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A PRELIMINARY REPORT ON STUDIES OF ESTERASE PHENOTYPES IN HERRING

by
Gunnar Nævdal
Fiskeridirektoratets Havforskningsinstitutt, Bergen

and
Didrik S. Danielsen
Statens Biologiske Stasjon Flødevigen, Arendal

Introduction

Transferrin types of herring, described by Nævdal and Haraldsvik (1966), appear to be useful in studies of segregation between herring populations. Also intraspecific variations in the enzyms lactate dehydrogenase and aspartate aminotransferrase have been applied in population studies of herring (Odense, Allen, and Leung 1966).

Electrophoretic studies on serum esterase were started in 1966. In this preliminary report an attempt is made to describe phenotypes and reveal the genetic basis of the observed variations.

Material and methods

Blood samples were obtained by cardiac puncture or cutting the tail. The samples were sent on ice in thermo bottles to the laboratory where they were cantrifuged and the sera were pipetted off. When possible the sera were analysed fresh, but some samples had to be stored for some weeks in the deep freeze before the analyses could be carried out.

The sera were analysed in combined starch and agar gel electrophoresis (Sick 1965, Møller 1966) for 75 minutes. For identification of the esterase phenotypes, the gels were immersed in a freshly prepared solution containing 4 ml 1% solution of 1-naphthyl acetate in acetone and 200 mg Fast Blue Salt BB in 100 ml of destilled water. The bands of esterase activity were then developed within a few minutes.

Sampling localities, numbers in samples, and date of sampling are listed

in Table 1.

Results and discussion

By combined starch and agar gel electrophoresis at pH 9.0 the esterase molecules moved towards the anode. One or two strong and maximum seven weak bands which represent esterase activity, were found. The electrophoretograms obtained are outlined in Fig. 1.

In most specimens only one strong esterase band was present. This band and the esterase molecular type which it represent, was named Es M(iddle). In some specimens a strong component, named Es F(ast), occurred at the anodic side of Es M, and a more cathodic component, designed Es S(low), appeared in a few specimens. Phenotypes with only the Es M-band were called Es MM, and phenotypes posessing in addition the Es F- or Es S-band, were called Es FM and Es MS respectively.

The four weak bands of greatest anodic mobility were supposed to belong to one group of esterase molecules and designed Es f_1, Es f_2, Es f_3, and Es f_4 respectiviely. The two middle weak bands were designed Es m_1 and Es m_2 and the slowest moving weak band, Es s. This band was screened by the stronger Es F band when the latter was present. The relative mobilities of the various bands are shown in Fig. 1. Especially the Es f_1 and Es f_2-bands varied considerably in strength, and occasionally they were nearly as strong as the Es M-component.

The phenotypes of the strong components could be determined from sera which had been frozen and thawed, but the patterns were clearer when fresh sera were used. The weak components, however, often were too diffuse to be determined with a reasonable degree of reliability in frozen sera.

The variations in the strong components might be explained by a hypothesis of three genes (probably allelic) called $\underline{Es^F}$, $\underline{Es^M}$, and $\underline{Es^S}$, which control the synthesis of Es F, Es M, and Es S respectively. Thus the phenotype Es MM is the phenotypic expression of the genotype $\underline{Es^M Es^M}$, and the phenotypes Es FM and Es MS represent the genotypes $\underline{Es^F Es^M}$ and $\underline{Es^M Es^S}$ respectively.

The hypothetical homozygotes Es FF and Es SS and the heterozygote Es FS were not found. However, it appears from the observed distributions in Table 1 that the Es F- and Es S-band and the hypothetical genes $\underline{Es^F}$ and $\underline{Es^S}$, are so rare that they cannot be expected in a homozygotous state or together in the same specimen in the present material.

No theory of a simple genetical control of the variations in the weak components is applicable. If these components are genetically controlled, several gene loci must be involved, or the variations must be caused by

formation of stable polymeres. For use in segragation studies of herring populations, the components Es m_1 and Es m_2 may have some value. One or both of these occurred in nearly all specimens, and when they were absent, it was always in specimens with weak total patterns. Therefore their absence may be due to low total concentration of esterase or low enzymatic activity in the sera. Three phenotypes occurred, and they were called Es m_1m_1, Es m_1m_2, and Es m_2m_2, according to which of the weak esterase bands they contained. Two allelomorphic genes, called $\underline{Es}^{m}1$ and $Es^{m}2$ respectively, were assumed to control these phenotypes. In Table 1 observed distributions of the m_1m_2-phenotypes are shown together with calculated frequencies of the hypothetical gene $Es^{m}1$. The numbers of specimens classified for Es m-types were lower than the numbers classified for types of strong components, because the weak bands were too weak for reliable classification in some specimens. Thus a great part of the specimens in sample 1 showed diffuse weak bands and therefore could not be classified in Es m-types, whereas the strong bands were clear enough. When observed distribution of phenotypes were compared to expected distribution of genotypes according to the Hardy-Weinberg law, fairly good accordance was found (Table 1), and except for sample 2 the deviations were not significant when tested by common χ^2-test. Sample 2 gave a significant excess of hypothetical heterozygotes, and therefore contradict the hypothesis, However, the good accordance between expected and observed distributions in the other samples supports the hypothesis. The problem cannot be solved by the present material, but analyses of new samples may give valuable population data. Breeding experiments have been planned to test the hypothesis of genetical control of the esterase phenotypes in herring.

It also follows from Table 1 that there was no great variations among samples in distributions of strong esterase phenotypes. Although the difference Es S- band was found only in sample 1, this do not show significant from the rest of the samples because the Es S-band occur at very low frequency also in sample 1. The Es F-band occurred at a low frequency in all samples, and it was not represented in sample 5.

However, the distributions of the Es m-types varied considerably, and Es m_1m_1 occurred at higher frequencies among the autumn spawners from the North Sea than among spring spawners from the Norwegian coast. Sample 1 and 5, collected at adjacent localities, but at different times of the year, differed somewhat, but here it should be taken into account that sample 1 contained spring spawners in spawning condition, while in sample 5 the spring spawners seemed to be mixed with some autumn spawners.

Summary

Herring serum esterase has been studied by combined starch and agar

gel electrophoresis. Both strong and weak bands which represented esterase activity, occurred. Most specimens contained one strong band of intermediate anodic mobility. Two bands of respectively higher and lower mobility, occurred at low frequencies, and always in combination with the middle band.

Maximum seven weak bands were found. Considerable variations among specimens occurred in these bands. No theory of genetic control of the total variations can be given at present, but two co-dominant alleles may be responsible for the variations in two of the weak bands.

Frequency variations among some of the samples were indicated.

References

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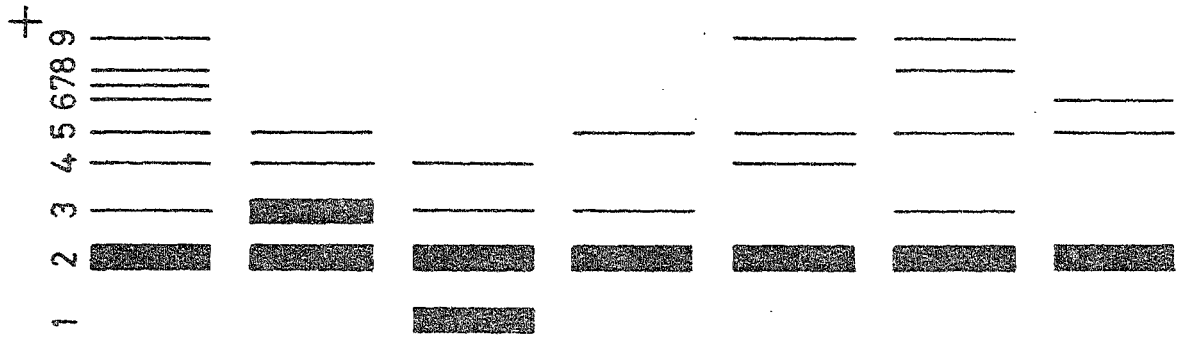
Table 1. Distribution of esterase phenotypes in herring samples from the Norwegian coast and the North Sea.

Sample no., locality and date		Types of weak esterase zones					Types of strong esterase zones						Indications of sample
		Es m_1m_1	Es m_1m_2	Es m_2m_2	No.	Frequency of Es^{m1}	Es FM	Es M	Es MS	No.	Frequency of Es^F	Frequency of Es^S	
1. Jomfruland 4.IV.67	obs.	13	26	5	44	0.59	2	86	2	90	0.01	0.01	Matured spring spawners
	exp.	15.3	21.3	7.4	44.0								
2. Austfjorden, Hordaland. April 67	obs.	30	49	7	86	0.63	4	93	-	97	0.02	-	Matured spring spawners
	exp.	34.1	40.1	11.8	86.0								
3. Flødevigen, Arendal. 24.IV.67	obs.	36	40	14	90	0.62	4	96	-	100	0.02	-	Matured spring spawners
	exp.	34.6	42.4	13.0	90.0								
4. Egersundbanken, North Sea. 20.V.67 57°30'N 06°00'E	obs.	91	21	1	113	0.90	7	131	-	138	0.03	-	Mainly autumn spawners
	exp.	91.5	20.3	1.1	112.9								
5. Langesund. 11.VIII.67	obs.	68	14	2	84	0.89	-	84	-	84	-	-	Probably mixed (spring and autumn spawners)
	exp.	66.5	16.4	1.0									
6. Bløden, North Sea. 24.-25.VIII.67 56°10'N 05°20'E	obs.	78	30	1	109	0.85	6	112	-	118	0.03	-	Mainly autumn spawners
	exp.	78.8	27.8	2.5									



ig. I. Esterase patterns in herring by combined starch and agar gel electrophoresis at pH 9.0 for 90 minutes.
Legend: 1: Es S, 2:Es M, 3: Es s(weak) and Es F(strong), 4: Es m_2, 5: Es m_1, 6: Es f_4, 7: Es f_3, 8: Es f_2, 9: Es f_1.