GENOTYPE DISTRIBUTIONS OF COD FROM THE NORWEGIAN SKAGERRAK COAST

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The genotype distributions for cod, *Gadus morhua* L., from the southeastern coast of Norway are studied as part of a more extensive study on cod enhancement and the possible effects of mass liberation of 0-group cod on endemic cod populations. This paper focus on possible geographical variation in allele frequencies, temporal variations in haemoglobin type frequencies and mean length of age of various genotypes. Twelve samples were analysed for genotype distribution of haemoglobins and the tissue enzymes LDH, PGI, IDH, PGM, and GPD by agar-gel and starch gel electrophoresis. Some intersample variations were found indicating heterogeneity of the total cod stock structure in the area. The haemoglobin-controlling genes occurred with similar frequencies in this material as in corresponding material analysed more than 25 years earlier. Possible associations between life history traits and individual genotypes were investigated, and genotype-dependent growth rate was indicated.

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INTRODUCTION

Frequency distributions of haemoglobin types in cod, *Gadus morhua* L., from the Skagerrak/Kattegat area, including the southeastern coast of Norway, were first published by FRYDENBERG & al. (1965). The frequencies of the gene *HbI(1)* was higher in this area than in any other areas studied at that time or later, differing both from corresponding frequencies in the Baltic and in the North Sea including the west coast of Norway. Later the dynamics and evolution of genetic variation in fish, including the cod haemoglobins, have been widely debated. The discovery by KARPOV & NOVIKOV (1980) of specific temperature dependence of oxygen dissociation curves for cod of the different haemoglobin types, and also the reports by MORK, GISKEØDEGÅRD & SUNDNES (1984a,b) on genotypic growth differences contributed to the discussion on the dynamics of genetic variation in this species.

In the present report gene frequencies in cod samples from the southeastern part of the Norwegian coast are dealt with. The study is part of a more extensive investigation on cod enhancement and the effect of mass liberation of 0-group cod on endemic cod populations. The material was collected for baseline studies on the native cod in the area. However, in the presentation it is focused on association between life history traits (growth rate and age) and genotype, as well as on gene frequency changes over a period of about 25 years, for testing of the following hypotheses:

1. No temporal variation in haemoglobin type frequencies exists.
2. No difference in mean length at age of the various genotypes at specified loci exists.

MATERIAL AND METHODS

Sampling of blood and muscle tissue was described by MØLLER (1968) and JØRSTAD (1984). For analyses of haemoglobins the method described by SICK (1965) was applied with modifications (JØRSTAD 1984). Starch gel electrophoresis was applied for analyses of muscle enzymes (JØRSTAD 1984).

Fig. 1 shows the sampling localities. The sampling took place from October 1986 to May 1991. 0-group cod were sampled by beach seine and older cod were caught by trap nets or gill nets. Numbers in samples, sampling dates and sample characteristics are given in Table 1.

A test based on Wright's fixation index of subpopulations (CHRISTIANSEN & al. 1976) was used to test for Hardy-Weinberg proportions. G-tests or χ^2-tests were used to test for homogeneity of gene distributions. Length distributions for fish of different genotypes within samples were compared using one-way ANOVA. For samples and genotypes chosen for such comparisons, see below.

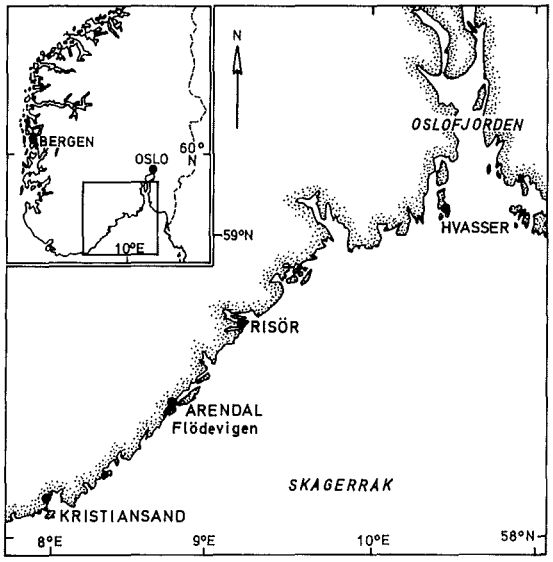

Fig. 1. Sampling localities along the Norwegian Skagerrak coast.

RESULTS

The following loci were chosen for routine analysis: haemoglobin (HB-1), lactate dehydrogenase (LDH-3), phosphoglucomutase (PGM), glucose-6-phosphate dehydrogenase (GPD), phosphoglucose isomerase (PGI-1), and isocitrate dehydrogenase (IDH-2). Loci coding for these enzymes and the alleles found in natural cod populations have been described and designated elsewhere (SICK 1961; CROSS & PAYNE 1978; MOTH-POULSEN 1982; MORK & al. 1982; JØRSTAD 1984).

Allele frequencies, calculated from observed distributions of genotypes, are presented in Table 1. Appendix 1 shows the observed genotype distributions.

No significant deviation from Hardy-Weinberg proportion was found in any sample. Thus no evidence for possible sampling of mixed populations or existence of non-directional selection was found from the distribution of genotypes.

Test of total heterogeneity among the samples by G-tests indicated no significant sample differences:

HB	:	G = 25.1	d.f. 22	P = 0.29
LDH	:	G = 31.2	d.f. 22	P = 0.09
PGI	:	G = 27.5	d.f. 22	P = 0.20

However, some samples gave a rather high contribution to the G-values indicating that intersample differences may exist.

Both the gene frequencies and the range of intersample variation for PGI-1 and LDH-3 as well as the other tissue enzymes were similar to corresponding values from other parts of the Norwegian coast (JØRSTAD & NÆVDAL 1989), inclusive one sample from Oslofjorden, (MORK & al. 1985).

Frequencies of the *Hb-1 (1)* gene varied between 0.49 and 0.68. The lower values are similar to corresponding values found in western Norway while the higher values are in accordance with previous samples from the same area collected more than 25 years earlier (FRYDENBERG & al. 1965). Five samples collected from the southeastern coast of Norway in

Table 1. Allele frequencies in samples of cod from southeastern Norway. n.a. = not analysed. * = rare allels included.

					Hb -1		*Ldh-3*			*Gpd*			*Pgi-1*				*Pgm*				*IDH-2*		
Sample	Age	Locality	Date	N	*1*	*2*	*70*	*100*	*150*	*90*	*100*	*120*	*30*	*70*	*100*	*150*	*0*	*30*	*70*	*100*	*70*	*100*	*130*
1	0+	Flødevigen	Nov.86	111	0.68	0.32	0.35	0.65	-	0.02	0.98	*	0.01	-	0.70	0.28 *	-	0.01	-	0.99	0.01	0.99	-
2	0+	Flødevigen	Sep.87	96	0.60	0.40	0.45	0.55	-		n.a.		-	-	0.63	0.37	-	0.02	0.01	0.97		n.a.	
3	>0+	Flødevigen	Apr.89	78	0.64	0.36	0.41	0.58	0.01	0.03	0.95	0.03	0.04	0.02	0.67	0.27	-	0.03	-	0.97	0.03	0.96	0.01
4	>0+	Risør	Jun.89	117	0.55	0.45	0.39	0.61	*	0.06	0.93	0.01	0.03	0.01	0.69	0.27	0.02	0.01	-	0.97	0.02	0.98	0.01
5	0+	Risør	Nov.89	171	0.60	0.40	0.36	0.64	-	0.02	0.98	*	0.02	0.01	0.67	0.30	0.01	0.01	-	0.98		n.a.	
6	>0+	Risør	Nov.89	94	0.62	0.38	0.40	0.59	0.01	0.02	0.97	0.01	0.01	0.02	0.68	0.28 *	-	0.03	-	0.97	-	1.00	-
7	>0+	Hvasser	Nov.89	102	0.58	0.42	0.39	0.61	-	0.05	0.94	0.01	0.05	-	0.64	0.31	0.01	0.01	-	0.98	0.02	0.98	-
8	1+	Flødevigen	Jan.90	46	0.49	0.51	0.36	0.64	-	0.01	0.98	0.01	0.03	-	0.62	0.35	0.01	-	-	0.99		n.a.	
9	1+	Risør	Aug.90	95	0.63	0.37	0.37	0.62	0.01	0.04	0.93	0.02	0.04	-	0.65	0.30 *	-	0.03	-	0.96 *	0.02	0.98	-
10	0+	Kristiansand	Oct.90	39	0.60	0.40	0.43	0.57	-		n.a.		0.04	-	0.57	0.39	-	0.03	-	0.97	-	1.00	-
11	0+	Hvasser	Oct.90	60	0.60	0.40	0.47	0.53	-		n.a.		0.01	-	0.76	0.23	-	0.01	-	0.98 *	-	1.00	-
12	>0+	Flødevigen	May 91	192	0.61	0.39	0.39	0.61	*	0.02	0.97	0.01	0.03	*	0.65	0.32	*	0.01	-	0.99	0.02	0.98	

1961–62 ranged from 0.59 to 0.69. Of the 12 samples in the present report, three are outside this range. When comparing haemoglobin allelic proportions in the present material and the relevant samples in FRYDENBERG & al. (1985) by a 2 × 2 contingency table test, the following results were obtained:

Pooled material this study N = 1201 q_1 = 0.60
'Old' material N = 393 q_1 = 0.65
χ^2 = 4.82 d.f. = 1 P < 0.05

This test indicates that some significant changes have taken place in frequencies of the haemoglobin-controlling genes since the 60's. When performing similar tests on the Oslofjord and the Flødevigen samples respectively, the following results were obtained:

Oslofjorden
Pooled material this study: N = 152 q_1 = 0.62
'Old' material: N = 157 q_1 = 0.62
χ^2 = 0.0 d.f. = 1 P ~ 1

Flødevigen
Pooled material this study: N = 445 q_1 = 0.63
'Old' material: N = 236 q_1 = 0.67
χ^2 = 4.03 d.f. = 1 P < 0.05

However, when comparing the recent samples from Flødevigen and Risør in a similar way (no test is needed for the Hvasser samples – which are very similar) the following results were obtained:

Flødevigen: χ^2 = 10.3 d.f. = 4 P < 0.01
Risør: χ^2 = 3.30 d.f. = 3 P < 0.01

Thus it seems clear that both the deviation between old and recent samples and the haemoglobin variations among recent samples are mainly due to the Flødevigen samples, and sample no. 8 is the main contributor to this variation. These results indicate that either have the samples been drawn from different populations or the haemoglobin type distributions are influenced by dynamic (selective) forces.

It was not possible to see any differences in gene frequency variation between 0-group cod and older fish. The between-sample variations seemed to be independent of the fish age.

To see whether it was possible to detect any differences in size at age (which would reflect differences in previous growth rate), the size of different phenotypes within samples were compared. Reasonably high numbers within such subgroups were obtained for the 0-group samples nos 1, 2, 5, 10, and 11, one year old fish of samples nos 9 and 12, and for two years old fish in samples nos 3, 4, 7, and 12. Frequent genotypes of haemoglobins, LDH and PGI could be used in these tests.

Table 2 shows the mean lengths of the different genotypes within samples. The underlying length distributions were tested by one-way ANOVA-tests. Only for haemoglobin types of sample 1 (F = 3.2, P = 0.047) and 10 (F = 77.6, P ~ 0.0), and Ldh types of 1+ fish of sample 12 (F = 3.7, P = 0.038) significant differences in mean lengths were found. It was not possible to see any trend in the non-significant differences (see Table 2), and no overall genotype dependent growth rate as far as it could be measured by size at age observations.

Table 2. Mean length (cm) of different genotypes within samples. Samples showing statistically significant variation (one-way ANOVA test) between genotypes are underlined. For disignation of genotypes see JØRSTAD & NÆVDAL (1989).

	Hb-1			*Ldh-3*			*Pgi-1*		
Samples	*11*	*12*	*22*	*70/70*	*70/100*	*100/100*	*100/100*	*100/150*	*150/150*
1	10.5	9.6	9.3	10.5	9.5	10	9.9	9.7	9.9
2	9.7	9.9	9.7	9.5	9.9	9.7	9.7	9.6	10.1
5	12.8	12.6	12.5	11.5	11.9	11.4	11.6	11.3	12.9
10	12.2	10.7	9.3	11	10.6	10.6	11.1	10.5	10.4
11	9.6	9.7	10.1	9.7	9.3	9.9	9.8	9.5	9.3
9	24.6	24	25.2	23.6	24.8	24.7	25.2	23.4	26.4
12 (1+)	22.2	21.9	19	23.2	22.2	20	20.9	22.4	23.3
3	39.8	39.7	36.9	38.8	39.2	39.9	39.6	38.8	40.8
4	39.4	40.7	40.5	40.4	40.4	40.2	39.7	40.6	40.4
7	44.9	46.9	46.2	46	45.8	46.2	45.8	46.4	44.8
12(2+)	34.7	34	36.7	34.7	35.5	33.9	34.3	34.7	34.7

DISCUSSION

The results gave little new knowledge about the structure of the cod population along the south-eastern coast of Norway. For the tissue enzymes variations within and between samples were found to exist on about the same level as found in other parts of the Norwegian coast. This was not unexpected in relation to previous results (MORK & al. 1982, 1985; MOTH-POULSEN 1982; JØRSTAD & NÆVDAL 1989). Gene frequencies of the tissue enzymes give no clear evidence of the existence of population units of cod in this area. The variation found among samples could reflect real interpopulation variation, but they are too small for utilization in population studies, and their biological significances are also unknown.

Concerning haemoglobins, the results of the study indicate that the gene frequencies may stay apparently uneffected through several fish generations. With the exception of one or two samples gene frequencies on the same level as expected on basis of analyses carried out 25 years earlier were found, indicating that no net directional selection has taken place. Nor were any clear indications of stabilizing selection found, but it should be brought in mind that the selection pressure should be high to result in detectable deviations from expected Hardy-Weinberg distributions by the present sample sizes. However, the samples which deviated both from the main part of the samples and from the samples analysed previously, indicate dynamic forces working on haemoglobin type distributions.

The genotype/growth rate covariation indicate that at least for some systems (e.g. haemoglobin) biochemical genetic variation have biological significance. The mean growth rate seems to some extent to be associated with genotype. However, the inconsistency of this observation indicates that the effect differs from time to time or from cohort to cohort, possibly reflecting natural variation in the environment. The different temperature dependent oxygen binding capacity of the haemoglobin variants (KARPOV & NOVIKOV 1980) indicate that the 'benefit' of being of a particular genotype may be temperature dependent.

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Appendix 1. Observed distribution of cod samples from the southeastern coast of Norway. For designation of genotypes see JØRSTAD & NÆVDAL (1989).

	Hb-1			*Ldh-3*					*Gpd*			*Idh-2*		
Sample	*1/1*	*1/2*	*2/2*	*70/70*	*70/100*	*70/150*	*100/100*	*100/150*	*90/100*	*100/100*	*100/120*	*70/100*	*100/100*	*100/130*
1	41	30	11	13	51	0	47	0	4	106	1	1	110	
2	36	44	16	16	55	0	25	0		n.a.			n.a.	
3	30	37	9	18	28	0	31	1	4	65	4	5	72	1
4	41	71	24	21	66	1	53	0	16	122	3	4	136	1
5	64	78	29	38	100	0	110	0	9	237	2		n.a.	
6	36	44	14	15	46	0	32	1	4	88	1	0	76	0
7	38	43	21	19	42	0	41	0	10	88	2	4	97	0
8	10	24	11	6	20	0	18	0	1	44	1		n.a.	
9	35	50	10	16	36	1	39	0	8	79	4	3	87	0
10	17	13	9	8	20	0	14	0		n.a.		0	43	0
11	19	21	9	14	26	0	18	0		n.a.		0	60	0
12	67	99	26	30	86	1	74	0	5	155	4	3	82	0

	Pgi-1									*Pgm*				
Sample	*30/30*	*30/100*	*30/150*	*70/70*	*70/100*	*70/150*	*100/100*	*100/150*	*150/150*	*0/100*	*30/100*	*70/100*	*100/100*	*100/150*
1	0	3	0	0	0	0	53	46	9	0	1	0	110	0
2	0	0	0	0	0	0	37	28	16	0	2	2	63	0
3	0	4	2	1	1	1	35	29	5	0	4	0	74	0
4	1	5	3	1	0	0	66	57	8	4	4	0	133	0
5	0	7	3	0	3	3	111	98	20	2	6	0	232	0
6	0	0	2	0	3	1	45	35	8	0	5	0	88	0
7	0	10	1	0	0	0	42	37	12	1	2	0	95	0
8	0	1	2	0	0	0	17	22	4	1	0	0	45	0
9	0	6	2	0	0	0	37	38	8	0	5	0	85	2
10	0	2	0	0	0	0	7	16	3	0	2	0	37	0
11	0	0	1	0	0	0	28	12	4	0	1	0	48	1
12	0	5	7	0	1	0	76	90	13	1	3	0	187	0