and BAKER (1970) cod haemoglobins are synthesized by three loci. MANWELL and BAKER (1970) postulated polymorphism at two of them to explain the common HB variation (SICK 1961) and the rare haemoglobin patterns (FRYDENBERG et al. 1965), respectively. In the present nomenclature, *HB-1* will account for the rare HB patterns described by FRYDENBERG et al. (1965), while the common polymorphism (SICK 1961) is attributed to *HB-2*. Variation was only observed at the latter locus which segregated for two alleles, *HB-2* (*100* and *83*), apparently identical to the alleles designated Hbl^2 and Hbl^1, respectively, in SICK's (1961) nomenclature.

IDH. — Two loci with different tissue predominance were observed. The most anodal one, *IDH-2*, appeared monomorphic, while *IDH-1* segregated for a fast allele, *IDH-1(140)*, in addition to the common *100* allele. No heterodimers between *IDH-1* and *2* were observed. The electrophoretic pattern at *IDH-1* was consistent with that expected from allelic segregation at a locus coding for a dimeric enzyme. Best activity and resolution for *IDH-1* was usually obtained using liver tissue; prolonged storage occasionally resulted in additional bands but they did not prevent reliable scoring.

LDH. — The electrophoretic expression of this tetrameric enzyme has been described previously (e.g., ODENSE and LEUNG (1975) and MORK et al.

(1980)). There are three loci, designated *LDH-1, 2,* and *3* in the present nomenclature, which correspond to LDH_C, LDH_A, LDH_B as designated by ODENSE and LEUNG (1975) and MORK et al. (1980); they are predominantly expressed in liver, muscle, and heart, respectively. The most anodal locus (*LDH-3*) segregated for two alleles, *LDH-3(100* and *70)*, in the present material. The variant allele *LDH-3(70)* is identical to the allele designated $LDH_B^{B'}$ in previous reports (cf. MORK et al. 1980).

MDH. — Three loci coding for this dimeric enzyme were identified in the present study. The cathodal locus (*MDH-1*) was monomorphic while two alleles were observed at each of *MDH-2* and *3*. Heterodimers consisting of subunits from different loci were not observed but the variability pattern at each of the polymorphic loci *MDH-2* and *3* was perfectly consistent with that expected at locus coding for a dimeric enzyme, i.e., heterozygous fish expressed a three-banded pattern in the typical 1:2:1 intensity ratio.

PGI. — DANDO (1974) and CROSS and PAYNE (1978) have reported this dimeric enzyme to be coded for by two loci in the cod. In both of those previous investigations the locus that is predominantly expressed in muscle tissue (*PGI-1* in our nomenclature, and designated Gpi_B and *PGI-2* by DANDO (1974) and CROSS and PAYNE (1978), respectively) was reported to be highly polymorphic. It segregated for five alleles in a sample from the English Channel (DANDO 1974) and for six alleles in samples collected off eastern North America (CROSS and PAYNE 1978). In addition to the most common allele at this locus, *PGI-1 (100)*, two variant alleles designated *PGI-1 (65* and *135)* were observed in our present study. At the more anodal locus *PGI-2* a single copy of a slow variant allele designated *PGI-2 (90)* was also observed; the presumed heterozygote exhibited a three-banded phenotype with the 1:2:1 staining intensity pattern expected for a dimeric enzyme.

PGM. — The electrophoretic pattern, either one or two bands, may be read from skeletal muscle, liver or heart extracts. The consistent expression of variants in different tissues supports our interpretation of a single locus segregating for two alleles. The rather simple electrophoretic expression of PGM in this study contrasts to the more complex cod PGM zymograms reported by TILLS et al. (1971). However, because of different analytical procedures the results are not comparable.

SDH (Polyol dehydrogenase). — Initially, D-Sorbitol was used as substrate when staining for this enzyme. Later it was found that it acted even better on Xylitol, the other staining conditions being unchanged. However, further analyses on the specificity of this enzyme have not been performed. Pending further notice, the initial designation SDH will therefore be used as a trivial name.

This enzyme was active in liver extracts only and was represented by two zones assumed to represent the expression of two loci. The cathodal zone (*SDH-1*) was invariant while single- and five-banded phenotypes appeared in the anodal zone (*SDH-2*). We interpret this variation in terms of two alleles (*100* and *80*) segregating at the *SDH-2* locus coding for the more anodal form of this tetrameric enzyme. Some sub-banding, anodal to the position of the *SDH-2(100)* homotetramer, was frequently observed; this sub-banding is likely to obscure reliable scoring of possible allelic variants migrating faster than the common *100* allele at this locus.

XDH. — Activity for XDH was only observed in skeletal muscle extracts. Some variation in width and relative position of bands, which may reflect genetic polymorphism, was observed. We were, however, not able to interpret this variation genetically.

Amount of variation

We have identified a minimum number of 38 loci, 30 of which were considered potentially usable in population genetic surveys. The majority of these loci have not been previously described in the open scientific literature; they may provide a powerful tool for detailed studies of the genetic population structure of the cod.

There was no heterogeneity of allele frequencies among age groups at any locus, and the point estimates of allele frequency differences between sexes were small and statistically unsignificant at all loci coding for enzymes. At *HB-2* there was a conspicuous difference beetween sexes (the frequency of the *HB-2 (83)* allele was 0.55 and 0.36 in males and females, respectively; $\chi_1^2 = 5.67$; $P=0.017$). At present, we cannot determine whether this significance reflects a true difference or a type I statistical error; the suggested heterogeneity will be examined in more detail in future studies. We observed no deviations from the genotypic distributions expected under random mating

Table 2. Allele frequencies (variant alleles only) at ten polymorphic loci in cod from a restricted area of Trondheimsfjorden, Norway. The exact probability (P) from tests for deviations from the expected genotypic distributions under random mating (VITHAYASAI 1973) are also given

Locus	Allele	Allele frequency	Exact P for observed genotypic distribution
EST-5	*93*	0.019	1
HB-2	*83*	0.456	1
IDH-1	*140*	0.194	0.339
LDH-3	*70*	0.481	0.654
MDH-2	*280*	0.013	1
MDH-3	*60*	0.013	1
PGI-1	*135*	0.344	0.479
	65	0.044	
PGI-2	*90*	0.006	1
PGM	*80*	0.044	1
	70	0.013	
SDH-2	*80*	0.319	0.132

(Table 2), and there were no indications of linkage disequilibrium (HILL 1974) between any pair of loci (out of 45 pairwise comparisons the largest χ_1^2 was 3.29).

The quantitative estimates of the amount of genetic variability were based on the 30 loci (Table 1) considered usable for population surveys. Depending on the criterion used for polymorphism (the frequency of the most common allele not exceeding 0.95 or 0.99) the frequency of polymorphic loci (P) in the present material was $P_{0.95}=0.20$ and $P_{0.99}=0.30$, respectively. The average heterozygosity ($\bar{H}$) was 0.082 with standard error 0.029 (NEI 1975, p. 131). The serum transferrin system (TF) could not be analyzed in the present material, for technical reasons. The TF-variability was among the first protein polymorphisms described in Atlantic cod (MØLLER 1966), and *TF* is the locus most frequently used in cod population genetic research (CROSS and PAYNE 1978; JAMIESON and TURNER 1978; and references therein). Allele frequency estimates at the *TF* locus were available, however, from an independent sample of 80 cod collected in October 1978 at the same location as the present sample, i.e., Borgenfjorden. The frequencies of four alleles at the *TF* locus in that sample were *A*: 0.04, *B*: 0.15, *C*: 0.80, and *D*: 0.01 (MORK, unpublished; electrophoretic techniques and allele designation following MØLLER (1966)). Assuming that the two samples represent the same population, and including the transferrin data in the computations of H and P do not alter the values of these quantities considerably (the new estimates, based on 31 loci are $P_{0.95}=0.23$, $P_{0.99}=0.32$, and $\bar{H}=0.090$ (± 0.028)).

Information on genetic variation in natural populations of fish is accumulating rapidly, and as judged from the present sample, the cod appears to exhibit a high level of genetic variability. NEVO (1978) estimated the mean of the frequency of polymorphic loci and average heterozygosity for bony fishes in general to be of the order of 0.15 and 0.05, respectively. CROSS and WARD (1980) computed mean heterozygosity over all salmonids examined to be about 0.03; they suggested that the low level of heterozygosity in these species might be explained by population bottlenecks during the glaciations and by the general tendency of the salmonids to form up in genetically disctinct subpopulations of limited size (e.g., RYMAN et al. 1979; RYMAN 1981; STÅHL 1981; RYMAN and STÅHL in press). They contrasted the salmonid situation to that of some flatfish species which are generally assumed to have very large effective population sizes. These species exhibited high levels of heterozygosity (average of six Atlantic species of the family Pleuronectidae was 0.09). The present results fit into this pattern as the cod appears to be more similar to the Pleuronectidae than to the salmonids both with respect to ecology and the level of genetic variability. It is premature to conclude anything from these observations, but it is interesting to note that the Atlantic herring (*Clupea harengus*), which is assumed to be characterized by large effective population sizes, also exhibits high levels of genetic variation, at least with regard to the frequency of polymorphic loci ($P_{0.99} \approx 0.40$ and $\bar{H} \approx 0.07$; ANDERSSON et al. 1981).

Current knowledge of the genetic population structure of cod has been obtained through electrophoretic investigations of either one or a few selected polymorphic loci (e.g., SICK 1965a, b; FRYDENBERG et al. 1965; MØLLER 1966, 1968; JAMIESON 1975; WILKINS 1971; ODENSE et al. 1969; CROSS and PAYNE 1978; JAMIESON and TURNER 1978; MORK et al. 1980; and references therein). Although their approach appears to contribute a practical description of units of stock as understood by fisheries biologists, larger samples of randomly chosen genes should expose a more typical collection of genes with which to quantify genetic variability and compare the genetic composition of cod in the different north Atlantic fisheries. Other cod populations along the Norwegian coast are currently studied.

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