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DISTRIBUTIONS OF MULTIPLE FORMS OF LACTATE DEHYDROGENASE, ASPARTATE AMINOTRANSFERASE AND SERUM ESTERASE IN HERRING SAMPLES FROM NORWEGIAN WATERS

By

Gunnar Nævdal
Institute of Marine Research, Bergen

INTRODUCTION

Polymorphisms in the enzymes lactate dehydrogenase (LDH) and aspartate aminotransferase (AAT) of herring, Clupea harengus, were described by Odense, Allan and Leung (1966a, b). In LDH five different phenotypes were found. The LDH molecule consists of two kinds of monomers designated A and B under control by separate loci. The five different phenotypes represent two mutant alleles at the B locus and one at the A locus. In AAT two rare mutant alleles were observed.

Intraspecific variation both in weak and in strong components of serum esterase have been described, and the two groups of components were assumed to be controlled by genes at separate loci (Nævdal 1969).

The present paper deals with electrophoretic analyses of LDH and esterase phenotypes in herring samples from Norwegian waters, especially variation among samples from different localities. Analyses of AAT based on a limited material is reported.

MATERIAL AND METHODS

Sampling localities are shown in Fig. I and listed in Tables I and III together with sampling dates, numbers of specimens in each sample and other available data.

LDH types were determined from analyses of sera in samples numbered 10-15 and 20, and from muscle extract in the other samples. Sample 19 was analyzed for AAT types from muscle extract. Analyses of esterase were performed on all samples of which sera were available.

Combined starch and agar gel electrophoresis (Møller 1967) for three hours was carried out to reveal the LDH types and for two hours to reveal the AAT types. The zones of enzyme activity were stained as described by Odense et al. (1966a, b). Analyses of esterase were carried out as described previously (Nævdal 1969).

RESULTS AND DISCUSSION

The relative mobility of the esterase component and the observed phenotypes are outlined in Fig. II. Distributions of esterase phenotypes are shown in Tables I and II. Relatively good accordance between observed distributions and expected Hardy-Weinberg distributions were found, but in some samples there was an excess of hypothetical homozygotes. This may be caused by mixing of individuals from populations which differ in frequencies of the esterase controlling genes. The hypothesis of genetical control (Nævdal 1969) still seems to be valid, but modifications or other explanations cannot be excluded.

Several bands in the region of the Es m_1 and Es m_2 components are indicated on some electrophoretograms (Fig. II), but they showed only small differences in electrophoretic mobility, and separation into more phenotypes is hardly possible by the present method. Destinction between the m_1 and m_2 phenotypes only is therefore a simplification which may reduce the reliability of the type determinations. In addition the weakness of the components may cause errors. However, differences of the order observed among some of the present samples (see below), cannot be explained as a result of incorrect type determinations alone. Due to the weakness of the zones the numbers of specimens determined for m_1m_2 types were often lower than the numbers determined for types of strong esterase components and LDH.

Patterns similar to the LDH phenotypes described by Odense et al. (1966 a, b) were found in the present material, and they were interpreted to represent the same genotypes. The observed LDH phenotypes were named AABB, AABB', AAB'B', AABB'' and AA'BB by Odense et al. (1966a, b), where B' and B'' represent mutant alleles at the B locus and A' represent a mutant allele at the A locus. The LDH components were well separated by the present method, and the phenotypes usually easily recognized.

The distributions of the phenotypes are given in Table III.

Muscle extract showed stronger LDH activity than sera, and all specimens could be termined for LDH phenotypes from muscle extract. When sera

were analyzed a few specimens of some samples could not be determined due to the weakness of the zones, and thus the numbers of specimens determined for LDH types were lower than the numbers determined for esterase types in some samples. When store frozen the LDH activity of sera was reduced considerably after a few weeks, while it persisted for at least half a year in muscle.

Of the 100 specimens analyzed for AAT types, two showed a three band pattern which was interpreted as the phenotype SS' of Odense et al. (1966 a, b), while all the others showed a one band pattern, probably the one named SS.

Comparing the results of the present study with corresponding results from the West Atlantic (Odense et al. 1966a, b), the B' gene seems to be somewhat more frequent on the European side, while the S' gene seems to occur at about the same frequency on both sided of the Atlantic as far as can be stated from the present limited material. The LDH genes B'' and A' were found only in two and one specimens respectively, and the AAT gene S'' were not found at all in the samples from Norwegian waters, but they were also very rare in the samples from the West Atlantic.

It appears from Tables I, II and III that variations in frequency distributions of phenotypes among samples occurred in both groups of esterase and in LDH. The significance of these variations have been tested by χ^2-homogeneity tests.

Among the total samples the variations were significant at the one per cent level in both groups of esterase, while the variations in distributions of LDH phenotypes were insignificant.

When treating the samples of North Sea autumn spawners separately, the differences in distributions of the types of weak esterase zones were found to be significant, while the variations in distributions of types of strong esterase components and LDH types were insignificant. This variation was largely contributed to by sample 7 which were collected at Bløden Ground and probably represent the Down herring stock, while the other samples more likely represent either the Bank herring stock or Kattegat autumn spawners (Haraldsvik 1969). Sample 10, collected in Kattegat, deviated from the North Sea samples with regard to distributions of types of strong esterase zones (low numbers of specimens prevented statistical tests), but not in types of weak esterase components.

All specimens in sample 5 of North Sea spring spawners were of the Es m_1m_1 phenotype, and the sample contained one specimen with the rare Es S_2 component. However, the small numbers of specimens in this sample prevented further conclusions. Unfortunately, analyses of LDH phenotypes were not undertaken on the first ten samples.

When treating the samples collected from inshore waters separately, the variation among samples was found to be significant at the one per cent level in distributions of types of both groups of esterase, and at the 5 per cent level in distribution of LDH phenotypes. Some samples, coincided with the North Sea samples (for instance no. 14 and 15 from the Oslofjord) or with samples from Norwegian spring spawners (for instance no. 18), while others showed frequency distributions not found in samples from offshore waters. No marked geographical trend could be seen in the variations. The results indicate that the herring in Norwegian inshore waters may be recruited both by immigration from offshore waters (by drift of larvae or by active immigration) or from local spawning, and that herring in these areas, especially the small herring, may represent different groups in different years and areas. However, for a complete account of the herring stocks in inshore waters and the origin of the young herring which occur in the fjords, the present material is too limited.

It may be concluded that the present study on herring enzymes has shown that among groups of herring in Norwegian waters and the North Sea significant variations excist in characteristics which problably are genetically controlled and not affected by environmental factors.

SUMMARY

1. By use of combined starch and agar gel electrophoresis, 20 samples (totally 2191 specimens) were studied for serum esterase polymorphism, 13 samples (1454 specimens) for lactate dehydrogenase (LDH) polymorphisms and one sample (100 specimens) for asparate aminotransferrase (AAT) polymorphism.
   The samples were collected in Norwegian waters and the North Sea.

2. In two groups of esterase (weak and strong components) and in LDH and AAT intraspecific, hereditable variations were observed.

3. Frequencies of LDH and AAT phenotypes were found to be similar to corresponding frequencies observed in samples from Canadian waters.

4. Statistically significant variations among samples were observed in distributions of the phenotypes, especially the esterase phenotypes.

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Table I. Observed distributions of esterase phenotypes in herring compared with expected distributions according to the Hardy-Weinberg law (Nævdal 1969).

Sample no	Locality and date of sampling	Indications of sample		Types of weak esterase zones					Types of strong esterase zones								
				Es m_1m_1	Es m_1m_2	Es m_2m_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, Hordaland	Spring spawners	obs.	30	49	7	86	0.63	-	4	93	-	-	97	0.02	-	-
	30 March-15 May 1967	in spawning condition	exp.	34	40.1	11.8			0.04	3.8	93.2	-	-		-	-	-
2	57°30'N, 06°00'E,	Mainly immatured	obs.	91	21	1	113	0.90	-	7	131	-	-	138	0.03	-	-
	North Sea 20 May 1967	autumn spawners	exp.	91.5	20.3	1.1			0.1	8.0	129.8	-	-				
3	Masfjorden, Hordaland	Immatured	obs.	67	16	3	86	0.87	1	5	89	2	-	97	0.04	0.01	-
	12 June 1967		exp.	65.0	19.5	1.5			0.2	7.4	87.5	1.8	-				
4	61°10'N, 00°35'W,	Adult autumn	obs.	43	1	-	44	0.99	-	2	47	-	-	49	0.02	-	-
	North Sea 17 June 1967	spawners	exp.	43.1	0.9	0.0			0.02	1.9	47.1	-	-				
5	61°10'N, 00°35'W,	Adult spring	obs.	47	-	-	47	1.00	-	3	46	-	1	50	0.03	-	0.01
	North Sea 17 June 1967	spawners	exp.	47.0	-	-			0.05	2.9	46.1	-	1.0				
6	58°11'N, 03°48'E,	Adult autumn	obs.	87	3	-	90	0.98	-	1	96	-	-	97	0.05	-	-
	North Sea 1 July 1967	spawners	exp.	86.4	3.5	0.0			0.0	1.0	96.0						
7	55°00'N, 06°00'E,	Adult autumn	obs.	78	30	1	109	0.85	-	6	112	-	-	118	0.03	-	-
	North Sea 24/25 Aug.67	spawners	exp.	78.8	27.8	2.5			0.1	6.9	111.0	-	-				
8	Tistam, Nordfjord	0-group	obs.	79	3	-	82	0.98	-	13	85	-	-	98	0.07	-	-
	14 Oct. 1967		exp.	78.8	3.2	0.04			0.5	12.8	84.8	-	-				
9	Borgenfj., Trøndelag	0-group	obs.	98	5	-	103	0.98	-	7	95	1	-	103	0.03	0.005	-
	26 Oct. 1967		exp.	98.9	4.0	0.04			0.1	6.0	95.9	1.0					
10	57°35'N, 10°55'E,	1-group	obs.	68	8	1	77	0.94	3	7	76	-	-	86	0.08	-	-
	Kattegat 8 Nov. 1967	autumn spawners	exp.	68.0	8.7	0.3			0.6	12.7	72.8	-	-				

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

Sample no.		Types of weak esterase zones					Types of strong esterase zones								
		Es m_1m_1	Es m_1m_2	Es m_2m_2	No.	q_1	Es FF	Es FM	Es MM	Es MS	Es MS_2	No.	q_F	q_{S_1}	q_{S_2}
11	obs.	87	8	-	95	0.96	-	6	90	-	-	96	0.03	-	-
	exp.	87.6	7.3	0.2			0.1	5.6	90.3	-	-				
12	obs.	67	12	1	80	0.91	1	16	83	-	-	100	0.09	-	-
	exp.	66.2	13.1	0.6			0.8	16,4	82,8	-	-				
13	obs.	81	10	3	94	0.91		3	103	-	-	106	0.01	-	-
	exp.	77.8	15.4	0.8			0.01	2.9	103.1	-	-				
14	obs.	106	14	1	121	0.93	-	1	123	-	-	124	0.004	-	-
	exp.	104.7	15.8	0.6			0.0	1.0	123.0	-	-				
15	obs.	68	2	1	71	0.96	-	1	79	-	-	80	0.01	-	-
	exp.	65.4	5.5	0.1			0.01	1.6	78.4	-	-				
16	obs.	40	21	3	64	0.79	5	16	72	-	-	93	0.14	-	-
	exp.	39.9	21.2	2.8			1.8	22.4	68.8	-	-				
17	obs.	51	31	5	87	0.76	4	11	87	2	-	104	0.09	0.01	-
	exp.	50.3	31.7	4.9			0.8	16.8	84.2	1.9	-				
18	obs.	69	16	6	91	0.85	-	11	89	-	-	100	0.06	-	-
	exp.	65.7	23.2	2.0			0.4	11.3	88.3	-	-				
20	obs.	183	88	9	280	0.81	1	15	276	3	1	296	0.03	0.01	0.002
	exp.	183.7	86.2	10.1			0.3	17.0	274.5	2.8	1.1				
23	obs.	139	16	3	158	0.93	1	3	152	3	-	159	0.02	0.01	-
	exp.	136.7	20.6	0.8			0.04	5.0	150.8	3.1	-				

Table III. Distribution of LDH phenotypes in samples of herring from the Norwegian coast and the North Sea.

Sample no	Locality and date of sampling	Indications of sample	LDH phenotypes AABB	AABB'	AAB'B'	AABB''	AA'BB	No.	Gene frequency B'	B''	A'
11	60°31'N, 00°05'E, North Sea, 19-20 June 68	Adult autumn spawners	81	11	1	-	-	93	0.07	-	-
12	Eid, Nordfjord, 1 July 68	1-group	93	4	-	-	-	97	0.02	-	-
13	57°10'N, 07°40'E, North Sea 2 Aug. 68	Immatured autumn spawners	76	9	1	-	-	86	0.06	-	-
14	Bastøy, Oslofjorden 30 Aug. 68	Immatured, about 20 cm	113	9	-	-	-	122	0.04	-	-
15	Nøtterøy, Oslofjorden 30 Aug. 68	0-group	72	5	-	-	-	77	0.03	-	-
16	Langfjorden, Romsdal 21 Sept.68	0-group	102	18	-	-	-	120	0.08	-	-
17	Langfjorden, Romsdal 21 Sept.68	Immatured, 13-16 cm	86	11	-	-	-	97	0.06	-	-
18	Langfjorden, Romsdal 21 Sept.68	Immatured, 16 cm	91	8	-	-	-	99	0.04	-	-
19	64°25'N, 08°25'E, off Trøndelag 20 Febr. 69	Norwegian spring spawners	92	7	1	-	-	100	0.05	-	-
20	64°02'N, 08°25'E, off Trøndelag 26 Febr. 69	Norwegian spring spawners	196	20	1	-	-	217	0.05	-	-
21	60°40'N, 02°30'E, North Sea 24 March 69	Mainly autumn spawners	88	5	1	1	-	95	0.04	0.005	-
22	Langesundsfjorden near Porsgrunn 26 March 69	Adult spring spawners	87	6	-	1	1	95	0.03	0.005	0.005
23	Ålf[illegible]n, Nordfjord 2 June 69	Immatured	131	25	-	-	-	156	0.08	-	-

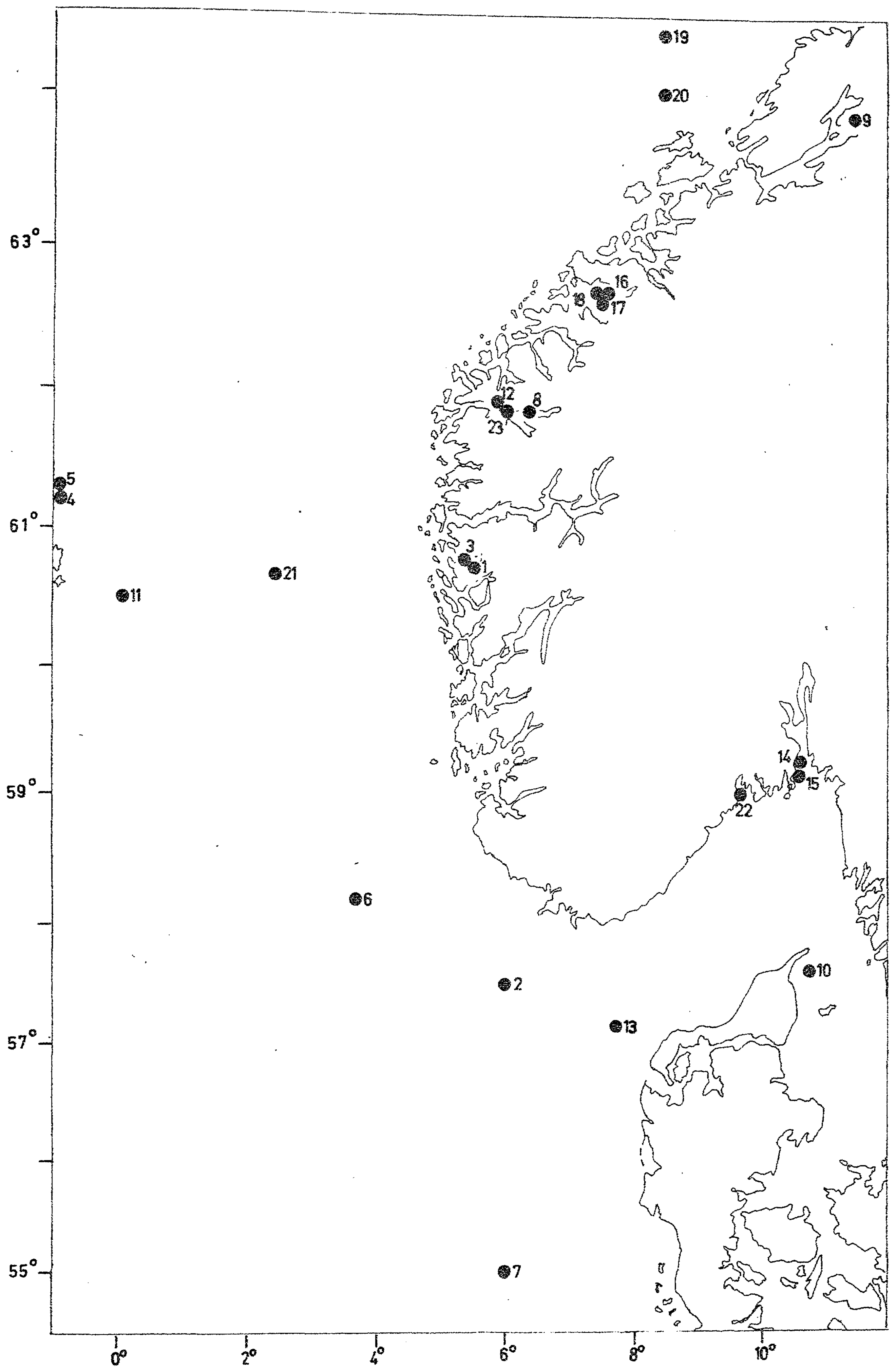

Fig. I. Sampling localities of blood samples of herring from the Norwegian coast and the North Sea.

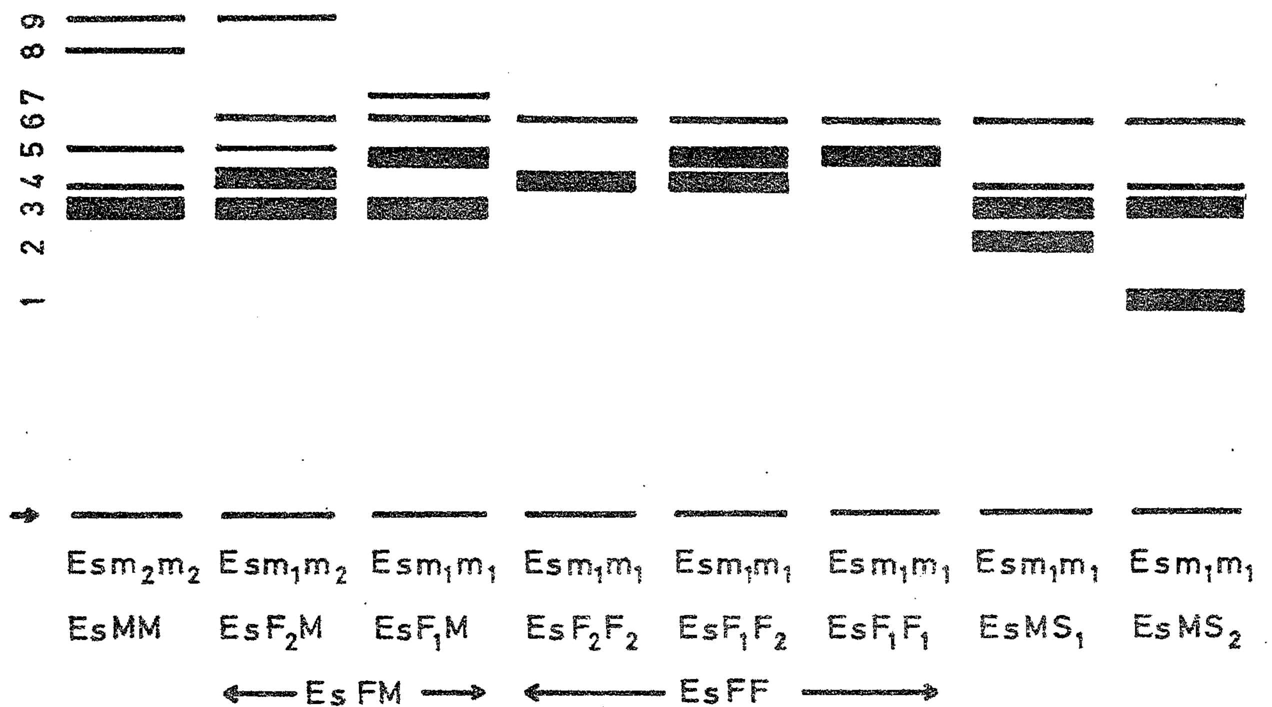

Fig. II. Outline of serum esterase phenotypes in herring by combined starch and agar gel electrophoresis at pH 9.0. Legend: Filled in bars: Strong bands. Single lines: Weak bands. Arrow indicates the point of application. 1: Es S_2, 2: Es S_1, 3: Es M, 4: Es s(weak) and Es F_2(strong), 5: Es m_2(weak)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