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Haemoglobin polymorphism in *Gadus morhua*: Genotypic differences in haematocrit*

J. Mork & G. Sundnes

Biological Station; N-7000 Trondheim, Norway

ABSTRACT: Haematocrit (erythrocyte/plasma ratio) values of the three common *HbI* genotypes were recorded in two samples totalling 149 specimens of Atlantic cod (*Gadus morhua* L.) caught in the Trondheimsfjord, Norway, during March-April 1984. The haematocrit values were shown to depend on both *HbI* genotype and sex; the females revealed higher average haematocrit values than males, and among the males the *HbI-22* genotypes displayed higher average haematocrit values than the two other HbI genotypes.

INTRODUCTION

The haemoglobins of the Atlantic cod (*Gadus morhua* L.) are polymorphic. The three most common phenotypes, as revealed by electrophoresis, represent combinations of two alleles at one autosomal locus, designated *HbI* by Sick (1961). These two alleles are present in virtually all current stocks of cod, but their relative proportions may differ considerably, even between adjacent stocks (Frydenberg et al., 1965; Sick, 1965a, b). Generally, however, there is a clinal reduction in the frequency of the *HbI-1* allele with increasing latitude on both sides of the Atlantic as well as in the Baltic Sea. Kirpichnikov (1981) considered the clines along the Norwegian coast and in the Baltic, and concluded that they were probably supported by environmental selection.

A possible physiological basis for such a selection was provided by the study of Karpov & Novikov (1980). Their results from a study of oxygen disassociation curves using erythrocytes from the three most common *HbI* genotypes suggest that temperature may be a potent selection factor; the HbI-22 molecule is by far the most efficient oxygen carrier at low temperatures while the HbI-11 molecule has similar advantages at higher temperatures. Red cells from the heterozygote *HbI-12* (both molecules in equal proportions) were consistently intermediate in performance (Karpov & Novikov, 1980). Water temperatures generally decline South-North on the northern hemisphere; thus the observed South-North increase in the frequency of the *HbI-2* allele might reflect the better performance of the HbI-22 molecule in cold environments.

Subsequent studies on Norwegian coastal cod have revealed results which seem to be biological manifestations of a strong environmental selection acting on cod haemoglobin genotypes. Thus Mork et al. (1983; 1984a, b) reported observations on *HbI* genotypic differences in growth, mortality, age at maturation, and within-season gonad

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development. As judged from the observed rank of genotypes in those comparisons, the *HbI-2*-possessing genotypes were best fitted for the environment on the actual sampling locations. Among both male and female cod the *HbI-11* homozygotes were shown to be consistently inferior with respect to growth; among males the *HbI-11* specimens were also inferior with respect to age for sexual maturation, and within-season gonad development rates (Mork et al., 1983; 1984a, b).

As pointed out by Mork et al. (1983) all these differing traits (cf. above) in the *HbI* genotypes are related to general body growth. Recently, we have expanded our routine handling of *HbI* genotyped cod specimens to include measurements of haematocrit (erythrocyte/plasma ratio in blood), which can give valuable information on the general physiological condition of individuals. The present paper deals with haematocrit observations in two samples of cod caught during March-April 1984 in the Trondheimsfjord, Norway.

MATERIAL AND METHODS

Two samples, taken at two different times and locations in the Trondheimsfjord, Norway, were analyzed. Sample 1, containing 58 male and 25 female cod, were taken by shrimp trawl and lure at 55–105 m depth at the main cod spawning place (location 1) in the Trondheimsfjord on March 27, 1984. This sample was heterogeneous with respect to sexual maturation, size (27–97 cm), and age (not recorded; deduced from size composition).

Sample 2 was taken by shrimp trawl on April 27, 1984, in a shallow (max depth 40 m) side-arm (location 2) of the main fjord. It consisted of 33 immature specimens of each sex. They were all 1 year of age as determined by otolith readings according to Rollefsen (1933).

The two sampling locations differ considerably with respect to hydrography. Location 1 (Verrabotn) is part of the main fjord with its estuarine type of hydrography including relatively stable deep-water temperatures (6–8 °C) throughout the year (Jacobson, 1983). Location 2 (Borgenfjord) is quite different. Due to extensive water turbulence set up by strong tidal currents through a narrow and shallow entrance, the water masses in this side-fjord are nearly meta-stable at all times of the year; temperature and salinity display homogeneous values from surface to bottom. Thus the cod present there will experience temperatures which normally range from 0–2 °C in winter to 16–20 °C in summer (Gulliksen, 1972). Tagging experiments (Mork, unpublished) have not indicated annual migrations to and from location 2; the cod is present there throughout the whole year (Denstadli, 1972).

Blood samples for haematocrit determination and haemoglobin genotyping were obtained by heart puncture. Haemoglobin *(HbI)* genotyping by agar gel electrophoresis followed the analytical procedures and genetic nomenclature of Sick (1961). Haematocrit values were measured after centrifuging whole blood in heparinized tubes for 5 min on a standard micro haematocrit centrifuge.

RESULTS AND DISCUSSION

Haematocrit values differed significantly between HbI genotypes. Both *HbI-11* and *HbI-12* specimens displayed relative anemia compared to the *HbI-22* specimens in our

samples taken in the cold season of the year. This result, which was statistically significant in Sample 1, was confirmed by additional sampling (Sample 2). In both samples, the *HbI* genotype effect on haematocrit was detected in males only. In Sample 2 the average haematocrit was higher in females than in males. Details from the analyses of the two samples are discussed below.

Sample 1. A statistically significant heterogeneity of haematocrit for the *HbI* genotypes in pooled sexes (1-factor analysis of variance: $F(2,80) = 4.799$, : $P = 0.011$) was shown to be due mainly to the genotypic variation of haematocrit in males, while no heterogeneity was detected in females (Table 1). In males, Newman-Keuls tests revealed a significant difference between *HbI-22* and the other genotypes. There was no detectable effect of sex on haematocrit in Sample 1.

Table 1. *Gadus morhua*. Mean haematocrit of *HbI* genotypes in Sample 1. Results from 1-factor analyses of variance, and Newman-Keuls test procedures for differences between means are given

	Males			Females		
	HbI-11	*HbI-12*	*HbI-22*	*HbI-11*	*HbI-12*	*HbI-22*
No. of specimens	18	28	12	5	15	5
Mean haematocrit	0.2733	0.2635	0.3302	0.2708	0.2900	0.2944
Standard deviation of mean	0.0398	0.0500	0.0655	0.0572	0.0608	0.0867

Males: $F(2,55) = 7.5052$, $P = 0.0013$, and *HbI-11* = *HbI-12* ≠ *HbI-22*
Females: $F(2,22) = 0.1995$, $P = 0.8206$

Sample 2. As in Sample 1, a significant *HbI* genotypic heterogeneity of mean haematocrit in pooled sexes (1-factor analysis of variance: $F(2,63) = 3.816$, $P = 0.027$) was shown to be caused mainly by the male part of the sample (Table 2). In males, a multiple range test (Newman-Keuls) showed that the mean haematocrit of *HbI-12* specimens was significantly ($P < 0.05$) lower than that of *HbI-22* specimens, while the mean of *HbI-11* genotypes did not differ significantly from either of the two others. In addition to the component of total variation contributed by *HbI* genotype, there was also a component caused by sex (Table 3).

Table 2. *Gadus morhua*. Mean haematocrit of *HbI* genotypes in Sample 2. Results from 1-factor analyses of variance are given. Cf text for results from Newman-Keuls test for differences between means

	Males			Females		
	HbI-11	*HbI-12*	*HbI-22*	*HbI-11*	*HbI-12*	*HbI-22*
No. of specimens	6	14	13	4	19	10
Mean haematocrit	0.3087	0.2785	0.3402	0.3668	0.3224	0.3411
Standard deviation of mean	0.0323	0.0514	0.0453	0.0544	0.0566	0.0335

Males: $F(2,30) = 6.002$, $P = 0.006$
Females: $F(2,30) = 1.439$, $P = 0.253$

Table 3. *Gadus morhua*. Effect of *HbI* genotype and sex on haematocrit in Sample 2 (age: 1 year). Summary of results from 2-factor analysis of variance

Source of variation		Sum of squares	d.f.	Mean square	F-value
A	HbI genotype	0.02615	2	0.01308	5.312
B	Sex	0.01410	1	0.01410	5.729
A*B	Interaction	0.00778	2	0.00389	1.581
	Within (error)	0.14770	60	0.00246	
	Total	0.19573	65		

Factor A: P = 0.008, Factor B: P = 0.020, A*B: P = 0.214

Both in nominal ranking and in terms of the statistical significance of the *HbI* genotypic haematocrit differences, Sample 2 confirmed the observations in Sample 1 (Tables 1 and 2). Apparently thus, the different hydrographical conditions on the two sampling locations did not cause a change in the main pattern of *HbI* genotypic effects on haematocrit. The overall, joint result in the two samples is that the male *HbI-22* specimens on average have higher haematocrit than the two other genotype groups.

This result is interesting in light of the fact that superiority of the male *HbI-22* individuals has been the main trait also in previous investigations considering e.g. growth rates, sexual maturation, and within-season gonad maturation (Mork et al., 1983; 1984a, b). In fact, a check of the mean lengths for *HbI* genotypes in Sample 2 confirmed those previous results in that the male *HbI-22* genotype was superior in this sample, too (Table 4).

Table 4. *Gadus morhua*. Mean lengths of *HbI* genotypes (age: 1 year) in Sample 2. Results from 1-factor analyses of variance, and Newman-Keuls test procedures for differences between means are given

	Males			Females		
	HbI-11	*HbI-12*	*HbI-22*	*HbI-11*	*HbI-12*	*HbI-22*
No. of specimens	6	14	13	4	19	10
Mean length	13.85	14.08	17.51	14.00	15.54	15.85
Standard deviation of mean	2.75	2.15	3.22	1.91	3.05	3.46

Males: $F(2,30) = 6.505$, $P = 0.005$, and *HbI-11* = *HbI-12* ≠ *HbI-22*
Females: $F(2,30) = 0.532$, $P = 0.593$

It could be that the higher haematocrit value of male *HbI-22* specimens was caused by a covariation between length and haematocrit; i.e. increasing haematocrit with increasing length. We tested this possibility by considering the correlation coefficients (r) between body length and haematocrit for all 12 combinations of sex and *HbI* genotype in the two samples. However, none of these r-values were significantly different from zero. Since no *HbI* genotypic heterogeneity of haematocrit had been observed in females

we were justified in pooling *HbI* groups in this sex to enhance test power. Doing so in Sample 1, the overall (pooled HbI genotype groups) correlation coefficient between length and haematocrit in females was −0.159, which is not significantly different from zero ($t = -0.774$, $df = 23$, $P = 0.447$). Anyway, if the "length effect" had been significant we would not have been able to discriminate between the effects of age and length in this sample.

This problem did not exist in Sample 2, in which the specimens were aged. In this sample the overall (33 specimens; pooled *HbI* groups) r-value for the correlation between length and haematocrit in females was 0.042 which is not significantly different from zero ($t = 0.234$, $df = 31$, $P = 0.816$). Considering both male and female *HbI* groups, the 6 calculated r-values in Sample 2 ranged between −0.018 (female *HbI-11*) and 0.416 (male *HbI-22*). None of them were significantly different from zero, and as tested by the chi-square procedure of Sokal & Rohlf (1981; p. 588) there was no significant heterogeneity among these values ($\chi^2 = 1.823$, $df = 5$, $P = 0.873$). Thus, as far as these test results can express, the haematocrit values may be regarded as independent of body length, and the observed *HbI* genotypic differences in haematocrit is therefore not likely to be just a side-effect of size differences. Instead, it may be assumed that in males the *HbI-22* specimens tend to have higher haematocrit than the two other *HbI* genotypes regardless of individual size.

At present we do not have sufficient information for drawing conclusions on the causal relationships behind the observed *HbI* genotypic haematocrit differences. Formally, the male *HbI-11* and *HbI-12* genotypes suffer from relative anemia compared to the *HbI-22* genotypes in the present samples. This could, or could not, be as simple as an iron deficiency anemia, but the explanation may be more complex. If, as seems plausible, the haematocrit differences are related to the temperature-dependent functional properties of the haemoglobins, there might be a seasonal (temperature) variation component of current unknown magnitude. Additionally, it is uncertain to what degree haematocrit reflects current or past environment effects since, for example, the turnover rate of cod erythrocytes is not known. One should also be aware that compensatory haemoglobin production (cf. high altitude adaption in humans) may take place, and tend to mask causal relationships. Finally: it is known that the proper structure of the haemoglobin molecule can affect erythrocyte shape, and thereby haematocrit (cf. the sickle-cells in humans). However, since the *HbI* effect on haematocrit seems to be restricted to male specimens in the present samples, such effects are not indicated although formally they remain to be studied, e.g. microscopically.

Although sampling area (despite hydrographical differences) did not appear to affect the main results substantially for the present two samples from the same fjord, it seems reasonable to expect that the nature, and detectability of *HbI* genotypic effects on haematocrit will depend on the environment to which cod specimens have been exposed. The effects might be quite different in different regions in the distribution range. The present location 2 displays an extensive annual temperature cyclus. Probably, a sampling program which covered this location at multiple times through the year could indicate whether or not season plays a role for the actual effect of *HbI* genotype on haematocrit. It would also be desirable to record additional parameters from each blood sample (e.g. cell count, average cell size, single cell haematocrit) in order to study potential compensatory effects.

For the present, we will refrain from extensive speculations on the physiological realities behind our observations. The most important result here is the actual demonstration that haemoglobin genotype can affect individuals physiologically to the degree that their haematocrit values become different. It is likely that this may under certain circumstances affect their physical performance and fitness.

Both with respect to the sexual difference in manifestation, and the intrinsic rank of *HbI* genotypes the haematocrit results resembles the patterns observed in studies of growth rates, age at maturation, and within-season gonad maturation in cod from the same areas (Mork et al., 1983; 1984a, b); the *HbI* effect is most predominant in males, and (at the time of sample collection) the male *HbI-22* genotype seems superior to the two others. Together with those previous studies, the present results demonstrate the profound effect that *HbI* genotype can have on the adaptive properties of individual cod.

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