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INTER- AND INTRASPECIFIC VARIATIONS IN
HEMOGLOBINS OF SOME GADOID FISHES

by

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Introduction

During the last few years it has been shown that in fish hemoglobins there exist intraspecific variations of at least two types, namely ontogenetic as in salmon (Kock, Evans, and Bergström 1964, Vanstone, Roberts, and Tsuyuki 1964) and herring (Wilkins and Iles 1966), and genetically controlled polymorphism as first found in whiting and cod (Sick 1961). In sprat (Wilkins and Iles 1966, Nævdal 1966) and some other clupeoid fishes (Simpson and Simon Schlothfeldt 1967) intraspecific hemoglobin variations have been described, but these variations were neither found to be associated with age or length, nor has the genetic basis of the variations been worked out completely.

The present work is part of a search program to find polymorphic characteristics for use in segregation studies of fish populations. Most interest have been paid to gadoid fishes of commercial value, but bloods of species of minor economical importance have also been analysed for comparison.

Material and methods

Blood samples have been obtained by cardiac puncture. Heparin or citrate was used as anticoagulant.. The blood specimens were stored cold, (0-4°C) and analyses were carried out within a few days from the time of sampling..

Agar-gel electrophoresis, described by Sick (1961) was applied. Each run lasted for 60 minutes with about 50 volts between ends of gel.

Part of the material was also analysed by combined starch-/agar-gel electrophoresis (Møller 1966) with 65 volts between ends of gel for 90 minutes, to control the results obtained by the other method.

Blood samples were collected from gadoid fishes from localities on the Norwegian coast and the North Sea. Species, localities, date of sampling and numbers of samples are listed in Table I where the results also are presented.

Results and discussion

The hemoglobin components of all the species concerned here, moved towards the cathode in agar gel at pH 7.3. Fig. I shows the relative mobilities of the hemoglobin components, and the hemoglobin pattern found for each species. For comparison the cod hemoglobin type Hb 1-1-2 is shown.

In all species one or two strong and at least two weak or moderately strong components were observed. The electrophoretic mobility of the strong components did not differ very much from the mobility of the strong hemoglobin components of cod. The highest cathodic mobility of strong components was found in coalfish, and the lowest in blue ling. The mobilities of the strong components of the other species were found within this range.

Intraspecific variation occurred in most species. In the following the same designations are used for corresponding patterns (phenotypes) of different species, although the mobilities of the components were not identical (see Fig. I).

In ling, blue ling and tusk hemoglobin patterns with either one or both of two strong hemoglobin components were found. These patterns were similar to the hemoglobin patterns of whiting and cod (Sick 1961), and a similar nomenclature was chosen. Thus the components were named Hb 1-1 and Hb 1-2 in order of decreasing cathodic mobility, and the hemoglobin types were called Hb 1-1, Hb 1-2 and Hb 1-1-2, according to which of the components they possessed.

No evidence of ontogenetic variation in the hemoglobin of these species was found. The variations may be explained, however, by assuming that two allelomorphous genes, named HbI^1 and HbI^2 , control the synthesis of the components Hb 1-1 and Hb 1-2 respectively. This corresponds to the genetical control of whiting and cod hemoglobin types (Sick 1961). The accordance between observed distributions of hemoglobin types (phenotypes) and calculated Hardy-Weinberg distribution of genotypes, Table I, greatly supports this hypothesis.

Two strong hemoglobin components, named Hb I-1 and Hb I-2, also occurred in haddock, coalfish, pollack and blue whiting, but only one of the single-banded phenotypes was found in each species. However, it follows from the distribution and the calculated gene frequencies, Table I, that one of the hypothetical genes is too rare to be expected in a homozygous state in the present material. Therefore a corresponding mode of inheritance of hemoglobin types may exist also in these species.

In coalfish variations were very rare as only one specimen out of 288 differed from the normal hemoglobin pattern of this species. In Norway pout and hake no individual variation in the strong hemoglobin components were found.

In all species the strong components were accompanied on their cathodic side by weaker components named Hb I-1 and Hb I-2. Sick (1961) found that corresponding components in whiting and cod increased in strength upon storing and this was evidently also the case in the species concerned here.

In ling and blue ling other weak components of still higher cathodic mobility were present (see Fig. 1). Also these components varied among specimens, but they often have a rather diffuse appearance, and therefore further studies have not been undertaken. In hake a moderately strong component with about twice as great cathodic mobility as the major hemoglobin component was present in some of the specimens.

Components similar to the Hb II components of whiting and cod were found present in haddock, coalfish, pollack, Norway pout and hake at positions between the point of application and the major components. The relative mobilities of these ^{complements} / differed among species, and also intraspecific variations were indicated, although not clear enough for classification of specimens into well defined groups. Except for some very faint bands, no components comparable to Hb II components could be detected in ling, blue ling and tusk.

The intraspecific variations described here could also be found by combined starch-/agar gel electrophoresis at pH 9.0 (anodal movement). This confirms that the variations are real molecular differences and not methodical artifacts.

Although the material was limited, the present observations clearly showed that intraspecific variations, probably genetically controlled, are present in several gadoid species.

Summary

Hemoglobin of haddock, coalfish, Norway pout, blue whiting, hake, ling, blue ling and tusk were studied by agar gel electrophoresis. Both inter- and intraspecific variations in hemoglobin patterns occurred. Intraspecific variations were found to be present in haddock, coalfish (only one specimen differed from the "normal" pattern), pollack, blue whiting, ling, blue ling and tusk. A hypothesis of genetical control involving two allelomorphic genes is proposed to explain the variations within each species. The population data coincided with this hypothesis. No indication of ontogenetic variation in hemoglobin patterns have been found.

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Table I. Observed distributions of hemoglobin types in samples of gadoid fishes compared to expected Hardy-Weinberg distribution.

Species, localities and dates of sampling.	Hemoglobin types			No.	χ^2	
	HbI-1	HbI-1-2	HbI-2			
<u>Haddock, <i>Gadus aeglefinus</i></u>						
Hjelmsøy, Finnmark 28. V. 65	obs	-	1	115	116	
Malanger, Tromsø 28. X. 65	obs	-	1	97	98	
Total, Northerh Norway	obs	-	2	212	214	0.004
Myking, Hordaland April 67	obs	-	5	80	85	0.03
	exp	0.1	4.9	80.0	85.0	
<u>Goalfish, <i>G. virens</i></u>						
Hordaland 16. XII. 65	obs	-	-	114	114	
Veidholmen, Nordmøre 17. XII. 65	obs	-	1	33	34	
Røstbanken, Lofoten 8-9. III. 65	obs	-	-	140	140	
Total	obs	-	1	287	288	0.002
<u>Pollack, <i>G. pollachius</i></u>						
Myking, Hordaland April 67	obs	76	5	-	81	0.97
	exp	76.2	4.7	0.01	81.0	
<u>Blue whiting, <i>G. poutassou</i></u>						
Tennholmen, Nordland 6. X. 67	obs	-	-	10	10	
25 n.m. NW marsteinen 24. V. 67	obs	-	-	50	50	
50 n.m. W Utsira 19. VIII. 67	obs	-	-	87	87	
30 n.m. S. Kristiansand 21-22. VIII. 67	obs	-	4	70	74	
W Ona, Møre 6. IX. 67	obs	-	-	91	91	
Total	obs	-	4	308	312	0.006

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Species, localities, and dates of sampling.		Hemoglobin types			No.	q ₁
		Hb I-1	Hb I-1-2	Hb I-2		
Norway pout, <u>G. esmarki</u>						
W Ona, Møre	obs	no variation			50	
6. IX. 67						
Hake, <u>Merlucius merlucius</u>						
25 n.m. NW Marsteinen	obs	no variation			15	
24. v. 67						
8. IX. 67	obs	no variation			37	
Ling, <u>Loiva molva</u>						
Tenn holmen, Nordland	obs	-	3	4	7	
6. X. 66						
Myking, Hordaland	obs	-	5	9	14	
April 67						
25 n.m. NW Marsteinen	obs	-	-	3	3	
24. V. 67						
Bressay Ground	obs	1	7	9	17	
8.-9 IX. 67						
Total	obs	1	15	25	41	0.21
	exp	1.8	13.6	25.6	41.0	
Blue ling, <u>M. byrkelang</u>						
25 n.m. NW Marsteinen	obs	1	12	38	51	0.14
24. V. 67						
	exp	1.0	12.3	37.7	51.0	
Bressay Ground	obs	2	24	89	115	0.12
8. IX. 67						
	exp	1.7	24.3	89.1	115.1	
Tusk, <u>Brosmius brosme</u>						
25.n.m. NW Marsteinen	obs	5	9	4	18	
24. V. 67						
W Ona, Møre	obs	1	4	2	7	
6. IX. 67						
Bressy Ground	obs	6	19	8	33	
8.-9. IX. 67						
Total	obs	12	32	14	58	0.48
	exp	13.4	28.9	15.7	58.0	

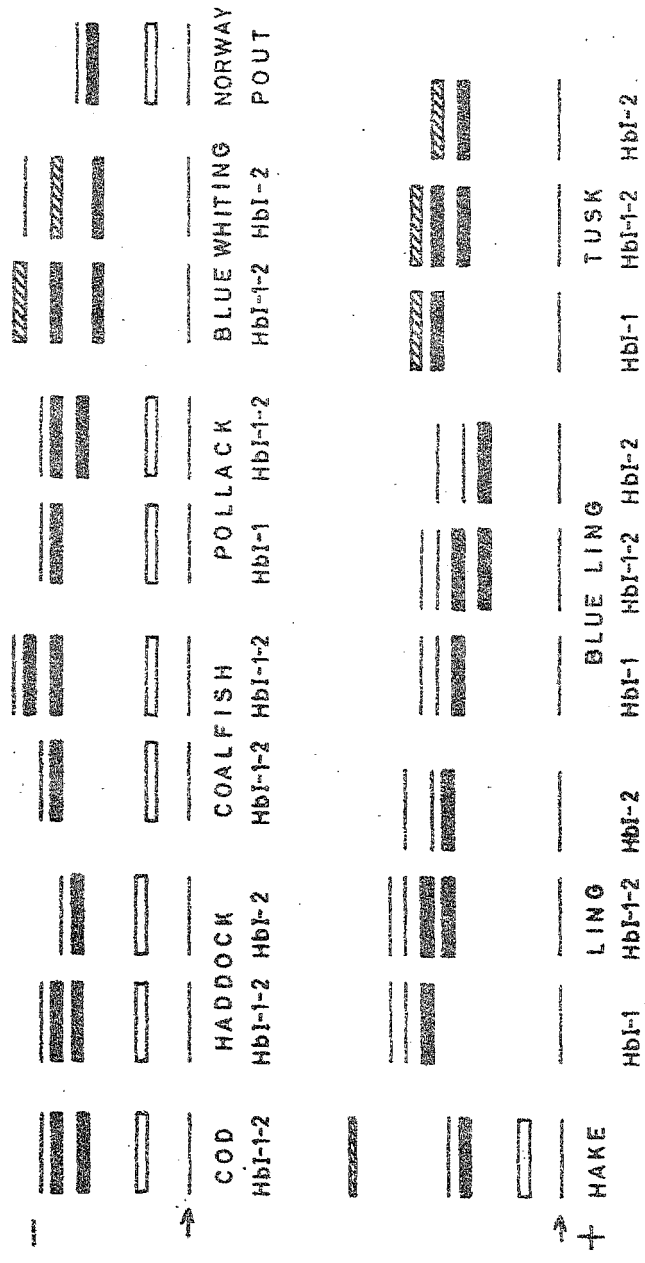


Fig. 1. Hemoglobin patterns of some gadoid fishes obtained by agar gel electrophoresis at pH 7.2. Legend: Filled in bars: Strong bands. Open bars: Hb II-components (moderately strong bands). Cross hatched bars: Other moderately strong bands. Single lines: Weak bands. Arrows indicate the points of application.