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LDH gene frequencies in cod samples from two locations on the Norwegian coast*

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In a sample of 88 cod (*Gadus morhua* L.) caught in the Lofoten area during the 1978 spawning the frequency of Ldh_B^B was 0·62 for the Arcto-Norwegian cod stock ("skrei"). The corresponding frequency in a sample of 203 coastal cod from Trondheimsfjorden was 0·60. In no sample did the observed genotype distribution differ significantly from the expected proportions under genetic equilibrium. The analyses were carried out by isoelectric focussing on thin layers of polyacrylamide gel. The value of the actual isozyme system as a tool in cod stock discrimination is discussed.

Introduction

The synthesis of the lactate dehydrogenase isozymes (E.C.1.1.1.27) in cod is governed by genes at three loci, designated Ldh_A, Ldh_B and Ldh_C by Odense et al. (1969), whose nomenclature is used here. The Ldh_B locus, which has gene products predominating in the heart muscle, has shown extensive polymorphism in cod from the areas hitherto studied, exhibiting at least three mutant forms $Ldh_B^{B'}$, $Ldh_B^{B''}$ and $Ldh_B^{B'''}$ in addition to the normal form Ldh_B^B (Odense et al., 1969). However, the gene freqencies of Ldh_B^B have shown little variability among Canadian cod stocks, although the genotype distribution in a sample of 98 cod from St. Johns, Newfoundland indicated a mixture of two or more populations with different gene frequencies, having a significant deficit of the $Ldh_B^{BB'}$ heterozygotes (Odense et al., 1969). Gene frequencies and genotype distributions in cod samples from offshore from Aberdeen, Scotland, reported by Odense et al. (1969) and Lush (1970) are given in Table 1. The difference between the two observed gene frequencies in Table 1 is significant at the 5% level by a t-test (t = 2·007, d.f. = ∞, P = 0·045). Moreover, the heterozygote deficiency in the sample from 1970 is significant by a chi-square goodness of fit test (chi-square = 4.645, d.f. = 1, $P \approx$ 0·03).

The classical interpretation of these test data would be that they clearly indicate the existence of two or more populations with different gene frequencies of Ldh_B^B. However, Jamieson (1975) states that his unpublished data on cod from Greenland and Iceland show Ldh_B^B frequencies similar to those reported for North American cod stocks, and that the occasional discrepancies between observed and expected genotype proportions may be due to differences in genotype viability, i.e. fitness, since he on one occation actually observed an excess of heterozygotes in the sample.

To the authors' knowledge, nothing has been published with respect to the LDH gene frequencies in two comparatively large cod stocks, namely the Arcto-Norwegian cod stock, "skrei", and the Norwegian coastal cod. This fact, in addition to the rather heterogeneous image of the samples taken offshore from Aberdeen (Table 1), initiated the present study, which is also part of a project concerning the dynamics of the cod in Trondheimsfjorden, Norway.

Material and methods

Specimens

Samples were drawn from net-caught cod landed by fishermen. The 88 cod in the Lofoten sample were picked out as "skrei" by morphological characters amoung thousands of cod caught during the spawning fishery for "skrei" in this area, and may therefore belong to the Arcto-Norwegian cod stock. However, since the coastal cod, although in much less number, spawn in the same area at the same time, a parallel sample of 15 cod was analysed for haemoglobin by agar gel electrophoresis (Sick, 1961), as the frequencies of the Hbl^1 allele are known to differ between these two cod stocks (Frydenberg et al., 1965).

The 203 cod in the Trondheimsfjord sample were caught during the local cod fishery and are assumed to be coastal cod.

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Table 1. Data from earlier investigations on cod LDH isozymes in the North Sea.

Investigators	Area		Genotypes				N	Estimated gene frequency of the Ldh_B^B allele
			Ldh_B^{BB}	$Ldh_B^{BB'}$	$Ldh_B^{B'B'}$	Others		
Odense et al. (1969)	Aberdeen	Obs.	28	43	23	1	95	0·52
		Exp.	26·7	46·8	21·0	0·5	95·0	
Lush (1970)	Aberdeen	Obs.	76	73	35	0	184	0·61
		Exp.	68·6	87·6	27·8	0	184·0	

Tissue extracts

The "skrei" hearts were cut out within 6 h after death, kept on ice for 24 h while being shipped to the laboratory, washed for 30 min in ice-cold fresh water and then stored at −20°C for up to 60 days before the analyses. Hearts from coastal cod were frozen immediately at −20°C and analysed within 14 days. The different handling of "skrei" and coastal cod hearts did not seem to induce any notable differences in LDH patterns or staining intensity. After thawing, the cod hearts were homogenized with 2 to 4 volumes of one % glycine in a mortar placed on crushed ice. Cell debris in the fluid part of the homogenate was roughly brought down by centrifugation, whereafter the supernatant was dialyzed 10 to 24 h against ice-cold distilled water. The dialysate was then centrifuged for 5 min at 9000 **g** in a Beckman[R] Spinco 152 microfuge before use. The desalting process could be omitted by using 10 volumes one % glycine for homogenization, but because of lower protein concentration the colour development in the bands of enzyme activity was then slower resulting in more diffuse banding patterns.

Isoelectric focussing in polyacrylamide gel (IFPAG)

IFPAG was carried out on flat beds with equipment from LKB (Stockholm, Sweden), consisting of a Multiphor[R] electrofocussing unit connected to a LKB 2103 power supply capable of delivering constant power and a LKB 2209 Multicool[R] cryostat with cooling plate. With the latter a constant temperature of 1°C was set. The acrylamide gel was moulded as described in an application note from the manufacturer of the equipment (Karlsson et al., 1973). An ampholyte concentration of 2% (w/v) consisting of 6 parts Ampholine[R] 3–10 (LKB) to 4 parts Bio–Lyte 4–6 (Bio-Rad) was used in a preliminary study. Once the LDH patterns became familiar they could easily be read using a one % concentration of carrier ampholytes. With this recipe all the actual LDH bands were localized to the anodic half of the gel. The pH gradient formed in the gel was measured at one cm intervals with an Ingold LOT 403-30-M8 micro surface electrode connected to a Radiometer[R] 27 pH meter while the gel was still placed on the cooling plate.

For a 25 × 10 × 0·2 cm gel and constant power set at 25 W (final voltage 2000 V) the analyses time was 3 h when focussing across the width of the gel. Fifteen mm^3 of sample solution was applied on 1 × 0·5 cm pieces of filter paper 3 cm from the cathode. The paper pieces were removed after 20 to 30 min run. Twenty-four samples were analysed each run. The staining procedure was essentially that described by Shaw and Prasad (1970) except that MTT was substituted for NBT and the gel was pre-equilibrated for 10 min in a 0·2 M phosphate buffer (pH = 7·25) which also substituted the tris-HCl buffer in Shaw and Prasad's recipe. The gels were incubated in the dark at 10° to 16°C and read after 1 to 3 h.

Results and discussion

The Hbl^1 frequency calculated for the mentioned parallel sample from Lofoten was 0·12, indicating cod of mainly "skrei" origin in the Lofoten samples. With the ampholyte mixture described in the recipe above the pH gradient obtained was not quite linear. This may have affected the isoelectric point (pI) estimates given in Figure 1 which were obtained by interpolation. However, the similarity between the pIs in Figure 1 and the approximate values reported by Odense and Leung (1975); pI = 5·3 and 5·4 for $LDH_B^{B_4}$ and $LDH_B^{B'_4}$ respectively, makes it reasonable to assume that the same isozymes are involved.

The functional LDH molecule is a tetramer. The subunits coded by various alleles at various loci can combine in different ways to tetramers with different pIs. In addition to the main isozyme bands which are drawn in Figure 1, extra bands often occurred both on the cathodic and anodic side of the $LDH_A^{A_4}$ band. These are believed to represent various combinations of the gene products from the Ldh_C locus with those from the Ldh_A and Ldh_B loci. As a rule these bands were faint and did not interfere with the genotype interpretations at the Ldh_B locus. Based on the assumption that the Ldh_B^B and $Ldh_B^{B'}$ alleles in this survey are in accordance with the nomenclature of Odense et al., (1969) the genotype distributions and gene frequencies for Ldh_B^B are calculated in Table 2. As can be seen, the observed difference in frequency of Ldh_B^B in samples of arctic and

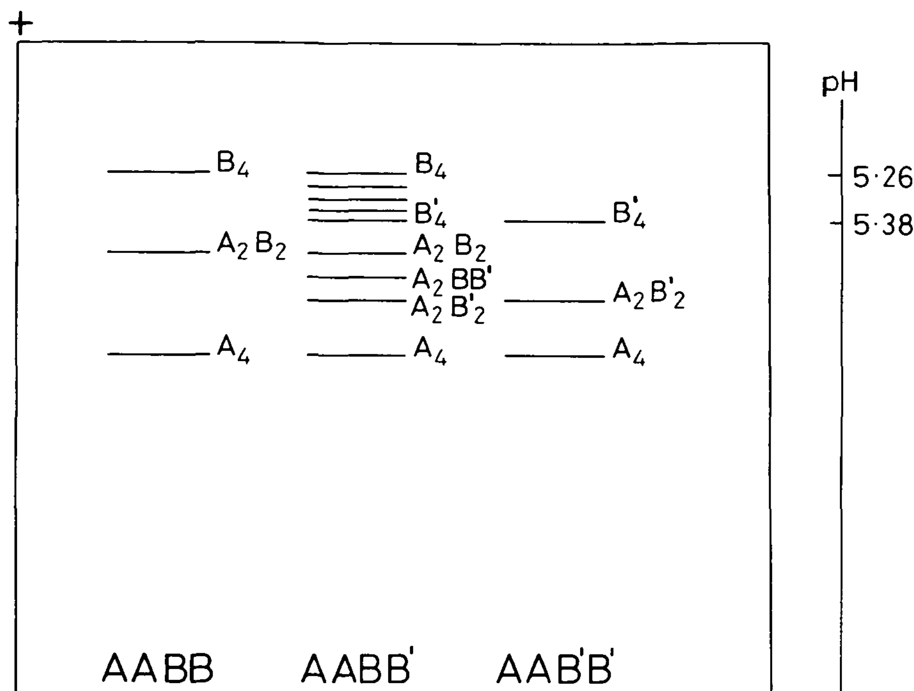

Figure 1. Drawing of LDH zymogram with interpretation of the three common banding patterns and estimated pIs of $LDH_B^{B_4}$ and $LDH_B^{B'_4}$. In the heterozygote the three intermediate bands between $LDH_B^{B_4}$ and $LDH_B^{B'_4}$ are interpreted as the heterotetramers $LDH_B^{B_3B'_1}$, $LDH_B^{B_2B'_1}$ and $LDH_B^{B_1B'_3}$ respectively. Experimental conditions are given in the text.

coastal cod is small and not significant by a *t*-test (t = 0·269, d.f. = ∞, P = 0·79). Hence it seems that if any difference exists between arctic and coastal cod with respect to the frequency of Ldh_B^B it must be small, i.e. not detectable with the present sample sizes. This fact sets further limits for the value of this polymorphism as a tool in cod stock discrimination, and the two populations sampled in the present study join the group of cod stocks exhibiting rather similar frequencies for the actual genes. With regard to the gene frequencies observed for cod caught offshore from Aberdeen, our calculated frequencies resemble those of Lush (1970, cf. Table 1). Note that if the deficit of heterozygotes in his sample is caused by a population mixture, one or more of the involved populations must have a frequency much higher than 0·61. It can easily be shown that for a 50/50 mixture of two populations exhibiting a mean frequency of the observed 0·61, the difference in gene frequency must be of the order 0·4 to account for the observed heterozygote deficiency in his sample, i.e. the involved populations must have gene frequencies of ~ 0·8 and ~ 0·4. So far cod populations with such Ldh_B^B frequencies have not been discovered. The present investigations extend the geographic survey of the gene locus Ldh_B in cod stocks. This new information for cod at Norway reduces the chance of finding examples of stocks showing divergent allele frequencies at the Ldh_B locus although it is of course not expected that Nor-

Table 2. Sampling dates and areas, observed and expected (under genetic equilibrium) genotype distribution and calculated gene frequencies for two samples of cod from the Norwegian coast 1978. Chi-square for goodness of fit and probability (P) of worse fit are given.

Date 1978	Area		Genotypes			N	χ^2	P	Estimated gene frequency of the
			Ldh_B^{BB}	$Ldh_B^{BB'}$	$Ldh_B^{B'B'}$				Ldh_B^B allele
7 Mar	Lofoten	Obs.	34	41	13	88	0·0128	0·91	0·62
		Exp.	33·75	41·5	12·75	88·0			
Apr–May	Trondheims-fjorden	Obs.	77	91	35	203	0·8209	0·365	0·60
		Exp.	73·1	97·4	32·5	203·0			

wegian arctic or coastal cod should interfere in catches from Scottish waters. It should be mentioned that the Norwegian coastal cod cannot be regarded as homogeneous. Segregation analyses for haemoglobin indicates subdivision into several local populations (Frydenberg et al., 1965). The sample from Trondheimsfjorden in this study is therefore not representative for the entire Norwegian coastal cod stock. Another subpopulation may display diverging frequencies for the actual genes, as may other, not investigated cod stocks as well. However, the present investigation gives support to the statement of Jamieson (1975) that the frequency of alleles at the Ldh_B locus in cod are monotonously uniform throughout the geographic range of this species.

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