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DISTRIBUTIONS OF MULTIPLE FORMS OF LACTATE DEHYDROGENASE, ASPARTATE AMINOTRANSFERASE AND SERUM ESTERASE IN HERRING 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. 1 and listed in Table 1 together with sampling dates, numbers of specimens in each sample and other available data. Fig. 1 also shows sampling localities of the samples (no. 1—10) previously analysed for serum esterase (NÆVDAL 1969). The results of these analyses are included in the following discussion.

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 analysed 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 1966) 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

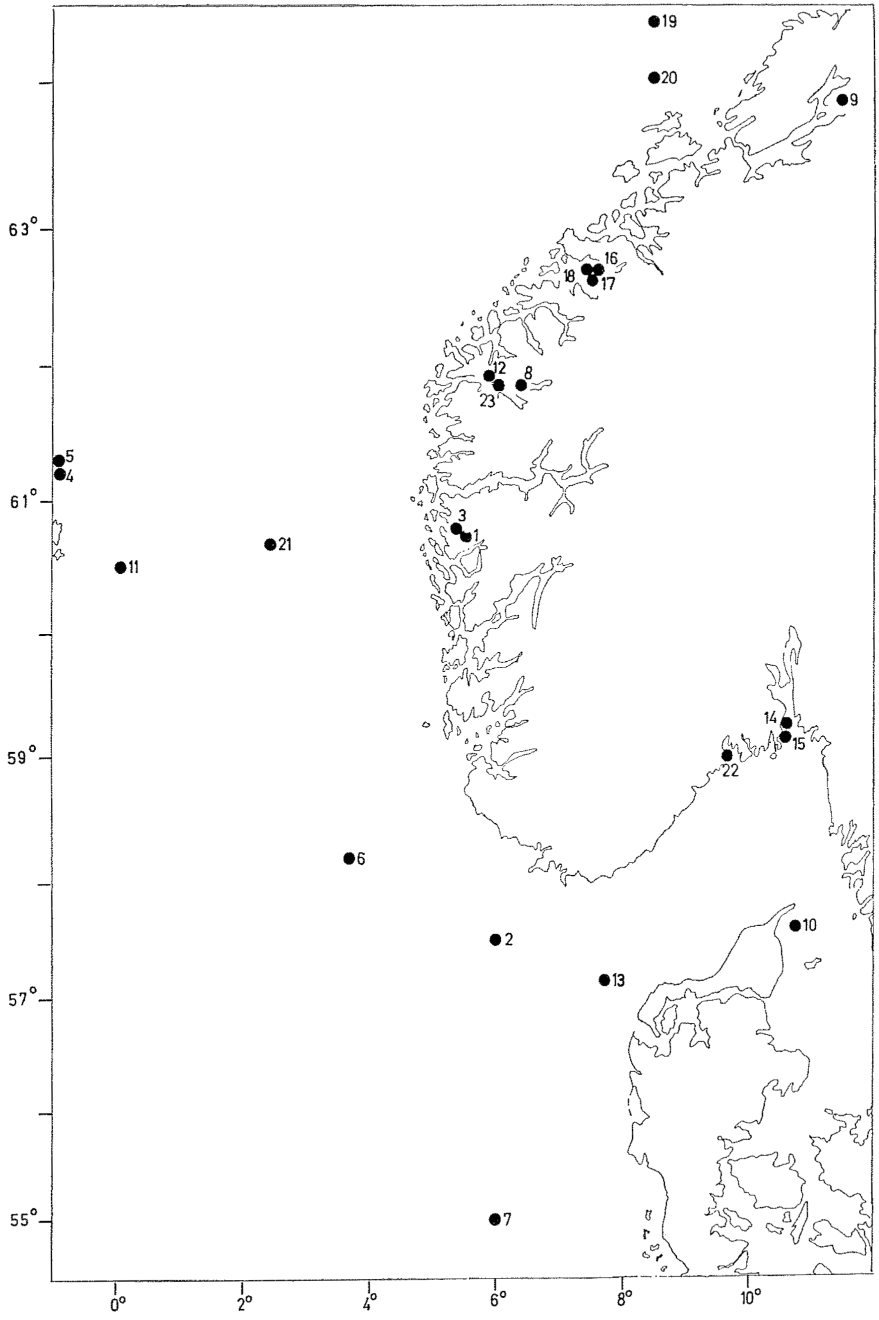

Fig. 1. Sampling localities of blood samples of herring from the Norwegian coast and the North Sea.

Table 1. 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					No.	Gene frequency		
			AABB	AABB′	AAB′B′	AABB″	AA′BB		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	Immature autumn spawners	76	9	1	—	—	86	0.06	—	—
14	Bastøy, Oslofjorden, 30 Aug. 68	Immature, 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	Immature, 13—16 cm	86	11	—	—	—	97	0.06	—	—
18	Langfjorden, Romsdal, 21 Sept. 68	Immature, 16 cm	91	8	—	—	—	99	0.04	—	—
19	64°25′N, 08°25′E, off Trøndelag, 20 Feb. 69	Norwegian spring spawners	92	7	1	—	—	100	0.05	—	—
20	64°02′N, 08°25′E, off Trøndelag, 26 Feb. 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	87	6	—	1	1	95	0.03	0.005	0.005
23	Ålfoten, Nordfjord, 2 June 69	Immature	131	25	—	—	—	156	0.08	—	—

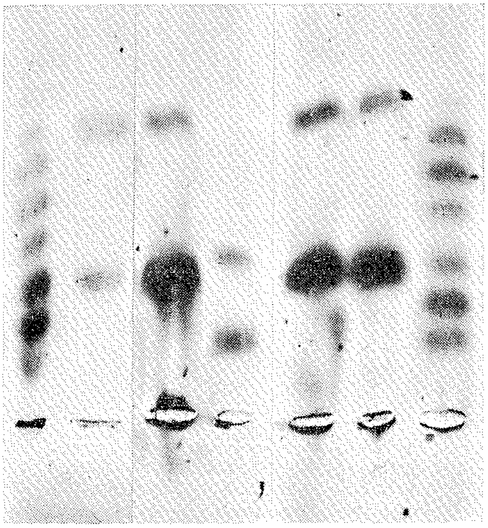

Fig. 2. Photograph of herring lactate dehydrogenase activity after combined starch and agar gel electrophoresis at pH 9.0 for three hours. From left to right the types are BB′, BB, BB, B′B′, BB, BB, and BB′.

described by ODENSE *et al.* (1966a, b). Analyses of esterase were carried out as described previously (NÆVDAL 1969).

RESULTS AND DISCUSSION

Patterns similar to the LDH phenotypes described by ODENSE *et al.* (1966a, b) were found in the present material (Fig. 2), 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 1.

Muscle extract showed stronger LDH activity than sera, and all specimens could be determined for LDH phenotypes from muscle extract. When sera were analysed 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 stored 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 analysed 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 (Fig. 3).

Table 2. 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_{S1}	q_{S2}
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	—				

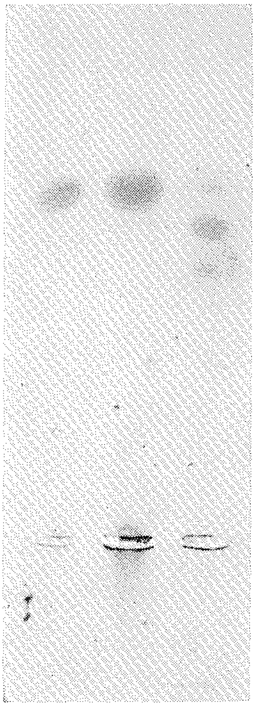

Fig. 3. Photograph of herring aspartate aminotransferase activity after combined starch and agar gel electrophoresis at pH 9.0 for two hours. From left to right the types are SS, SS, and SS′.

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 sides of the Atlantic as far as can be stated from the present 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. They were also very rare in the samples from the West Atlantic.

Distributions of esterase phenotypes are shown in Table 2. 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 were indicated on some electrophoretograms, but they showed only small differences in electrophoretic mobility, and separation into more phenotypes is hardly possible by the present method. Distinction 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.

Tables 1 and 2 show 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. The results of the previous analyses (NÆVDAL 1969) of samples numbered one to ten are included in the 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 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 Oslo fjord) 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 in the North Sea significant variations exist in characteristics which probably are genetically controlled and not affected by environmental factors.

SUMMARY

1. By use of combined starch and agar gel electrophoresis 10 samples (1258 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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