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STUDIES ON SERUM ESTERASE IN HERRING AND SPRAT

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INTRODUCTION

Serology and molecular biology have been used in segregation studies of herring, *Clupea harengus* L., on several occasions. SINDERMANN and MAIRS (1959) found two herring populations in the Gulf of Main by frequencies of erythrocyte antigenes. They also found variability in serum protein patterns. Some variations were found to be connected with diseases, some were probably related to age, but some offered hope of electrophoretic characterization of the populations (SINDERMANN and MAIRS 1958, MAIRS and SINDERMANN 1960). SINDERMANN and HONEY (1963) did not find intraspecific variations in electrophoretic mobility of herring hemoglobins, and WILKINS and ILES (1965) found hemoglobin types related to body length. Transferrin types were described by NÆVDAL and HARALDSVIK (1966), and ODENSE, ALLEN and LEUNG (1966) applied types of the enzymes lactate dehydrogenase and aspartate aminotransferrase in studies on Canadian herring populations.

In the present paper intraspecific variations in herring esterase phenotypes are described, and an attempt is made to reveal the genetic basis of the variations. Intraspecific variations in esterase phenotypes of sprat, *Sprattus sprattus* (L.), are also studied.

Part of the results concerning herring have been presented in a preliminary report (NÆVDAL and DANIELSEN 1967).

MATERIAL AND METHODS

Blood was sampled by cardiac puncture or by cutting the tail. The samples were sent on ice in thermo bottles to the laboratory where they were centrifuged. Most sera were analysed fresh, but some samples had to be stored deep frozen for some weeks before the analyses could be carried out.

The sera were analysed by combined starch and agar gel electrophoresis (SICK 1965, MØLLER 1966) for 75 minutes. For identification of

the esterase components, the gels were immersed in a freshly prepared solution containing 4 ml 1 % solution of 1-naphtyl 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 few minutes.

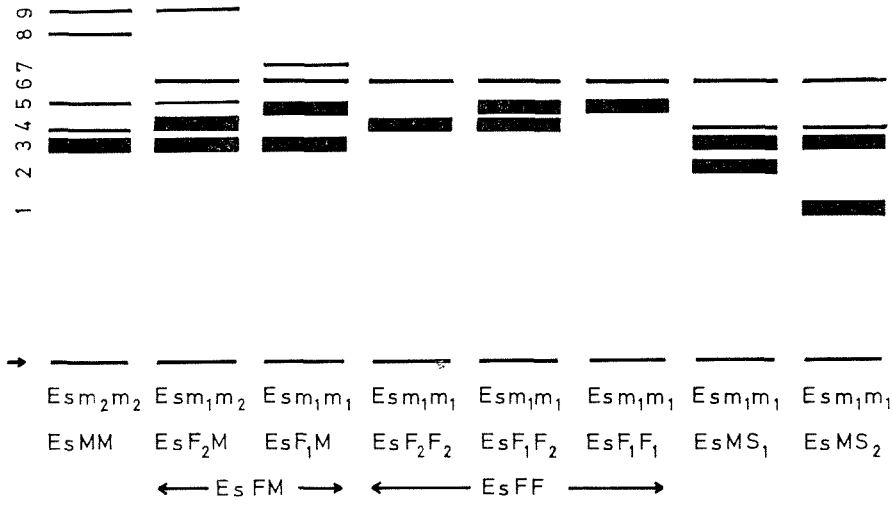
Sampling localities, numbers in samples and date of sampling are listed in Table 1 (herring) and Table 2 (sprat).

RESULTS AND DISCUSSION

HERRING

The esterase molecules moved towards the anode. One or two strong and up to six weak esterase bands were found. The electrophoretograms are outlined in Fig. 1.

In most specimens only one strong esterase band was present. This band and the corresponding esterase molecular type was named Es M (middle). Two bands of higher anodic mobility than Es M occurred



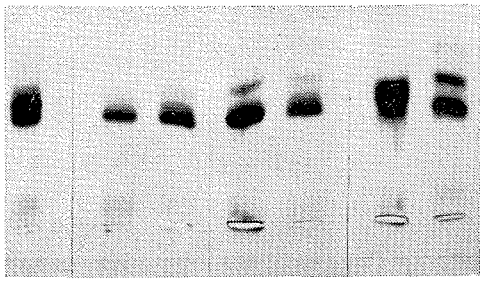


Fig. 1. Outline of serum esterase phenotypes in herring by combined starch and agar gel electrophoresis at pH 9.0, together with a photograph of patterns obtained by routine analyses. Legend: Filled in bars: Strong bands. Single lines: Weak bands. Arrow indicate the point of application.
1: Es S_2, 2: Es S_1, 3: Es M, 4: Es s(weak) and Es F_2(strong), 5: Es m_2 (weak) and Es F_1(strong), 6: Es m_1, 7: Es f_3, 8: Es f_2, 9: Es f_1.

Table 1. Observed distributions of esterase phenotypes in herring compared with expected distributions according to the Hardy-Weinberg law.

Sample no.	Locality and date of sampling	Indications of sample		Types of weak esterase zones					Types of strong esterase zones								
				Es $m_1 m_1$	Es $m_1 m_2$	Es $m_2 m_2$	No.	q_1	Es FF	Es FM	Es MM	Es MS_1	Es MS_2	No.	q_F	q_{S_1}	q_{S_2}
1.	Austfjorden, Hordal. 30 March—15 May 1967	Spring spawners in spawning condition	obs.	30	49	7	86	0.63	—	4	93	—	—	97	0.02	—	—
			exp.	34	40.1	11.8			0.04	3.8	93.2	—	—		—	—	—
2.	61°10' N, 06°00' E North Sea May 1967	Mainly immatured autumn spawners	obs.	91	21	1	113	0.90	—	7	131	—	—	138	0.03	—	—
			exp.	91.5	20.3	1.1			0.1	8.0	129.8	—	—				
3.	Masfjorden, Hordal. 12 June 1967	Immatured	obs.	67	16	3	86	0.87	1	5	89	2	—	97	0.04	0.01	—
			exp.	65.0	19.5	1.5			0.2	7.4	87.5	1.8	—				
4.	61°10' N, 00° 35' W, North Sea 17 June 1967	Adult autumn spawners	obs.	43	1	—	44	0.99	—	2	47	—	—	49	0.02	—	—
			exp.	43.1	0.9	0.0			0.02	1.9	47.1	—	—				
5.	61°10' N, 00°35' W, North Sea 17 June 1967	Adult spring spawners	obs.	47	—	—	47	1.00	—	3	46	—	1	50	0.03	—	0.01
			exp.	47.0	—	—			0.05	2.9	46.1	—	1.0				
6.	58°11' N, 03°48' E, North Sea 1 July 1967	Adult autumn spawners	obs.	87	3	—	90	0.98	—	1	96	—	—	97	0.05	—	—
			exp.	86.4	3.5	0.0			0.0	1.0	96.0						
7.	55°00' N, 06°00' E, North Sea 24/25 Aug. 1967 ..	Adult autumn spawners	obs.	78	30	1	109	0.85	—	6	112	—	—	118	0.03	—	—
			exp.	78.8	27.8	2.5			0.1	6.9	111.0	—	—				
8.	Tistam, Nordfjord . 14 Oct. 1967	0-gr.	obs.	79	3	—	82	0.98	—	13	85	—	—	98	0.07	—	—
			exp.	78.8	3.2	0.04			0.5	12.8	84.8	—	—				
9.	Borgenfj., Trøndelag 26 Oct. 1967	0-gr.	obs.	98	5	—	103	0.98	—	7	95	1	—	103	0.03	0.005	—
			exp.	98.9	4.0	0.04			0.1	6.0	95.9	1.0	—				
10.	57°35' N, 10°55' E, Kattegat 8 Nov. 1967.....	1-gr., autumn spawners	obs.	68	8	1	77	0.94	3	7	76	—	—	86	0.08	—	—
			exp.	68.0	8.7	0.3			0.6	12.7	72.8	—	—				

in some specimens. These components, named Es F_1 (fast) and Es F_2, were in most cases found together with Es M. In only one specimen Es F_1 and Es F_2 occurred together. Distinction between Es F_1 and Es F_2 was difficult in routine analyses, and therefore they were lumped together and named Es F. Two bands, named Es S_1 (slow) and Es S_2, of anodic mobility lower than Es M also occurred at low frequencies, and always in combination with Es M. The mobility of Es S_2 differed greatly from the mobility of Es M, while the mobilities of Es M and Es S_1 were little different.

The phenotype with only the Es M component was named Es MM, and the other phenotypes were named according to the components they contained, i.e. Es FM, Es FF, Es MS_1 and Es MS_2 (Fig. 1).

The three weak bands of greatest anodic mobility were supposed to belong to one group of esterase molecules and designed Es f_1, Es f_2 and Es f_3. The two weak bands of intermediate mobility were designed Es m_1 and Es m_2. Additional bands occurred near Es m_1 and Es m_2 in some specimens, but they could not be effectively separated from them by the present method, and therefore they have been omitted in the following discussion.

The slowest moving weak band, named Es s, (and partly also Es m_2) were screened by the stronger Es F bands when one of 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, but the patterns were clearer when fresh sera were used. The weak components, however, often were too diffuse to be determined from frozen sera.

The strong components may be explained as the product of separate genes (possibly allelic) named Es^F, Es^M, Es^{S_1}, and Es^{S_2} (where indices indicate the components which the genes are supposed to control). The hypothetical homozygotes Es S_1S_1 and Es S_2S_2 and the heterozygotes Es FS_1 and Es FS_2 were not found, but it appears from the distributions of phenotypes in Table 1 that the genes Es^F Es^{S_1} and Es^{S_2} were so rare that the lacking combinations should not be expected in the present samples. Except for sample 10 the population data are in fairly good accordance with expected Hardy-Weinberg distributions, and this supports the introduced hypothesis. Sample 10, however, show a surplus of hypothetical homozygotes and therefore to some extent contradict the hypothesis. If this sample is drawn from a panmixed population, the result indicates alternative explanations of the variations (genetic or non genetic).

If the variations in the weak components are genetically controlled, several gene loci must be involved, or the variations must be caused by formation of stable polymeres. For use in segregation studies of herring populations, the components Es m_1 and Es m_2 may have some importance. One or both of these occurred in nearly all specimens. When they were absent, it was always in specimens with weak total consentration of esterase or with 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. Two allelomorphic genes, called Es^{m_1} and Es^{m_2}, were assumed to control these phenotypes. In Table 1 are listed the observed distributions of the m_1m_2-phenotypes and the calculated frequencies of the hypothetical gene Es^{m_1}. The numbers of specimens classified as m_1m_2-types were lower than the numbers classified as types of strong components, because the m_1m_2-bands in some specimens were too weak for reliable classification. When observed distributions of phenotypes were compared to expected distributions of genotypes according to the Hardy-Weinberg law, fairly good accordance was found (Table 1,) and except for sample 1 the deviations were not significant when tested by common χ^2-tests. Sample 1 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.

Breeding experiments have been planned to test the hypothesis of genetical control of the esterase phenotypes in herring.

There was no evidence of dependence on factors other than genetic of the variations of esterase phenotypes. The variations occurred in all age groups and in both sexes. However, it should be emphasized that the present analyses were only qualitative, and that variations in strength of esterase activity may occur which were not recorded by the present method.

Table 1 shows that there was no great variations among samples in distributions of strong esterase phenotypes. Es S_1 and Es S_2 were only found at very low frequencies in two and one sample respectively, but this did not show significant differences from the rest of the samples. The Es F bands occurred at low frequencies in most samples, but in samples 8 and 10 their hypothetical controlling gene was found at a frequency of 0.07 and 0.08 respectively, indicating real differences among the populations from which the samples were drawn.

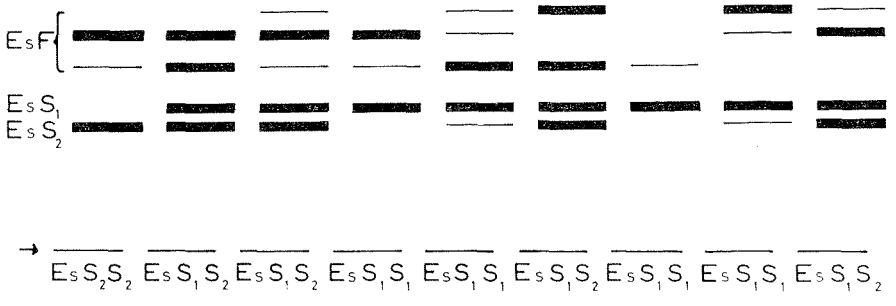
The distributions of the Es m types varied considerably, and although Es m_1m_1 occurred at high frequencies in most samples, it was found at lower frequencies in spring spawners from the coast (samples 1 and 3) and in one sample of autumn spawners from the North Sea (Sample 7).

SPRAT

Very extensive and complicated variations in serum esterase were revealed in sprat by combined starch and agar gel electrophoresis, and several patterns were found (Fig. 2). The patterns comprise variation in at least five zones of weak esterase activity. The three fastest moving components were supposed to belong to one group of esterase components and called Es F (fast) while the two slower moving were called Es S

Table 2. Observed distributions of esterase phenotypes in one year old sprat compared with expected distribution according to the Hardy-Weinberg law.

Sample no., locality and date of sampling		Esterase phenotypes			No.	q_1
		EsS_1S_1	EsS_1S_2	EsS_2S_2		
1. Kattøya, Langesund-fjorden	obs	15	35	33	83	0.39
3 Oct. 1966	exp	12.6	39.5	30.9		
2. Nå, Hardangerfjorden	obs	19	38	30	87	0.44
14 Oct. 1966	exp	16.8	42.9	27.3		
3. Risnes, Masfjorden	obs	10	44	30	84	0.38
4 June 1961	exp	12.1	39.6	32.3		
4. Skorpo, Hardangerfjorden.	obs	29	31	8	68	0.65
6 June 1967	exp	28.7	30.9	8.3		
5. Gjermundshamn, Hardangerfjorden	obs	22	21	6	49	0.66
6 June 1967	exp	21.3	22.0	5.7		



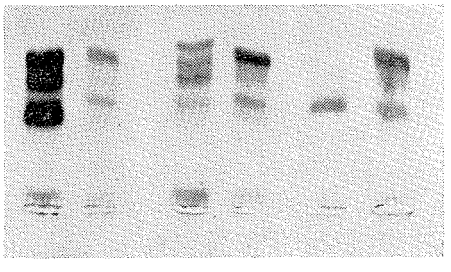


Fig. 2. Outline of serum esterase phenotypes in sprat by combined starch and agar gel electrophoresis at pH 9.0, together with a photograph of patterns obtained by routine analyses. Legend: Fig. 1.

(slow). A considerable part of the specimens showed diffuse electrophoretograms, and only the two slowest moving components, named Es S_1 and Es S_2 (which sometimes appeared double), were clear enough to form the basis for classification. One or both of these components were present in all specimens. When not taking into account the weak bands which often were present at position of lacking strong bands (Fig. 2), the specimens could be classified into three phenotypes on basis of the variations in Es S_1 and Es S_2 bands. These phenotypes were called Es S_1S_1, Es S_1S_2, and Es S_2S_2 according to which of the bands they posessed. The distributions of the phenotypes in five samples are shown in Table 2.

When a hypothesis of genetical control involving two allellomorphic genes, named Es^{S_1} and Es^{S_2}, is introduced, it appears that there are fairly good accordance between observed distributions of phenotypes and expected distributions of genotypes (Table 2). The hypothesis may accordingly explain the present variation in the zones Es S_1 and Es S_2. However, the variations in the other zones are still unexplained, and it is impossible to have any idea of the control of these variations as long as the specimens can not be classified with a reasonable degree of reliability.

It appears from Table 2 that there were considerable differences among samples in distributions of the phenotypes, and thus in frequencies of the hypothetical genes. Although the type determinations may be somewhat unreliable, the variations among samples were greater than may be explained by incorrect type determination or by errors of sampling. Thus the differences probably represent real differences between the populations from which the samples were drawn. This coincides with results from analyses of hemoglobins and transferrins (NÆVDAL 1968 and unpublished) which show significant frequency variations among samples of sprat from Norwegian waters. But because the type determination are somewhat unprecise, variations in esterase patterns in sprat appear at present to be of little value in segregation studies.

SUMMARY

1. Herring and sprat serum esterase has been studied by combined starch and agar gel electrophoresis at pH 9.0. Both strong and weak bands which represented esterase activity occurred in both species.
2. Most herring specimens contained one strong component of intermediate anodic mobility. Two strong components of higher and two of lower anodic mobility occurred at low frequencies. A hypothesis of genetical control by one gene controlling each of the components is proposed.

3. Maximum six weak bands of herring serum esterase 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 codominant alleles may be responsible for the variation in two of the weak bands.
4. The intraspecific variations in sprat serum esterase were complicated, and it was difficult to classify the specimens into well defined groups on basis of these variations. A hypothesis of control by two allelic genes of the variations in two of the components is introduced.
5. Frequency variations among some of the samples of both species were indicated.

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