International Council for the Exploration of the Sea

C.M.1975/F:22
Demersal Fish (Northern) Committee

A preliminary study of some enzyme polymorphisms of potential value for cod stock identification off eastern North America

by

T. F. Cross and R. H. Payne

Department of the Environment

Fisheries and Marine Service

Newfoundland Biological Station

St. John's, Newfoundland, Canada

INTRODUCTION

Prior to the middle of the last decade the only techniques available for fish stock discrimination were tag return studies and analysis of geographic variation in morphometric and meristic characters. More recently, electrophoretic analysis of protein polymorphism has been used to discriminate between stocks of various marine fish species (de Ligny 1969): assuming a genetic basis for protein polymorphism, stocks may be distinguished by locating discontinuities in the geographic variation of alleles at polymorphic loci.

In the case of the cod, Gadus morhua L., proteins investigated for polymorphism include hemoglobin (Sick 1965a and b; Møller 1968; Wilkins 1971), transferrin (Møller 1966; Jamieson 1967; Jamieson and Otterlind 1971; Jamieson and Jónsson 1971), general serum proteins (Ullrich 1968), and enzymes such as esterase (Nyman 1965) and lactate dehydrogenase (Odense et al. 1969). Most of these studies concentrated on cod stocks of the eastern North Atlantic.

Jamieson (1975) has made a preliminary electrophoretic investigation of cod stock composition in the western North Atlantic in which he analysed material from eleven provenances between Georges Bank and Ritu Bank (N.E. Newfoundland Shelf). Significant differences were found between samples in the frequencies of alleles at the transferrin locus. In addition, non-significant differences were found in the frequencies of variant alleles coding for serum esterase, hemoglobin and LDH-B (heart type).

This communication reports our preliminary results on the electrophoretic analysis of some enzymes in two western North Atlantic cod populations. Samples were collected

from Northern Grand Bank and Burgeo Bank. Tag return data (Templeman 1974) suggest that cod inhabiting these two banks belong to different stocks.

MATERIALS AND METHODS

Specimens were collected by otter trawling from the Northern Grand Bank (47°09' to 47°17'N, 47°27' to 47°48'W;N=110) in April 1974, and from Northern St. Pierre/Burgeo Banks (46°37' to 47°28'N, 56°38' to 58°04'W;N=108) in June 1975. A small piece of muscle was taken from each specimen as soon as possible after capture and stored at -20°C until required for electrophoresis. Otoliths were collected for age determination, and length and sex was recorded for each specimen.

Muscle samples were homogenized in an equal volume of cold 30% dimethyl sulphoxide in 0.7 M 'tris'-HCl, pH 7.5; centrifuged for 15 min at 4000 X G; and the supernatants used for electrophoresis.

The elctrophoresis buffer was 135 mM 'tris', 45 mM citric acid, pH 7.0 which was used full strength in the electrode vessels and diluted 1 to 15 for gel preparation (Ayala et al. 1972). Starch gels (15% starch; British Drug Houses) were prepared in 180 mm X 180 mm X 6 mm plastic moulds. Samples were applied to the gels on 5 mm X 5 mm squares of 'Whatman No. 3' filter paper, and horizontal electrophoresis was conducted at 150 v and room temperatures with forced air cooling for 5 h. All the enzymes investigated were found to migrate anodally.

The basic staining solution was 100 mM 'tris', 10 mM $MgCl_2$ containing 100 mM lithium DL-lactate (for LDH staining), or 50 mM L-malic acid (for NAD-MDH staining), or 100 mM DL-α-glycerophosphate, disodium salt (for α-GPDH staining), and adjusted to pH 8.0 with HCl or NaOH as required. Before use, 10 mg MTT tetrazolium and 30 mg β-NAD were added to 100 ml buffered substrate. Gel slices were incubated in the staining solution for 30 min in the dark, 3 mg phenazine methosulphate were added, and incubation in the dark continued until the bands had developed.

PGI was located by the method of Scopes (1968). A piece of Whatman 1 chromatography paper cut to appropriate size was soaked with 3 ml of 100 mM 'tris'-HCl, pH 8.0, containing 10 mM $MgCl_2$, 10 mg fructose-6-phosphate, 9 mg MTT tetrazolium, 5 mg NADP, 1.5 mg phenazine methosulphate and 10 units of glucose-6-phosphate dehydrogenase; laid on the gel surface and incubated in the dark for approximately 20 mins. When bands had developed, the paper overlay was peeled off, washed in water and dried in an oven at 50°C.

Abbreviations used in this report are: LDH, NAD-lactate dehydrogenase (E.C.1.1.1.27); NAD-MDH, NAD-malate dehydrogenase (E.C.1.1.1.37); α-GPDH, NAD-α-glycerophosphate dehydrogenase (E.C.1.1.1.8); PGI, phosphoglucose isomerase (E.C.5.3.1.9); 'tris', 2-amino-2(hydroxymethyl)-1, 3-propandiol.

RESULTS AND DISCUSSION

A single, invariant band of LDH activity was observed. This is interpreted as equivalent to the skeletal muscle homotetramer of cod designated A_4 by Odense et al. (1969) and K_4 by Lush (1970); and which they found to be electrophoretically-invariant in cod samples from both sides of the North Atlantic. The designation A_4 has historical precedent (Appella and Markert 1961) in vertebrate genetics.

NAD-MDH was observed as three equally-spaced bands and a fourth more diffuse band of slower mobility (Fig. 1). No variability was noted. This three-banded pattern is interpreted as the product of two independent loci coding for sub-units which associate as dimers. This interpretation (Bailey et al. 1970) is applicable to the cytoplasmic ("supernatant") NAD-MDH patterns of many fish species. The more diffuse band of lower mobility is considered to be mitochondrial NAD-MDH.

Four α-GPDH phenotypes were observed (Fig. 2). The commonest pattern, designated α-GPDH^2, consisted of a single band associated with two satellite bands of faster mobility. This phenotype is interpreted as the product of the homozygous genotype, α-GPDH^2/α-GPDH^2. The other phenotypes observed were patterns of three equally-spaced bands in which the central band stained more intensely: they are interpreted genetically as corresponding to the heterozygous genotypes; α-GPDH^1/α-GPDH^2, α-GPDH^2/α-GPDH^3 and α-GPDH^2/α-GPDH^4.

The quaternary structure, tissue distribution and genetic control of PGI isozymes in the teleosts has been described by Avise and Kitto (1973) and Dando (1974). The latter author showed that in cod two loci, Gpi_A and Gpi_B, code for the sub-units which associate as dimers; in a study of 100 cod specimens from the English Channel he observed polymorphism at the Gpi_B locus involving 5 alleles (labelled Gpi_B^1 to Gpi_B^5 in order of decreasing electrophoretic mobility). Following the principle of historical priority, we are treating Gpi_A and Gpi_B (Dando 1974) as junior synonyms of PGI-1 and PGI-2 (Avise and Kitto 1973) respectively.

In our experiments, three regions of activity were observed in the filter paper overlays stained for PGI; they were labelled 1, 2 and 3 in Fig. 3. Regions 1 and 3 are interpreted as the homodimeric products of PGI-1 and PGI-2 respectively, and region 2 as the heterodimers of these products. Two distinct patterns were observed in region 1, a single band and a group of three bands; they are designated PGI-1^a and PGI-1^a/PGI-1^b, two of the three theoretically-possible phenotypes produced by a diallelic polymorphism at PGI-1. In region 3, the strongest staining region in skeletal muscle electropherograms, we observed PGI-2^a, PGI-2^a/PGI-2^b, PGI-2^a/PGI-2^c PGI-2^b/PGI-2^c and PGI-2^b; five of the six theoretically-possible phenotypes generated by a triallelic polymorphism at PGI-2. The alleles at each locus have been labelled provisionally with letters as superscripts until we can establish homologies with phenotypes in European cod and adopt Dando's use of numerals as superscripts.

The observed phenotype frequencies at each provenance are given in Table 1, and the allele frequencies in Table 2 are estimated from these data. Table 2 also gives the estimated sample size required to establish significance at the 5% level with 50% probability of success if the differences in allele frequency estimates between the two areas are real (Sokal and Rohlf 1969).

The rare α-GPDH alleles are probably of no value for stock discrimination unless they are found to be much commoner in other populations. Two PGI-2 alleles, PGI-2^a and PGI-2^b, exhibited significant differences in frequency between the two populations despite the small size of our samples. This polymorphism may prove to be a valuable character for discriminating North American cod stocks. The other allele at this locus, PGI-2^c, is not rare ($p=0.016$), but no significant frequency difference was found between the two populations. Similarly, the polymorphism at the PGI-1 locus did not show significant frequency differences, but the differential could be significant if the sample sizes were increased to approximately 600.

REFERENCES

Avise, J. C., and G. B. Kitto (1973). Phosphoglucose isomerase gene duplication in the bony fishes: An Evolutionary History. Biochem. Genetics 8: 113-131.

Appella, E., and C. L. Markert (1961). Dissociation of lactate dehydrogenase into subunits with guanidine hydrochloride. Biochem. biophys. Res. Communic. 6: 171-176.

Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourao and S. Perez-Salas (1972). Enzyme variability in the Drosophila willistoni group. IV. Genic variation in natural populations of Drosophila willistoni. Genetics, 70: 113-139.

Bailey, G. S., A. C. Wilson, J. E. Halver, and C. L. Johnson (1970). Multiple forms of supernatant malate dehydrogenase in Salmonid fishes: Biochemical, immunological and genetic studies. J. biol. Chem., 245: 5927-5940.

Dando, P. R. (1974). Distribution of multiple glucosephosphate isomerases in teleostean fishes. Comp. Biochem. Physiol. 47B: 663-679.

Jamieson, A. (1967). New genotypes in cod at Greenland. Nature 215: 661-662.

(1975). Enzyme types of Atlantic cod stocks on the North American Banks. pp 585-591. In: Isozymes, IV. Genetics and Evolution, C. L. Markert ed., Academic Press.

Jamieson, A., and J. Jónsson (1971). The greenland component of spawning cod at Iceland. Rapp. P.-v. Reún. Cons. perm. int. Explor. Mer., 161: 65-72.

Jamieson, A., and G. Otterlind (1971). The use of cod blood protein polymorphisms in the Belt Sea, the Sound and the Baltic Sea. Rapp. P.-v. Reún. Cons. perm. int. Explor. Mer., 161: 55-59.

Ligny, W.de (1969). Serological and biochemical studies on fish populations. Oceanogr. Mar. Biol. Ann. Rev. 7: 411-513.

Lush, I. E. (1970). Lactate dehydrogenase isoenzymes and their genetic variation in coalfish, Gadus virens, and cod, Gadus morhua. Comp. Biochem. Physiol. 32: 23-32.

Møller, D. (1966). Polymorphism of serum transferrin in cod. Fisk Dir. Skr., Ser. HavUnders., 14: 51-60.

(1968). Genetic diversity in spawning cod along the Norwegian coast. Hereditas, 60: 1-32.

Nyman, O. L. (1965a). Inter- and intraspecific variations of protein in fishes. Ann. Acad. Regiae Scient. Upsaliensis 9: 1-18.

Numachi, K. (1971). Genetic polymorphism of α-glycerophosphate in saury.. Cololabis saira-I. Seven variant forms and genetic control. Bull. Jap. Soc. Sci. Fish. 37: 755-760.

Odense, P. H., T. C. Leung, T. M. Allen, and E. Parker (1969). Multiple forms of lactate dehydrogenase in the cod Gadus morhua L. Biochem. Genet. 3: 317-334.

Scopes, R. K. (1968). Methods of starch gel electrophoresis of sarcoplasmic proteins. Biochem. J. 107: 139-150.

Sick, K. (1965a). Haemoglobin polymorphism of cod in the Baltic and Danish Belt Sea. Hereditas, 54: 19-48.

(1965b). Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean. Hereditas 54: 49-69.

Sokal, R. R., and F. J. Rohlf (1969). Biometry: The principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco 776 p.

Templeman, W. (1974). Migrations and intermingling of Atlantic cod (Gadus morhua) stocks of the Newfoundland area. J. Fish. Res. Board Can. 31: 1073-1092.

Ullrich, H. (1968). Zweite Mitteilung zur Blutserumelektrophorese von Kabeljan des Nordwestatlantiks. Fisch.-Forsch., 6, 37-39.

Wilkins, N. P. (1971). Haemoglobin polymorphism in cod, whiting and pollack in Scottish waters. Rapp. P.-v. Reún. Cons. perm. int. Explor. Mer., 161: 60-63.

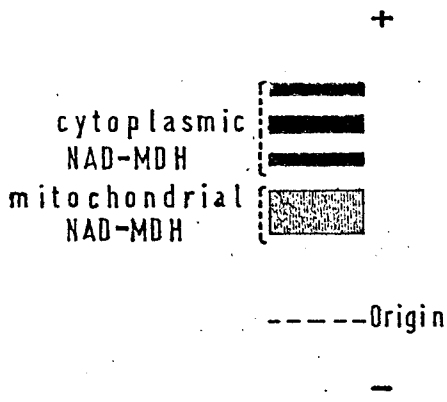

Fig. 1. Cod muscle NAD-MDH phenotype.

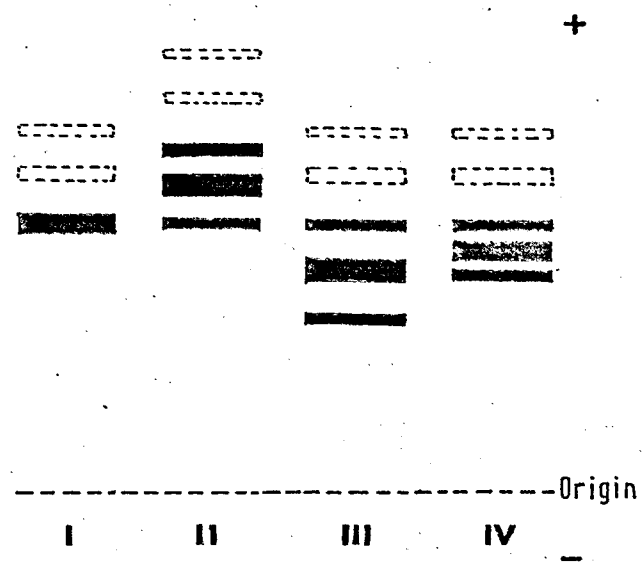

Fig. 2. Cod muscle α-GPDH phenotypes.

I. $\alpha\text{-GPDH}^2$
II. $\alpha\text{-GPDH}^1/\alpha\text{-GPDH}^2$.
III. $\alpha\text{-GPDH}^2/\alpha\text{-GPDH}^3$.
IV. $\alpha\text{-GPDH}^2/\alpha\text{-GPDH}^4$.

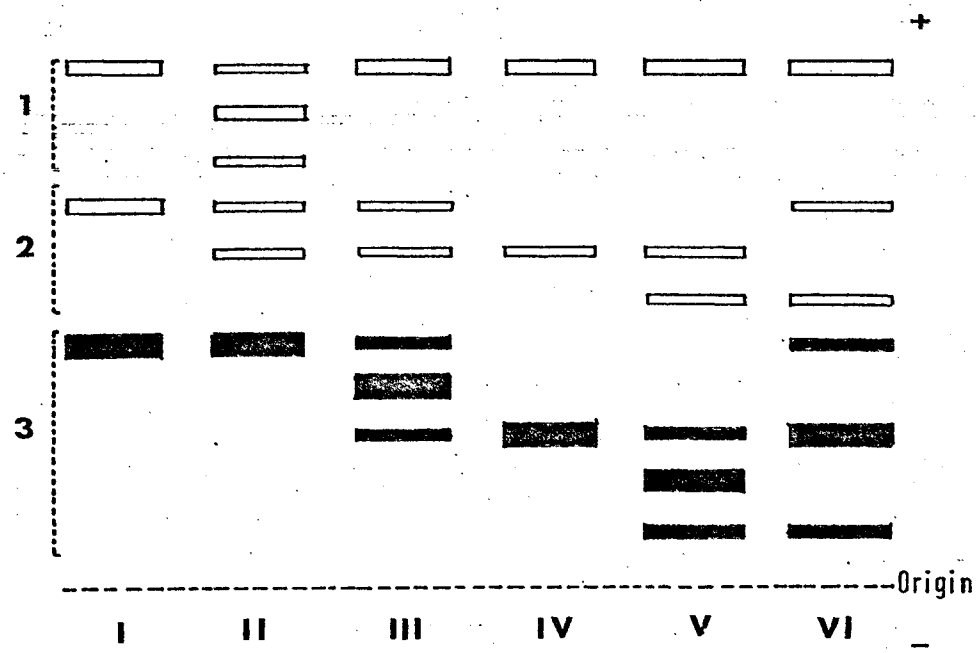

Fig. 3. Some cod muscle PGI phenotypes. I. $PGI\text{-}1^a$; $PGI\text{-}2^a$. II. $PGI\text{-}1^a/PGI\text{-}1^b$; $PGI\text{-}2^a$. III. $PGI\text{-}1^a$; $PGI\text{-}2^a/PGI\text{-}2^b$. IV. $PGI\text{-}1^a$; $PGI\text{-}2^b$. V. $PGI\text{-}1^a$; $PGI\text{-}2^b/PGI\text{-}2^c$. VI. $PGI\text{-}1^a$; $PGI\text{-}2^a/PGI\text{-}2^c$.

Regions 1, 2 and 3 are discussed in the text.

Table 1. The observed frequencies of α-GPDH, PGI-1 and PGI-2 phenotypes in cod muscle samples from Burgeo Bank and Northern Grand Bank. The frequencies in brackets are those expected from the allele frequency estimates in table 2 if a H-W-C equilibrium is assumed.

Locus	Phenotype	Burgeo Bank (N=108)	Northern Grand Bank (N=110)
α-GPDH	α-GPDH^1	0 (0.002)	0 (0.002)
	α-GPDH^1/α-GPDH^2	1 (0.989)	1 (0.977)
	α-GPDH^1/α-GPDH^3	0	0 (0.005)
	α-GPDH^1/α-GPDH^4	0	0 (0.005)
	α-GPDH^2	107 (107.009)	107 (107.028)
	α-GPDH^2/α-GPDH^3	0	1 (0.977)
	α-GPDH^2/α-GPDH^4	0	1 (0.977)
	α-GPDH^3	0	0 (0.002)
	α-GPDH^3/α-GPDH^4	0	0 (0.005)
	α-GPDH^4	0	0 (0.002)
PGI-1	PGI-1^a	104 (104.041)	108 (108.007)
	PGI-1^a/PGI-1^b	4 (3.922)	2 (1.984)
	PGI-1^b	0 (0.037)	0 (0.009)
PGI-2	PGI-2^a	38 (37.340)	49 (51.826)
	PGI-2^a/PGI-2^b	49 (50.562)	49 (44.623)
	PGI-2^a/PGI-2^c	2 (1.765)	4 (2.748)
	PGI-2^b	18 (17.116)	8 (9.605)
	PGI-2^b/PGI-2^c	1 (1.195)	0 (1.183)
	PGI-2^c	0 (0.021)	0 (0.036)

Table 2. Estimated allele frequencies at Burgeo Bank and Northern Grand Bank.

Allele	Frequency		P	Calculated sample size to establish significance*
	Burgeo Bank (N=108)	Northern Grand Bank (N=110)		
α-GPDH[1]	0.005	0.005	P>0.5	∞
α-GPDH[2]	0.995	0.986	0.5>P>0.2	420
α-GPDH[3]	0	0.005	0.2>P>0.1	192
α-GPDH[4]	0	0.005	0.2>P>0.1	192
PGI-1[a]	0.982	0.991	0.5>P>0.2	614
PGI-1[b]	0.019	0.009	0.5>P>0.2	513
PGI-2[a]	0.588	0.686	0.05>P>0.02	92
PGI-2[b]	0.398	0.296	0.05>P>0.02	83
PGI-2[c]	0.014	0.018	P>0.5	3766

* Calculated sample size to establish significance at 5% level with 50% probability of success (Sokal and Rohlf 1969).