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The Propagation of cod *Gadus morhua* L.

THE HAEMOGLOBIN POLYMORPHISM IN ATLANTIC COD (*Gadus morhua* L.); GENOTYPIC DIFFERENCES IN SOMATIC GROWTH AND IN MATURING AGE IN NATURAL POPULATIONS*

J. Mork, R. Giskeødegård and G. Sundnes

Biological Station, N-7001 TRONDHEIM, Norway

ABSTRACT

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The two common alleles at the polymorphic haemoglobin locus *HbI* (Sick, 1961) in the Atlantic cod (*Gadus morhua* L.) code for proteins whose functional properties are differentially influenced by environmental temperatures (Karpov and Novikov, 1980). In the present study *HbI* genotypic mean lengths in a total of 275 immature specimens (0.5 - 1.5 year of age) from 8 samples taken in the Trondheimsfjord and Oslofjord during 1977-1981 were investigated by rank correlation analysis. In males the overall genotypic mean length rank was $HbI^{2-2} > HbI^{1-2} > HbI^{1-1}$, while in females $HbI^{1-2} > HbI^{2-2} > HbI^{1-1}$; both ranks were highly significant.

The age at first spawning (A_m) was determined by otolith reading in a sample of 118 male cod caught during spawning in the Trondheimsfjord in 1981. In this sample the individual A_m ranged from 4-7 years with a significantly lower mean for the HbI^{2-2} genotype. The A_m of heterozygotes was intermediate but not significantly lower than for the HbI^{1-1} genotype. Some population genetic and aquacultural implications of these results are briefly discussed.

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*Contribution No. 221. Biological Station, N-7001 Trondheim Norway

INTRODUCTION

The haemoglobin locus *HbI* of the Atlantic cod is polymorphic (Sick, 1961). Two common alleles are widespread throughout the range of the species, although in proportion which differ considerably between stocks. The HbI^2 allele is most abundant in stocks inhabiting cold waters (West Atlantic, Greenland, Iceland, Barents Sea and the inner Baltic). The frequency of this allele increases from south to north among cod on the east coast of America, on the coast of Norway and in the Baltic (Frydenberg et al., 1965; Sick, 1965a; b). Is has been suggested that these clines are supported by natural selection (Kirpichnikov, 1981). A selection may act through temperature regimes. Karpov and Novikov (1980) presented experimental data on the effect of temperature on the functional properties of erythrocytes from the three common cod haemoglobin genotypes which fitted well to observations of the relative abundance of the alleles in natural populations. At low temperatures the HbI^{2-2} molecule was by far the most efficient oxygen carrier, while the HbI^{1-1} molecule showed similar advantages at high temperatures (>13°C). Erythrocytes from heterozygotes (HbI^{1-2}), which contain both molecules in equal proportions, were intermediate in performance at all temperatures. Seemingly in accordance with these experimental data, Mork et al (1983) reported a correlation between *HbI* genotype and size among immature cod from the Trondheimsfjord. In four succeeding age groups (0.5 - 3.5 year, pooled sexes) the rank of mean lengths was $HbI^{2-2} > HbI^{1-2} > HbI^{1-1}$.

Since the egg number in cod females is a linear function of body weight (Daan, 1975) these finding may point to an explanation for the observed distribution of *HbI* alleles in the various climate regions. They also indicate that the development of area-characteristic *HbI* allele frequencies may be, in evolutionary terms, an

extremely rapid process.

The existence of *HbI* genotypic size differences will be notorious in creating problems in obtaining unbiased samples for studies of, e.g., population *HbI* allele frequencies and genotypic growth rates, because usually the sampling gear employed is size-selective. Preferably therefore, reported field observations on genotypic growth differences should be checked experimentally to exclude such sampling errors (Mork et al., 1983), and also to see if growth rate differences are manifested in captive cod and may thus be utilized in cod aquaculture.

Pending results from such experiments, which are currently in progress, we present here additional population data which support earlier observations and clarify some aspects discussed by Mork et al. (1983) for the apparent selection at *HbI* in Atlantic cod. These aspects concern genotypic differences in growth and in age at first spawning, both of which are important factors which need to be clarified in relation to population genetic analyses as well to the developing field of cod aquaculture.

MATERIALS AND METHODS

The procedures for blood sampling and agar gel electrophoresis of haemoglobins followed Mork et al. (1983) and Sick (1965a), respectively. Our genetic nomenclature conform with Sick (1965a).

HbI genotypic mean length analyses

To avoid bias due to effects from commercial fisheries on the *HbI* genotypic length distribution among cod in the sampled areas (cf Mork et al., 1983), only 0.5 - 1.5 year old cod specimens were considered in the mean length analyses. The batches for analyses (grouped according

to sex) were compiled from eight samples taken at different times and locations in Norwegian waters during 1977-1981 (Table 1).

TABLE 1

Cod samples used for *HbI* genotypic mean length analyses. The sampling locations Beitstadfjord, Borgenfjord, and Verrabotn are situated within the Trondheimsfjord

Batch	Location	Date	Age (year)	Sex	Number of specimens	Gear
A	Beitstad-fjord	Oct.-77	0.5	Males	7	Beach seine
B	Beitstad-fjord	Oct.-81	0.5	Males	21	-"-
C	Beitstad-fjord	Oct.-81	0.5	Females	23	-"-
D	Borgen-fjord	Mar.-78	1.0	Males	17	Shrimp trawl
E	Borgen-fjord	Mar.-78	1.0	Females	27	-"-
F	Beitstad-fjord	Oct.-77	1.5	Males	4	Beach seine
G	Borgen-fjord	Oct.-78	1.5	Males	23	Shrimp trawl
H	Borgen-fjord	Oct.-78	1.5	Females	22	-"-
I	Borgen-fjord	Oct.-80	1.5	Males	27	-"-
J	Borgen-fjord	Oct.-80	1.5	Females	19	-"-
K	Verra-botn	Oct.-80	1.5	Males	5	-"-
L	Verra-botn	Oct.-80	1.5	Females	7	-"-
M	Oslo-fjord	Dec.-81	1.5	Males	31	Trap net
N	Oslo-fjord	Dec.-81	1.5	Females	42	-"-

Only batches where all the three common *HbI* genotypes were represented in a particular age/sex groups were included in the analyses. By treating the sexes separately a larger number of groups were available for trend analyses, biases due to sexual growth differences were avoided, and potential sexual differences with respect to genotypic mean length rank trends could be detected.

Age at maturation for *HbI* genotypes

The age at first spawning was recorded by otolith readings (Rollefsen, 1933) in a sample of 123 cod caught with shrimp trawl during spawning in Verrabotn on Apr. 23, 1981. This particular sample contained almost exclusively male cod; a total of 5 females were excluded from the statistical analyses, leaving 118 male cod for study.

RESULTS

HbI genotypic mean length analyses

The calculated mean lengths for *HbI* genotypes in samples A-N are shown in Table 2.

Within each batch the genotypic mean lengths are based on different number of specimens and are thus not equally reliable estimates of the respective population parameters. Before the application of trend tests, the mean lengths were therefore standardized according to Mork et al. (1983):

$$U_i = (\bar{x}_i - \mu)/(\sigma_i/\sqrt{n_i})$$

where

U_i = standardized mean length of specimens in the i-th genotype group

TABLE 2

Mean lengths of *HbI* genotypes calculated for immature cod grouped according to age and sex

Batch	Mean length of genotypes (cm)		
	HbI^{2-2}	HbI^{1-2}	HbI^{1-1}
A	15.60	12.30	9.95
B	9.04	8.78	7.40
C	9.30	10.06	9.08
D	20.33	18.82	17.44
E	18.70	19.22	18.32
F	32.00	28.00	26.50
G	30.50	29.18	28.50
H	29.00	28.56	28.20
I	23.25	21.00	21.20
J	20.00	20.80	19.60
K	25.00	24.00	23.00
L	17.20	17.89	17.10
M	33.00	32.91	30.75
N	33.60	33.70	33.57

$\bar{x}_i$ = calculated mean length of specimens in the i-th genotype group

μ = overall mean length (pooled genotypes) in the batch

σ_i = standard deviation of mean length in the i-th genotype group

n_i = number of specimens in the i-th genotype group

When, after proper standardization, the genotypic mean length in each sample (A-N) were ranked 1-3 from higher to lower values, the HbI^{1-1} genotype were inferior in all samples except one (13 out of 14 possible; Sign test; $P = 0.0017$). The intrinsic rank of HbI^{2-2} and HbI^{1-2} seemed less consistent until samples were grouped according to sex; then there appeared to be a consistent difference between sexes with respect to genotypic size rank (Table 3).

TABLE 3

Ranks of standardized *HbI* genotypic mean length in samples of immature male and female cod (1-3 from higher to lower value)

Batch	Rank of standardized mean length for genotypes		
	HbI^{2-2}	HbI^{1-2}	HbI^{1-1}
Males			
A	1	2	3
B	1	2	3
D	1	2	3
F	1	2	3
G	1	2	3
I	1	3	2
K	1	2	3
M	2	1	3
Females			
C	2	1	3
E	2	1	3
H	2	1	3
J	2	1	3
L	2	1	3
N	2	1	3

The statistical significance of the observed patterns for males and females in Table 3 may be tested by Kendall's τ-tests for rank correlation (Kendall, 1962), assuming that the test statistic S from the test on each sample will be normally distributed with expected value = 0. (The variance of S = [2N(N-1)(2N+5]/36, where N=3, the number of genotype groups in each test; cf Mork et al, 1983). From such tests it turns out that in males the *HbI* genotypic mean length rank; $HbI^{2-2} > HbI^{1-2} > HbI^{1-1}$ is highly significant (pooled S for 8 tests = 20; Student's t = 3.69, P = 0.00024), as

also is the rank in females; $HbI^{1-2} > HbI^{2-2} > HbI^{1-1}$ (pooled S from 6 tests = 18, t = 3.83, P = 0.00013).

Age at first spawning for *HbI* genotypes

As revealed by otolith readings the age at first spawning (A_m) in the sample of 118 male spawning cod varied between 4 and 7 years. The actual distributions of A_m within genotypes are shown in Table 4.

TABLE 4

Number of specimens maturing at various age within cod haemoglobin genotypes (cumulative percentages in parenthesis). Data obtained by otolith readings in 188 male spawners

Genotype	Number of recruit spawners and cumulative % at age (years)				Total number
	4	5	6	7	
HbI^{2-2}	11(37%)	14(83%)	5(100%)	-	30
HbI^{1-2}	6(12%)	22(54%)	19(90%)	5(100%)	52
HbI^{1-1}	3(8%)	17(56%)	9(81%)	7(100%)	36

There is a statistically significant heterogeneity in the genotypic distribution of A_m i Table 4 as revealed by a 4x3 χ^2 contingency table (expected values under the null hypothesis of no heterogeneity derived from column totals): $\chi^2_6 = 18.297$, P = 0.0055. The mean recruiting age for genotypes were: HbI^{2-2}: 4.80±0.71, HbI^{1-2}: 5.44±0.83, HbI^{1-1}: 5.56±0.91. By single factor analyses of variance and subsequent Newman-Keul test it was shown that the mean A_m for HbI^{2-2} was significantly lower than for the two other genotypes (Table 5).

TABLE 5

Analyses of variance of mean age at first spawning for the three common cod *HbI* genotypes. Data from 118 male spawners

Source of variation	Sum of squares	DF	Mean square
Between genotypes	10.884	2	5.442
Within genotypes	78.516	115	0.683
Total	89.400	117	

$F = 7.91$, $P = 0.00057$

Newman-Keul test (significance niveau = 0.001):

$HbI^{2-2} \neq HbI^{1-2} = HbI^{1-1}$

DISCUSSION

The existence of *HbI* genotypic differences with respect to growth and age at maturation, which were indicated by data from Norwegian coastal cod reported by Mork et al. (1983), are strongly supported in this study. The present analyses of extended materials has also uncovered a previously undetected sexual difference in the effect of *HbI* genotype on growth (Table 3). Potentially, a superior growth of HbI^{1-2} females will tend to stabilize the gene frequencies (balanced polymorphism) while a superior growth of HbI^{2-2} males will tend to break down the equilibrium. The reason for this sexual differences is at present not known. Presumably, behavioural differences (level of activity, habitat preference) are involved, since temperature-related O_2 demands at present seem to be the most probable explanation for the apparent differences for cod *HbI* genotypes.

For this reason it is difficult to predict the results from *HbI* genotypic growth experiments in the laboratory,

where the specimens have no habitat choice and do not have to hunt the prey. It is, nevertheless, important to clarify if any genotypic growth differences are manifested when the genotypes are kept at controlled, equal conditions. Such conditions can only be achieved in the laboratory, and the relevant experiments are in progress.

As discussed by Mork et al. (1983), current cod *HbI* allele frequencies, which seem to be in a state of equilibrium in the investigated areas, are, potentially, not balanced by natural selection alone, but are also significantly influenced by human impact in the form of size-selective fishing gear. Such gear will predictably prevent fast growing specimens from producing proportional amounts of offspring. Thus, observed allele frequencies may represent a kind of pseudo-equilibrium influenced by genotypic size (egg number), genotypic age at maturation, and intensity of commercial exploitation with size-selecting gear. A temporal and spatial variation in these factors and their relative influence on allele frequencies must be expected, but they may eventually reach a state of equilibrium where the various forces cancel each other out. Although artificial selection by fishing gear may retard the qHbI^1 lowering effect of better growth of HbI^2-possessing, it seems that the HbI^1-possessing genotypes nevertheless must have some advantages under natural conditions in order to explain the persistance of this allele in evolutionary frames. After all, human exploitation with selective gear is of recent origin. Such reasoning raises the question as to whether cod individuals can actively choose habitats suitable for their haemoglobin genotype. Karpov and Novikov (1980) reported such behaviour of cod haemoglobin genotypes in relation to environmental temperatures, but this has not been confirmed experimentally.

Obviously, there are questions which remain to be answered in connection with the haemoglobin polymorphism of cod. The present materials gave no information on the age

at first spawning for female *HbI* genotypes, and in males the superior growth of heterozygotes compared to the HbI^{1-1} genotypes was not reflected in a significantly lower age at maturation, although a connection between fast growth and low maturity age was indicated by the HbI^{2-2} homozygotes. Such inconsistencies might point to the existence of sampling artifacts: the very existence of genotypic selection by fishing gear will tend to level out potential differences and may create unpredictable sample artifacts. It was noted that our sample of 118 male spawning cod was probably a growth-selected part of the population; they were among the survivors after several years of commercial exploitation. Also it might be questioned how realistic samples collected with the present gear picture the real connection between growth rates and age at first spawning. It might be that the slower growing genotype in fact matures at the same age as the fastgrowing individuals, only that they were not caught by the present gear due to, e.g., gear-induced size-selection or behavioural differences like size-dependent shoaling. On the other hand, inconsistent results are not necessarily sampling artifacts; age at maturation is, in any case, not determined by growth rates alone, and our results are probably influenced by a number of currently unidentified factors, physiological, and perhaps also behavioural.

A low age at maturation would perhaps not be a desirable trait in cod aquaculture since it is sometimes connected with a pronounced growth retardation. If *HbI* genotypic growth differences are manifested in cod aquaculture as in the present material from natural populations, this problem may arise but can be dealt with by an adequate selection programme.

The present materials are too scarce and, for reasons discussed above, not adequate for reliable estimation of genotypic differences in production (growth) in nature, and definitely not in captivity. However, the consistency

of the trends observed (Table 3) indicates that the differences may be considerable in natural populations.

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