FiskDir. Skr. Ser. HavUnders., *15*: 91—97.

STUDIES ON HEMOGLOBINS OF SOME GADOID FISHES

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INTRODUCTION

During the last few years it has been shown that in fish hemoglobins there excist intraspecific variations of at least two types, namely ontogenetic as in salmon (KOCK, EVANS, and BERGSTRØM 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 1968) and some other clupeoid fishes (SIMPSON and SIMON SCHLOTHFELDT 1966) intraspecific hemoglobin variations have been described, but these variations were neither found to be assosiated 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 minor economic important species 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 after sampling.

Agar gel electrophoresis, described by (SICK 1965) was applied. Each run lasted for 60 minutes with about 50 volts between ends of gel. To control the results obtained by this method, part of the material was also analysed by combined starch and agar gel electrophoresis (MØLLER 1966) with 65 volts between ends of gel for 90 minutes.

Blood samples were collected from gadoid fishes from localities along the Norwegian coast and in the North Sea. Species, localities, date of

sampling and numbers are listed in Table 1 where the results also are presented. Scientific names and the order of the species are after SVETOVIDOV (1962).

RESULTS AND DISCUSSION

The hemoglobin components of all the species concerned here, moved towards the cathode in agar gel at pH 7.3. Fig. 1 shows the relative mobilities of the hemoglobin components and the hemoglobin pattern found for each species except forked hake. For comparison the cod hemoglobin type HbI–1–2 is shown. The hemoglobin of the forked hake moved only insignificantly by this method.

In all species, except forked hake, 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, 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 (Fig. 1).

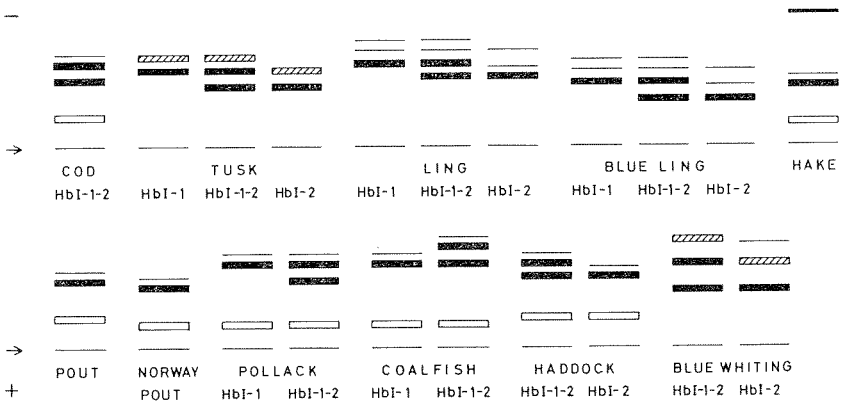

Fig. 1. Hemoglobin patterns of gadoid fishes obtained by agar-gel electrophoresis at pH 7,2. Filled in bars) strong bands, open bars) Hb II-components [moderately strong bands], cross hatched bars) other moderately strong bands, single lines) weak bands. Arrows indicate the points of application.

In tusk, ling and blue ling 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 HbI–1 and HbI–2 in order of decreasing cathodic mobility, and the hemoglobin types were called HbI–1, HbI–1–2, and HbI–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 allelomorphic genes, named HbI^1 and HbI^2, control the synthesis of the components HbI–1 and HbI–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 greatly supports this hypothesis (Table 1).

Two strong hemoglobin components, named HbI–1 and HbI–2, also occurred in pollack, coalfish, haddock and blue whiting, but only one of the single banded phenotypes was found in each species. However, the distribution and the calculated gene frequencies show that one of the hypothetical genes is too rare to be expected in a homozygotous state in the present material (Table 1). 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 of 288 differed from the normal hemoglobin pattern of this species. In hake, pout and Norway pout no individual variations in the strong hemoglobin components were found.

In all species the strong components were accompanied on their cathodic side by weaker components named HbI–1' and HbI–2'. SICK (1961) found that corresponding components in whiting and cod increased in strength upon storing. This was evidently also the case in the species concerned here.

In ling and blue ling other weak components, named HbI–1'' and HbI–2'', of still higher cathodic mobility were present (Fig. 1). Also these components varied among specimens, but they often had 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 HbII components of whiting and cod (SICK 1961) were found in hake, pout, Norway pout, pollack, coalfish and haddock at positions between the point of application and the major components. The relative mobilities of these components 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 HbII components could be detected in tusk, ling, blue ling and blue whiting.

The intraspecific variations described here could also be found by combined starch and agar gel electrophoresis at pH. 9.0 (anodal movement). This confirms that the variations are real molecular differences and not methodical artifacts. By this method also the hemoglobins of forked hake moved towards the anode, but no intraspecific variation were found in this species.

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

The different distribution of the hemoglobin types in the samples of ling (Table 1) indicate that different random mating populations excist in this species. However, the numbers of specimens are too low for reliable deductions.

The studies of protein polymorphism in fishes are increasing. Although yet insufficient to give a total survey, and despite different methods and lack of common reference, polymorph systems of one protein type often seem to be characteristic for one taxonomic group (family etc.), while systems of another protein are characteristc for others, for instance polymorphism in hemoglobins for *Gadidae* (Sick 1961) and in esterase for *Scombridae* (SPRAGUE 1967, NÆVDAL unpublished.).

SUMMARY

1. Hemoglobin of tusk, forked hake, ling, blue ling, hake, pout, Norway pout, pollach, coalfish, haddock and blue whiting were studied by agargel electrophoresis.
2. Both inter- and intraspecific variations in hemoglobin patterns occurred.
3. Intraspecific variations were found in tusk, ling, blue ling, pollack, coalfish (only one specimen differed from the «normal» pattern), haddock and blue whiting.
4. 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.
5. No indication of ontogenetic variation of hemoglobin patterns have been found.
6. Frequncy variations of the hemoglobin types in ling indicate segregetion in the population structure.

Table 1. Material analysed for hemoglobin polymorphism. Observed distributions of types compared with the expected distributions of genotypes according to Hardy-Weinberg law.

Species, locality, and date of sampling	Distribution of hemoglobin types						No.	Frequency of the *HbI*[1] allele
	HbI-1		HbI-1-2		HbI-2			
	obs.	exp.	obs.	exp.	obs.	exp.		
Tusk, *Brosme brosme* (Müll.)								
N. 60°20', E. 4°20' ..... 24/ 5—67	5		9		4		18	
N. 62°56', E. 6°07' ..... 6/ 9—67	1		4		2		7	
N. 61°52', E. 1°23' ..... 8/ 9—67	6		19		8		33	
Total	12	13.4	32	28.9	14	15.7	58	0.48
Forked hake, *Phycis blennoides* (Brünn.)								
N. 61°52', E. 1°23' ..... 8/ 9—67			monomorphic				38	
Ling, *Molva molva* (L).								
Tennholmen, Nordland ..... 6/10—66	—		3		4		7	
Myking, Hordaland ..... 4—67	—		5		9		14	
N. 61°52', E. 1°23' ..... 8/ 9—67	1		7		9		17	
Total	1	1.8	15	13.0	22	23.1	38	0.22
N. 60°51', E. 3°02' ..... 27/ 2—68	—		1		56		57	0.08
Blue ling, *M. dipterygia* (Penn.)								
N. 60°20', E. 4°20' ..... 24/ 5—67	1	1.0	12	12.3	38	37.7	51	0.14
N. 61°52', E. 1°23' ..... 8/ 9—67	2	1.7	24	24.3	89	89.1	115	0.12
Hake, *Merluccius merluccius* (L).								
N. 60°20', E. 4°20' ..... 24/ 5—67			monomorphic				15	
N. 61°52', E. 1°23' ..... 8/ 9—67			monomorphic				37	
N. 60°51', E. 3°02' ..... 27/ 2—68			monomorphic				9	

Pout, *Trisopterus luscus* (L.).									
N. 53°35', E. 2°56'	16/ 9—67			monomorphic				27	
Norway pout, *T. esmarkii* (Nilss.)									
N. 62°56', E. 6°07'	6/ 9—67			monomorphic				50	
Pollach, *Pollachius pollachius* (L.)									
Myking, Hordaland	4—67	76	76.2	5	4.7	—	0.01	81	0.97
Coalfish, *P. virens* (L.)									
Hordaland	16/12—65	—		—		114		114	
Veidholmen, Nordmøre	17/12—65	—		1		33		34	
Røstbanken, Lofoten	9/ 3—66	—		—		140		140	
Total		—		1		287		288	0.002
Haddock, *Melanogrammus eaglefinus* (L.)									
Hjelmsøy, Finnmark	28/ 5—65	—		1		115		116	
Malangen, Troms	28/10—65	—		1		97		98	
Total, Northern Norway		—		2		212		214	0.004
Myking, Hordaland	4—67	—	0.1	5	4.9	80	80.0	85	0.03
Blue whiting, *Micromesistius poutassou* (Risso)									
N. 60°20', E. 4°20'	24/ 5—67	—		—		50		50	
N. 59°15', E. 3°35'	19/ 8—67	—		—		87		87	
N. 57°32', E. 7°57'	22/ 8—67	—	0.1	4	4,3	70	69.9	74	0.03
N. 62°56', E. 6°07'	6/ 9—67	—		—		91		91	

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Received 30 April 1968

Printed 15 February 1969