HAEMOGLOBIN POLYMORPHISM OF COD IN THE BALTIC AND THE DANISH BELT SEA

By *KNUD SICK*

INSTITUTE OF GENETICS, UNIVERSITY OF COPENHAGEN, DENMARK

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I. INTRODUCTION

THE cod (*Gadus morhua* L) lives in shallow marine waters down to a depth of approximately 300 meters, i.e. border-seas, coastal areas, and banks. Geographically the species is widely distributed in the northern regions of the Atlantic Ocean from the coast of U.S.A. to Greenland, Iceland, and Norway, to the North Sea. A closely related species or subspecies lives in the North Pacific.

In the Danish Belt Sea and in the western Baltic the cod is abundant everywhere. In the Baltic proper it extends eastwards and northwards to the middle of the gulfs of Finland and Bothnia. Effective spawning does not take place north of Gotland, because of too low salinity of the water (OTTERLIND, 1961). Otherwise spawning occurs all over the area. Both with respect to season and locality, cod spawning seems rather diffuse in this area.

With the aid of morphological characters, the cod has been divided into a number of geographical races or subspecies. Within the area covered by the present study, SVETOVIDOV (1962) distinguishes between the Baltic cod (*Gadus morhua callarias*) which inhabits the eastern Baltic, and the Atlantic cod (*Gadus morhua morhua*) inhabiting the western Baltic and the other Danish waters. Also the results of SCHMIDT (1930), who counted vertebrae and fin ray numbers, revealed differences between cod from the Baltic and cod from the Belt Sea. The morphological differences reported by these authors are, however, very subtle and the heritability of these traits is unknown. It is therefore impossible to tell whether we are dealing with genetically different and reproductively isolated subspecies, or rather with environmentally de-

termined modifications of one and the same freely interbreeding population. Tagging experiments have shown that the exchange of cod specimens between the Belt Sea and the Baltic proper is rather limited (BAGGE, 1961; OTTERLIND, 1961), thus indicating the existence of separate populations. Some exchange does, however, occur and it is not possible from the tagging results alone to draw conclusions about the extent of interbreeding between cod from the two areas, especially since the pelagic eggs and larvae may be transported over long distances.

The genetic significance of the observed morphological variation cannot be evaluated by experimental breeding and rearing, because of the susceptibility of the early fry to gas-bubble disease (DANNEVIG and DANNEVIG, 1950). At present, the only way to obtain valid information about the population structure of cod from the genetical point of view, seems to be investigation of possible blood group differences or other biochemical variation, that may safely be considered hereditary. Such variation has been found to occur with respect to cod haemoglobins (SICK, 1961). The present report is an account of the results obtained by haemoglobin typing of a large number of cod collected in the Belt Sea and the Baltic.

II. MATERIAL AND METHODS

The haemoglobin type of 5439 cod specimens has been determined by agar electrophoresis. The material was collected at 42 different localities in the Kattegat, the Belt Sea, and the Baltic Sea. In some of the samples the length of the fish was also recorded.

The fish were procured by Danish and Swedish fishery research vessels or with the aid of commercial fishing boats. In some of the latter cases the geographical position of the sample cannot be given accurately. Blood was obtained by heart puncture of live or recently dead fish. Usually the fish was cut open and a Pasteur pipette was inserted into the heart. In some cases, when it was desirable that the fish survived the blood sampling, a hypodermic needle was inserted into the heart from between the pelvic fins, and the heart was allowed to pump out a little blood by its own force. This treatment did not seem to damage the fish to any significant degree, since the percentage of recaptures of specimens from which blood had been taken was approximately the same as for an untreated control group (BAGGE, OTTERLIND and SICK, unpublished).

As anticoagulant we have used sodium citrate solution, isotonic with cod blood, i.e. corresponding to 1.17 per cent sodium chloride (HALVOR-

TABLE 1. *Date and locality of collected samples.*

Sample number	Date	Locality
1	14-8-61	Göteborg, N. 57°45′, E. 11°15′
2	26-4-61	Æbeltoft
3	11-2-61	Sejerø Bay
4	15-11-61	Sejerø Bay
5	15-4-61	Isefjord
6	1-5-61	N. Sealand
7	1-5-61	Hven
8	6-5-61	S. Amager
9	15-11-61	S. Copenhagen
10	5-3-62	Åbenrå Fjord
11	2-8-61	Ærø
12	6-3-61	Fehmarn Belt
13	17-6-61	E. Falster
14	24-8-61	E. Møn, N. 55°08′, E. 12°59′
15	15-8-61	S. Trelleborg, N. 55°13′, E. 13°22′
16	28-10-63	S. Trelleborg, N. 55°12′, E. 13°17′
17	24-3-62	N. Rügen, N. 54°56′, E. 13°17′
18	27-4-62	N. Rügen, N. 54°56′, E. 13°16′
19	7-11-62	N. Rügen, N. 54°55′, E. 13°17′
20	12-11-62	S. Ystad, N. 55°13′, E. 13°40′
21	29-10-63	S. Ystad, N. 55°13′, E. 13°38′
22	24-8-61	N. Rügen, N. 54°49′, E. 13°25′
23	24-8-61	W. Bornholm, N. 54°53′, E. 13°57′
24	28-4-62	W. Bornholm, N. 55°01′, E. 13°57′
25	8-11-62	W. Bornholm, N. 55°02′, E. 13°57′
26	30-10-63	W. Bornholm, N. 55°02′, E. 14°04′
27	24-8-61	W. Bornholm, N. 55°11′, E. 14°15′
28	13-10-61	W. Bornholm, N. 55°11′, E. 14°15′
29	25-11-61	W. Bornholm, N. 55°11′, E. 14°15′
30	18-2-61	W. Bornholm
31	23-8-61	S. Bornholm, N. 54°45′, E. 14°58′
32	13-10-61	S. Bornholm, N. 54°45′, E. 14°58′
33	25-11-61	S. Bornholm, N. 54°45′, E. 14°58′
34	23-6-61	S.E. Bornholm, N. 54°45′, E. 15°10′
35	15-11-61	S.E. Bornholm, N. 54°51′, E. 15°15′
36	22-6-61	S.E. Bornholm, N. 54°50′, E. 15°28′
37	23-8-61	S.E. Bornholm, N. 54°55′, E. 15°22′
38	13-10-61	S.E. Bornholm, N. 54°55′, E. 15°22′
39	24-11-61	S.E. Bornholm, N. 54°55′, E. 15°22′
40	23-8-61	S.E. Bornholm, N. 55°02′, E. 15°22′
41	16-11-61	E. Simrishamn, N. 55°27′, E. 14°40′
42	26-3-62	E. Simrishamn, N. 55°27′, E. 14°40′
43	14-11-62	E. Simrishamn, N. 55°27′, E. 14°42′
44	15-8-61	E. Simrishamn, N. 55°34′, E. 14°44′

Sample number	Date	Locality
45	20-7-61	E. Simrishamn
46	16-8-61	N.E. Bornholm, N. 55°33′, E. 15°32′
47	16-8-61	N.E. Bornholm, N. 55°43′, E. 15°47′
48	17-8-61	E. Bornholm, N. 55°28′, E. 16°12′
49	18-8-61	E. Bornholm, N. 55°18′, E. 16°12′
50	18-8-61	E. Bornholm, N. 55°15′, E. 17°28′
51	27-3-62	Gulf of Danzig, N. 55°30′, E. 19°02′
52	14-11-61	S.E. Gotland, N. 57°10′, E. 18°50′
53	10-11-61	E. Gotland
54	15-11-61	Fårö, N. 57°51′, E. 19°27′
55	4-10-61	S.E. Åland Is., N. 59°44′, E. 21°19′
56	14-5-62	W. Åland Is., N. 60°13′, E. 18°57′
57	26-5-62	Gulf of Bothnia
58	10-10-62	Gulf of Bothnia

SEN and MØLLER, 1961). Cod blood as well as blood from all other fishes, of which we have experience, is much more difficult to store than blood from mammals. Thus, it is absolutely necessary to keep the blood cold (0—4° C) from the time of collection until the time of the electrophoretic analysis. At room temperature haemolysis will occur within a few hours. Even when kept cold the samples start to haemolyse after approximately 5 days. For transport of the samples from the locality of collection to the laboratory in Copenhagen, we have used thermos flasks filled with ice.

In the laboratory the erythrocytes are washed twice by centrifugation in 1.17 per cent sodium chloride solution. The cells are then haemolysed by adding approximately three volumes of the electrophoresis buffer to one volume of packed red cells. After ten minutes in the cold the haemolysis is completed and the cell debris is spun down at 1000 g for 5 minutes. The supernatant haemoglobin solution thus obtained is used for electrophoresis without further treatment. The haemolysate must be analysed the day it has been prepared. Unlike mammalian haemoglobins, fish haemoglobins cannot be stored in the freezer, even after it has been converted to the carbonmonoxy- or cyanmeth-form. In contrast to the experience of SINDERMANN and HONEY (1963) with herring haemoglobin, we find that freezing and thawing interferes with the cod haemoglobin molecules, so that the electrophoretic patterns become diffuse and difficult to read. We have therefore always used freshly prepared haemoglobin solutions, and in all the material presented here, the haemoglobin type of every single specimen in the sample could be determined without ambiguity.

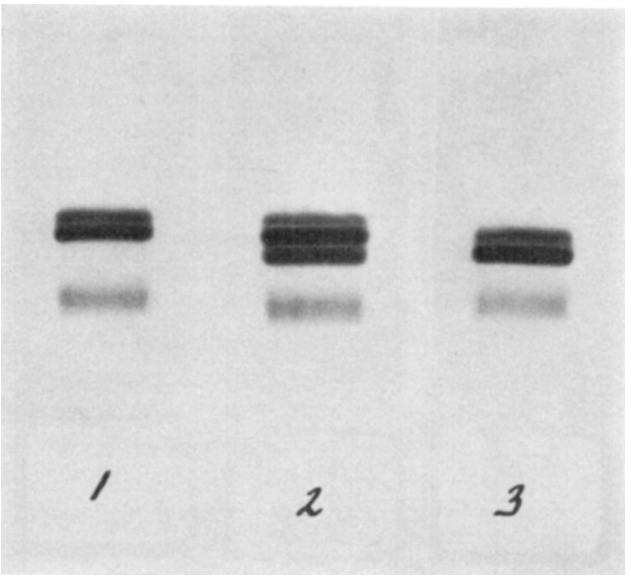


Fig. 1. Cod haemoglobin patterns obtained by electrophoresis in Difco purified agar for 60 minutes. 1: type Hb I-1. 2: type Hb I-1-2. 3: type Hb I-2.

The electrophoresis technique employed is a modified agar gel electrophoresis on microscope slides. A number of well-known electrophoresis buffers such as Veronal (pH 8.6) and Tris-EDTA-Boric acid (pH 9.0) have been tried. The best results were obtained with buffers prepared from mixtures of primary and secondary phosphates. In this buffer system many different combinations of pH and ionic strength values were tried. The buffer which gave the optimal results, i.e. sharp bands together with good separation, had a pH value of 7.3 and an ionic strength of 0.02. This buffer, which is made by dissolving 9.85 g of K_2HPO_4 and 4.75 g of $NaH_2PO_4+2\,H_2O$ in 10 litres of distilled water, has been used in all the haemoglobin type determinations presented here.

Several different brands of agar have been tested. Good results were obtained with a 1.0 per cent solution of Difco Purified Agar (Fig. 1). For most routine scoring we have, however, used Oxo Ionagar No. 2, which on account of its low electro-osmotic flow properties, allows to run four samples simultaneously on each slide. Most of the results presented here have been obtained by running 4 specimens on each slide. Fig. 2 shows 4 such slides from a single electrophoretic run.

The electrophoresis is carried out in the following way. By means of

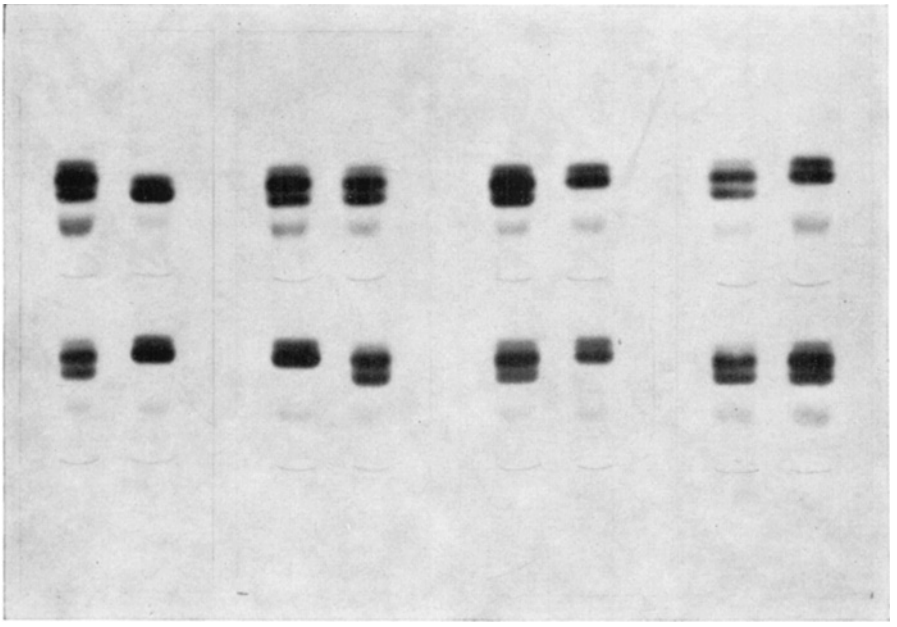
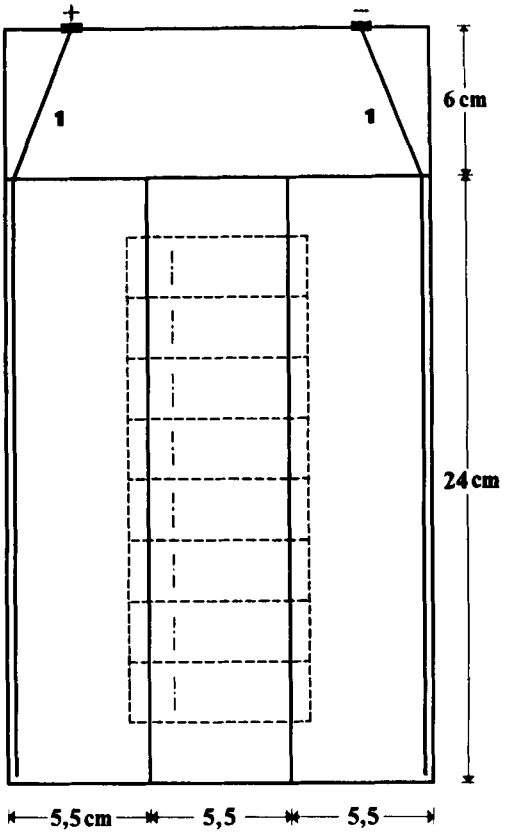


Fig. 2. Patterns obtained by routine scoring in Oxo Ion Agar. The electrophoretic conditions are described in the text. From left to right the types in the upper row are: Hb I-1-2, Hb I-2, Hb I-1-2, Hb I-1-2, Hb I-1-2, Hb I-1, Hb I-1-2, and Hb I-1. In the lower row the types are: Hb I-1-2, Hb I-1, Hb I-1, Hb I-1-2, Hb I-1-2, Hb I-1, Hb I-1-2, and Hb I-1-2.

a pipette 2 ml of an 0.8 per cent Oxo Ion Agar solution (at 95° C) in phosphate buffer (pH 7.3, i.s. 0.02) is layered on a 76×26 microscope slide, and allowed to gel. A piece of filter paper (S&S No. 2043 bmgl) is inserted into the gel at the line of application and allowed to soak up moisture to a height of approximately 4 mm. The filter paper is then gently removed, and the slit thus produced is filled from a capillary pipette containing the haemoglobin solution. In this way a very sharp line of application is obtained.

In our electrophoresis box 8 slides may be run at a time. The buffer compartments each hold 400 ml of the same buffer that was used for preparing the gel. Double layers of Whatman filter paper No. 1 are used as bridges between the gel and the buffer. By means of a current stabilized power pack, 40 mA (i.e. 5 mA per slide) are applied for 45 minutes. The resulting voltage gradient in the gel is approximately 13 V/cm. The considerable heat generation makes it necessary to cool the apparatus. Cooling is obtained by pouring cold buffer (4° C) into the jars



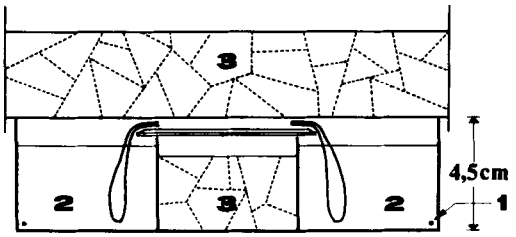


Fig. 3. Diagram of the electrophoresis apparatus: 1: platinum electrodes, 2: buffer solution, 3: ice.

and by having ice in the compartment underneath the slides as well as in the metal box that serves as a cover for the apparatus. Dimensions and general layout of the apparatus are shown in Fig. 3.

In this electrophoresis the cod haemoglobins migrate towards the cathode. The patterns can usually be read directly without staining. If,

however, for some reason or other the sample has become very diluted, it may be necessary to visualize the pattern by staining with Amido Black or Orthodianizidine.

III. RESULTS

1. Description of the haemoglobin types

Three different electrophoretic patterns were found. Their appearance, when electrophoresis was carried out in Difco Purified Agar, is shown in Fig. 1. They all have a slow-moving (i.e. anodic) component in common (Hb II). The relative intensity of this band varies a great deal from specimen to specimen, and it is often rather diffuse. In addition to the Hb II component the patterns may show either one or both of two faster moving bands (Hb I-1 and Hb I-2). Both the Hb I-1 component and the Hb I-2 component are accompanied on the cathodic side by a weaker band, designated Hb I-1′ and Hb I-2′ respectively. Since storing of the haemoglobin solutions tends to increase the relative amount of these minor components, they may represent denaturation products. They are, however, also present in completely fresh material, and their increase upon storing does not occur until the whole pattern tends to become diffuse. Thus it is likely that the minor components exist also *in vivo*.

The three types are called type Hb I-1, type Hb I-1-2, and type Hb I-2. The Hb I-1-2 type is indistinguishable from the pattern obtained by running an *in vitro* mixture of equal amounts of haemoglobin solutions from individuals of type Hb I-1 and Hb I-2. The Hb I-2′ component coincides electrophoretically with the Hb I-1 component, and is therefore not seen as a separate band in the *in vitro* mixture. In all probability the Hb I-2′ component also exists in type Hb I-1-2, but is here overlapped by the larger Hb I-1 band. The greater intensity of the Hb I-1 band as compared to the Hb I-2 band in the Hb I-1-2 pattern may be taken as evidence that this is so.

2. Genetic interpretation of the variation

Ontogenetic variation of haemoglobin patterns have been reported for the salmon (KOCH *et al.*, 1964). In this species the development from the juvenile stage to sexually mature adult is accompanied by gradual transitions from one haemoglobin pattern to another. This situation is comparable to the shift from fetal to adult haemoglobin known in mam-

mals (INGRAM, 1963), birds (MANWELL *et al.*, 1963), elasmobranchs (MANWELL, 1963), and cyclostomes (ADINOLFI *et al.*, 1959).

The haemoglobin variation of cod is clearly of a different nature. First, there were never observed types intermediary or transient between those already described. Second, the type of haemoglobin pattern was not found to be correlated with the size of the fish, except in a few samples, to be dealt with later.

The observed haemoglobin variation in cod is comparable to the genetic haemoglobin variation described in man (INGRAM, 1963), cattle (BANGHAM, 1957), and sheep (EVANS *et al.*, 1956), rather than to the ontogenetic variation found in the salmon. In human adults there are normally two electrophoretically different haemoglobins, a large component called Hb A and a much smaller component, named Hb A_2. Hb A is composed of two α- and two β-polypeptide chains, and Hb A_2 is composed of two α- and two δ-chains. Thus only the α-chain is common to these two haemoglobins. This explains why mutations of the β-chain locus, such as those resulting in the production of Hb S or Hb C instead of Hb A, do not affect the structure of the Hb A_2 component. The demonstration of "hybrid haemoglobins" in a number of fish species hybrids (MANWELL, 1963; SICK *et al.*, 1963) suggests that also fish haemoglobins are polymers. The relationship between the Hb I and Hb II components of cod could be similar to that between human Hb A and Hb A_2, and the observed variation could be due to alteration of a polypeptide chain specific for Hb I and not found in Hb II, which therefore remains unaffected by this variation. With respect to the minor components (Hb I-1′ and Hb I-2′) that invariably accompany the major ones (Hb I-1 and Hb I-2), an identical variation of electrophoretic patterns, i.e. the simultaneous variation of a major and an accompanying minor component, has been reported for the serum transferrins in the mouse (SCHREFFLER, 1960). Breeding experiments have shown that this variation in the mouse can be explained by the segregation of two allelic genes.

By analogy with these mammalian cases of protein polymorphism, it seems obvious to adopt a genetic hypothesis involving two codominant alleles, to explain the variation of cod haemoglobins. According to this hypothesis two codominant allelic genes *Hb* I^1 and *Hb* I^2 determine the haemoglobin components Hb I-1 and Hb I-2 respectively. Type Hb I-1 and type Hb I-2 are considered as homozygous for the corresponding allele, and type Hb I-1-2 would represent the heterozygote. This hypothesis is supported by the population data presented in the following, and also by the chemical studies by RATTAZZI and PIK (1965). These

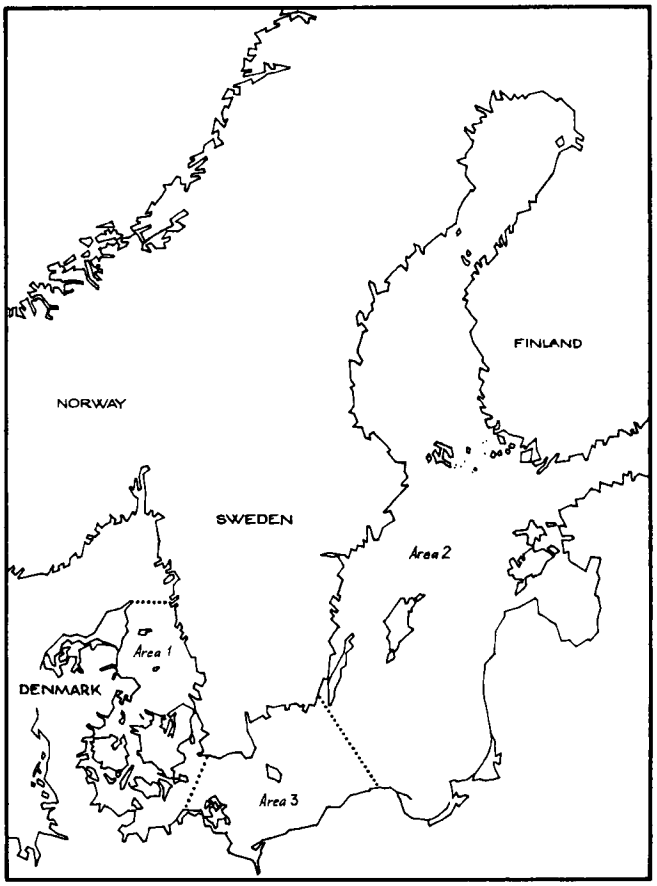


Fig. 4. Map of the Baltic and the Danish Belt Sea showing the adopted subdivision into three areas.

authors found the difference between Hb I-1 and Hb I-2 to reside in one single peptide, a result which strongly indicates that these proteins are determined by allelic genes.

3. The geographical distribution of the types

The geographical area covered by the present study comprises the Danish seas inside the Skaw and the whole of the Baltic Sea. As shown in Fig. 4 the area has been divided into three subareas, of which maps on a larger scale are shown in Figs. 5, 6, and 7. The border lines between the areas were chosen in order to obtain a convenient division for

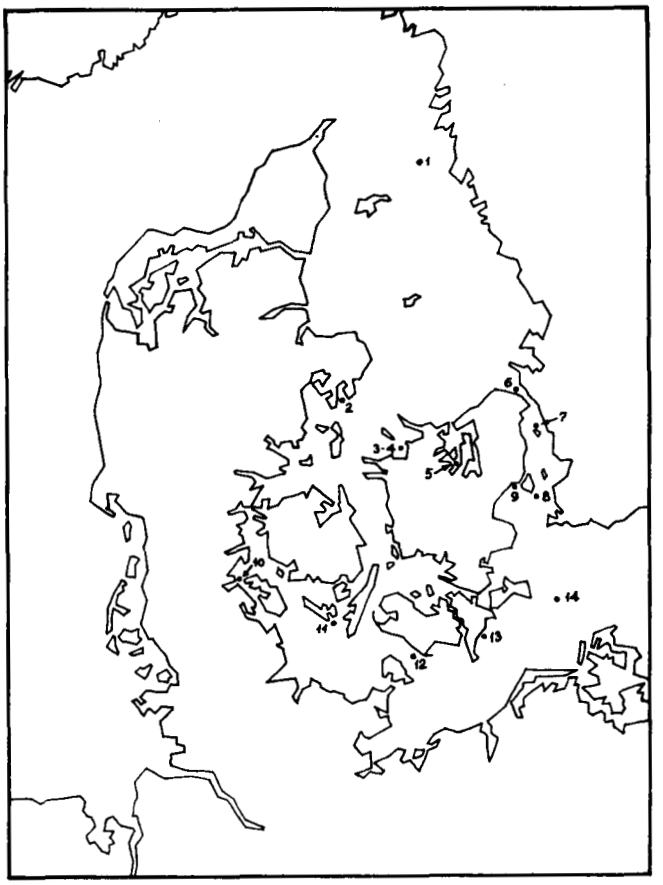


Fig. 5. Map showing the locations of the 14 samples collected in the Kattegat, the Belt Sea, and the western Baltic (Area 1).

the treatment of the results; they have no particular geographical or hydrographical significance. Table 1 gives the date and locality of the samples. The localities are marked out in Figs. 5, 6, and 7 together with the numbers designating the samples in the tables.

A. *Area 1. Kattegat, the Belt Sea and the western Baltic*

Within this area are located samples nos. 1—14 (Fig. 5). The results are given in Table 2. This table contains the number of the three types observed in each sample (obs.), and the frequency (q^1) of the inferred *Hb* I^1 allele. The calculation of q^1 was done by direct counting of the

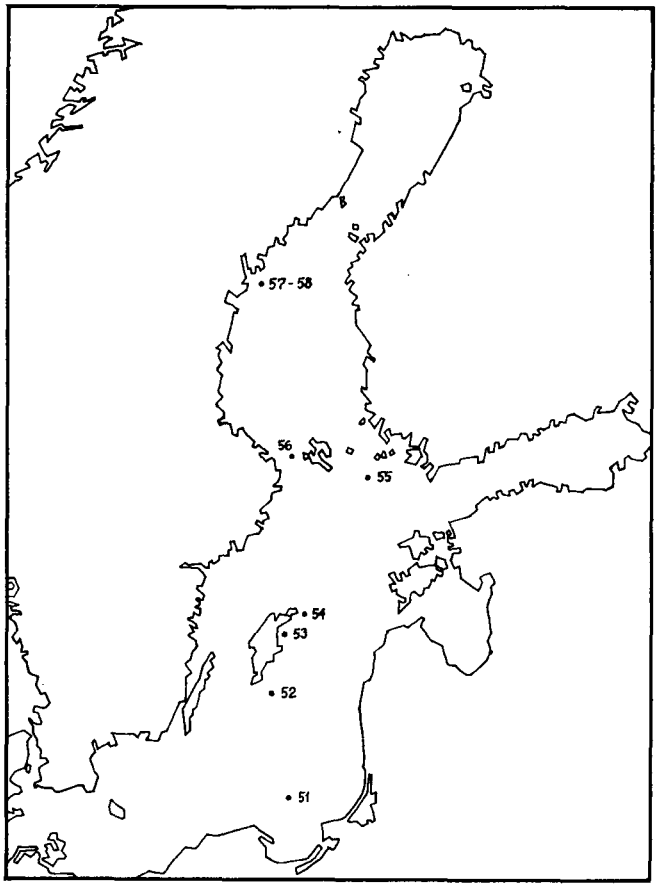


Fig. 6. Map showing the locations of the 8 samples collected in the eastern Baltic (Area 2).

genes, based on the genetic assumptions put forward above, namely that type Hb I-1 specimens are homozygous for this allele, type Hb I-1-2 is heterozygous, and type Hb I-2 is homozygous for the mutually exclusive *Hb* I^2 allele. The table also gives the numbers expected (exp.) according to the Hardy-Weinberg law of genotype distributions in large random-mating populations. The agreement between the observed and expected distributions has been tested by means of Fischer's χ^2 test and the values obtained as well as the corresponding probability intervals for one degree of freedom are shown.

The values of q^1 are very similar for these samples, varying only from 57 per cent to 66 per cent. The value for the total material of 1396 spec-

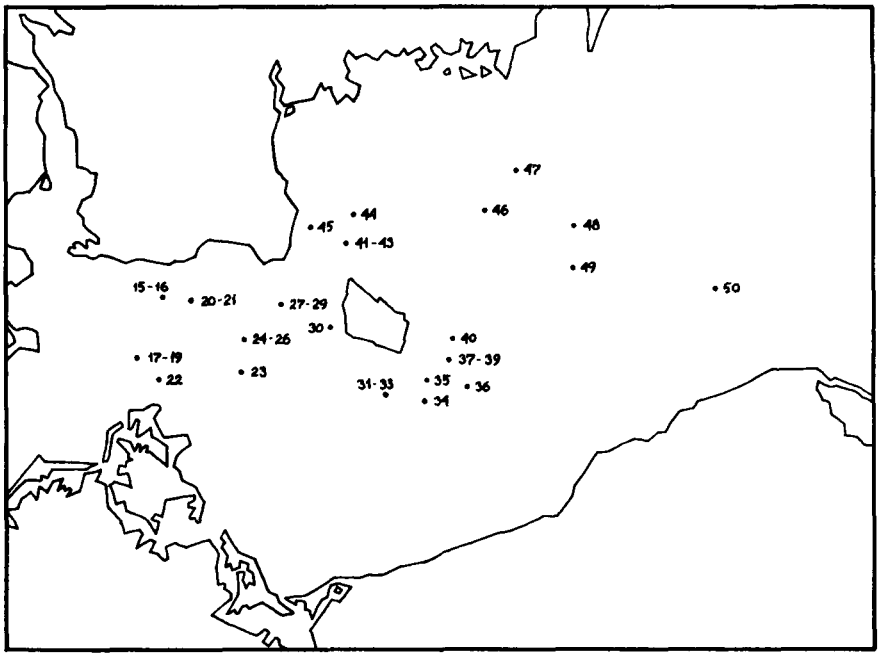


Fig. 7. Map showing the locations of the 36 samples collected in the central Baltic (Area 3).

imens from this area is 61 per cent, thus falling in the middle of this range. In none of the samples is the deviation from 61 per cent larger than twice the binomial error, as a matter of fact the deviation is never greater than this error. There is no observable tendency in the geographical or seasonal distribution of the q^1 values; actually two of the samples showing extreme values (no. 6 with 57 per cent and no. 7 with 66 per cent) were collected close to each other in the northern part of the Sound on the same day.

The distribution of the types is for all the samples in good agreement with that expected according to the Hardy-Weinberg law. This is also true for the totals for the area. In the majority of the samples and also in the totals, there is a slight excess of heterozygotes. This is the situation one would expect if the cod haemoglobin polymorphism is balanced by heterosis. Our material does not, however, permit any conclusions as to whether heterosis pertains to this system or not. First, the observed excess of heterozygotes is not statistically significant. Second, other mechanisms might account equally well for this result. The only

TABLE 2. *The genotypic composition of the 14 samples (Nos. 1—14) collected in area 1, compared to the Hardy-Weinberg distribution.*

Sample No.	1—1 homozygotes		1—2 heterozygotes		2—2 homozygotes		Total	χ^2	Probability of worse fit	Frequency of the Hbl^1 allele (q^1)
	obs.	exp.	obs.	exp.	obs.	exp.				
1	24	24.5	30	28.9	8	8.5	62	0.0839	0.70—0.80	0.63
2	24	25.8	37	33.4	9	10.8	70	0.8168	0.30—0.50	0.61
3	37	37.7	50	48.6	15	15.7	102	0.0813	0.70—0.80	0.61
4	7	8.2	14	11.7	3	4.2	24	0.9600	0.30—0.50	0.58
5	28	28.1	34	33.8	10	10.1	72	0.0040	0.90—0.95	0.63
6	30	30.9	49	47.1	17	17.9	96	0.1529	0.20—0.30	0.57
7	30	31.3	35	32.3	7	8.3	72	0.4925	0.30—0.50	0.66
8	33	29.9	28	34.3	13	9.9	74	2.4947	0.10—0.20	0.64
9	126	128.4	159	154.3	44	46.4	329	0.3078	0.50—0.70	0.62
10	43	47.0	71	62.9	17	21.0	131	2.1604	0.10—0.20	0.60
11	30	32.3	41	36.4	8	10.3	79	1.2391	0.20—0.30	0.64
12	27	25.6	30	32.9	12	10.6	69	0.5259	0.30—0.50	0.61
13	28	29.0	45	43.0	15	16.0	88	0.1825	0.10—0.20	0.57
14	44	43.9	62	62.1	22	21.9	128	0.0004	0.98—0.99	0.59
Totals 1—14	511	521.7	685	663.6	200	210.7	1396	1.4530	0.20—0.30	0.61

thing that can be said is that the selective forces involved must be of small magnitude. The subject of possible heterosis in this system is dealt with in more detail in a paper by FRYDENBERG *et al.* (1965) on the haemoglobin polymorphism of Norwegian cod populations.

In sample no. 9, which comprises 329 specimens, the length of each fish was recorded together with its haemoglobin type. The genotypic composition of the different size groups in this sample is given in Table 3. The gene frequency for the part of the sample that measured from 15 to 26 cm was 63 per cent, while the rest of the sample, measuring from 27 to 54 cm, had a q^1 value of 61 per cent. This small difference between the two groups is not statistically significant.

The results for area 1 may be summarized in the following way. All the samples have q^1 values close to 61 per cent. In all the samples the haemoglobin types are distributed in agreement with the Hardy-Weinberg expectations. There is no gross correlation between haemoglobin type and the size of the fish.

These observations lead to the conclusion that the cod in the Kattegat, the Belt Sea, and the western Baltic could form or be part of one single freely interbreeding (panmictic) unit. It could, however, also be, com-

TABLE 3. *The genotypic composition of different size groups in sample no. 9.*

Length in cm	Number of specimens	Haemoglobin type			q^1
		Hb I—1	Hb I—1—2	Hb I—2	
15—16	35	15	15	5	
17—18	79	35	35	9	
19—20	86	27	43	16	
21—22	59	23	30	6	
23—24	14	5	8	1	
25—26	6	3	3	0	
Sub-total 15—26	279	108	134	37	0.63
27—30	7	1	6	0	
31—34	9	4	3	2	
35—38	13	2	9	2	
39—42	5	3	1	1	
43—46	6	3	2	1	
47—50	9	4	4	1	
51—54	1	1	0	0	
Sub-total 27—54	50	18	25	7	0.61
Total 15—54	329	126	159	44	0.62

posed of a number of demes, i.e. small population units, only slightly isolated from each other which have almost identical genetic constitutions (SIMPSON, 1953). There is at present no means of distinguishing between these possibilities. Neither is it possible to tell whether this polymorphism is balanced or transient (*sensu* FORD, 1953). The facts that the different size groups of sample no. 9 had similar q^1 values, and that samples collected in 1965 also showed q^1 values close to 61 per cent (FRYDENBERG, unpublished data) suggest that this gene frequency is fairly stable in the population.

B. *Area 2. The Eastern Baltic*

Samples were collected at 7 different stations well spread over the area from the Gulf of Danzig in the south to the Gulf of Bothnia in the north (Fig. 6). The results are given in Table 4. The gene frequencies of these samples are radically different from those met with in the Belt

TABLE 4. *The genotypic composition of the 8 samples (Nos. 51—57) collected in area 2, compared to the Hardy-Weinberg distribution.*

Sample No.	1—1 homozygotes		1—2 heterozygotes		2—2 homozygotes		Total	χ^2	Probability of worse fit	Frequency of the Hbl^1 allele (q^1)
	obs.	exp.	obs.	exp.	obs.	exp.				
51	0	0.1	5	4.8	75	75.1	80	0.0832	0.70—0.80	0.03
52	2	0.2	5	8.6	104	102.2	111	19.6710	<0.001	0.04
53	1	0.2	4	5.7	54	53.2	59	5.2260	0.02—0.05	0.05
54	0	0.1	5	4.8	75	75.1	80	0.0832	0.70—0.80	0.03
55	0	0.1	5	4.8	75	75.1	80	0.0832	0.70—0.80	0.03
56	0	0.0	1	1.0	79	79.0	80	0.0032	0.95—0.98	0.01
57	0	0.1	3	2.9	37	37.1	40	0.0607	0.80—0.90	0.04
58	0	0.0	1	1.0	39	39.0	40	0.0063	0.90—0.95	0.01
Totals 51—58	3	0.5	29	33.9	538	535.5	570	13.2468	<0.001	0.03

Sea and the western part of the Baltic. They range from 1 per cent to 5 per cent, being 3 per cent for all 570 specimens taken together.

With a frequency of 3 per cent for the *Hb I^1* allele one would expect 0.09 per cent type Hb I-1, 5.82 per cent type Hb I-1-2, and 94.09 per cent Hb I-2 specimens in the samples if random-mating pertained. Six of the eight samples contained a small percentage of heterozygotes and no Hb I-1 homozygotes, and thus fit the expected values quite well. Sample no. 53 contains one Hb I-1 homozygote. In samples of this order of magnitude (40—100 specimens) one would expect to find one (or more) of this homozygote in approximately one twentieth of the samples, calculated according to the Poisson distribution with 0.09 per cent as the mean value. The occurrence of one such sample among eight does not therefore violate the idea of random-mating in this population. Sample no. 52, on the other hand, contains two type Hb I-1 individuals out of 111 specimens. In samples of that size only one out of 250 would be expected to contain two (or more) of this homozygote. The excess of homozygotes in this sample is therefore highly significant; especially when the deviation of sample no. 53 has been accepted as insignificant. Of the total of 570 specimens collected in this area, three were of type Hb I-1, only 0.5 being expected. In only one out of approx. 2000 cases would samples of 570 specimens be expected to contain 3 (or more) of this homozygote if the samples came from a random-mating population with a q^1 value of 3 per cent. The observed excess of homozygotes must therefore be considered significant.

In Table 4 the χ^2 values for goodness of fit have been given. In spite of the serious limitations of this test, when the expected number in any one group is small, its use leads to the same conclusions as put forward above, namely that sample no. 52 has a highly significant excess of homozygotes, sample no. 53 being at the limit of significance, and the rest of the samples conforming with expectations.

It seems natural to explain this unquestionable excess of type Hb I-1 specimens as being due to rare stray fish from an adjacent cod population possessing a higher frequency of the *Hb I^1* allele, such as that known from the Belt Sea. It may be more than a coincidence, that all the Hb I-1 specimens were found at two of the three southernmost localities. If we accept that at most one of the three rare homozygotes actually observed is inherent to the cod population of the eastern Baltic, the two others (and a similar number of heterozygotes) being visitors from outside, then our estimate of 3 per cent for the q^1 value of the Baltic proper population will tend to be a little too high.

In most of the samples the length of the fish was also recorded. No tendency of the Hb I-1 or Hb I-1-2 specimens to be larger or smaller than the rest of the material could be detected.

The picture that emerges from these results is, that the eastern Baltic is inhabited by a homogeneous cod population possessing a q^1 value of only 3 per cent, and that the southern part of the area is reached by exceptional visitors from the west. The possibility of a subdivision into a number of demes with similar haemoglobin gene frequencies, of course exists here as well as in the Belt Sea and in the western Baltic.

C. *Area 3. The Central Baltic*

A relatively large number (36) of samples has been collected in this area (Fig. 7) in order to elucidate the character of the transition between the Belt Sea population and the eastern Baltic population. By and large the western population is homogeneous with respect to the haemoglobin genes and characterized by a q^1 value of 61 per cent, while the eastern population has also been shown to be homogeneous (or nearly so) but with a q^1 value of only 3 per cent. Cod are abundant everywhere in the central Baltic, and no hydrographical barrier hindering migration of adult cod or transport of their eggs and fry from the eastern to the western Baltic or *vice versa* is known. The transition between these populations thus occurs in an area with no gaps in the distribution of the species.

TABLE 5. *The genotypic composition of the 36 samples (Nos. 15—50) collected in area 3, composed to the Hardy-Weinberg distribution.*

Sample No.	1—1 homozygotes		1—2 heterozygotes		2—2 homozygotes		Total	χ^2	Probability of worse fit	Frequency of the Hbl^1 allele (q^1)
	obs.	exp.	obs.	exp.	obs.	exp.				
15	20	11.4	25	42.3	48	39.4	93	15.5399	<0.001	0.35
16	29	17.9	48	70.2	80	68.9	157	15.7172	<0.001	0.34
17	17	15.1	35	38.9	27	25.1	79	0.7820	0.30—0.50	0.44
18	14	11.5	28	32.9	26	23.5	68	1.5300	0.20—0.30	0.41
19	36	33.9	55	59.2	28	25.9	119	0.6072	0.50—0.70	0.53
20	64	43.6	113	153.8	156	135.6	333	23.4269	<0.001	0.36
21	20	8.1	40	63.8	138	126.1	198	27.6093	<0.001	0.20
22	17	11.4	41	52.1	65	59.4	123	5.6101	0.01—0.02	0.30
23	15	11.2	26	33.6	29	25.2	70	3.5813	0.05—0.10	0.40
24	16	10.9	26	36.2	35	29.9	77	6.0753	0.01—0.02	0.38
25	7	3.3	14	21.4	38	34.3	59	6.9999	0.001—0.01	0.24
26	14	7.2	35	48.6	89	82.2	138	10.8289	<0.001	0.23
27	14	6.7	20	34.6	52	44.7	86	15.3183	<0.001	0.28
28	12	6.3	16	27.5	36	30.3	64	11.1921	<0.001	0.31
29	13	5.6	17	31.9	53	45.6	83	18.0580	<0.001	0.26
30	2	0.1	2	5.8	74	72.1	78	33.2939	<0.001	0.04
31	6	1.5	8	16.9	51	46.5	65	18.0711	<0.001	0.15
32	4	1.4	13	18.2	62	59.4	79	6.4647	0.01—0.02	0.13
33	4	1.6	9	13.7	31	28.6	44	5.2016	0.02—0.05	0.19
34	0	0.0	3	2.9	78	78.0	81	0.0288	0.80—0.90	0.02
35	12	4.2	12	27.7	54	46.2	78	25.0467	<0.001	0.23
36	0	0.1	5	4.9	112	112.1	117	0.0558	0.80—0.90	0.02
37	5	1.7	13	19.6	59	55.7	77	8.6698	0.001—0.01	0.15
38	0	0.1	5	4.8	65	65.1	70	0.0960	0.70—0.80	0.04
39	10	3.1	16	29.7	77	70.1	103	21.9313	<0.001	0.17
40	0	0.3	9	8.5	66	66.3	75	0.3056	0.50—0.70	0.06
41	14	5.8	15	31.4	51	42.8	80	21.8788	<0.001	0.27
42	6	2.3	15	22.3	57	53.3	78	8.4000	0.001—0.01	0.17
43	26	9.6	31	63.9	123	106.6	180	47.6650	<0.001	0.23
44	0	0.3	9	8.5	71	71.3	80	0.2842	0.50—0.70	0.06
45	17	9.5	20	35.1	40	32.5	77	14.2128	<0.001	0.35
46	1	0.2	5	6.6	49	48.2	55	3.0937	0.05—0.10	0.06
47	0	0.2	7	6.7	65	65.2	72	0.1880	0.50—0.70	0.05
48	0	0.0	4	3.9	81	81.0	85	0.0494	0.80—0.90	0.02
49	2	0.2	4	7.6	66	64.2	72	15.9446	<0.001	0.06
50	1	0.2	5	6.7	74	73.2	80	5.1221	0.02—0.05	0.04

As may be seen from Table 5, the value of q^1 varies greatly from sample to sample, the trend being a decrease of this value from west towards east. This trend is best illustrated when mean values for groups of neighbouring samples are compared. The westernmost samples (nos. 15—22) from the area between Rügen and Scania have a mean value for q^1 of 37 per cent. The samples from just west of Bornholm (nos. 23—30) have a mean value of 27 per cent. The five samples from north of Bornholm (nos. 41—45) show the value of 22 per cent, while the group of samples (nos. 31—40) from south-east of this island have 12 per cent of the *Hb* I^1 allele. Finally in the five samples (nos. 46—50) collected at a considerable distance east of Bornholm, this value has dropped down to only 5 per cent, and thus approaches the parameter established for the eastern Baltic cod population.

These geographical clusters of samples are, however, far from homogenous with respect to their q^1 values. The westernmost group, for instance, reveals values ranging from 20 per cent to 53 per cent, and in the group from south-east of Bornholm, values from 23 to only 2 per cent are met with. Even samples from one and the same locality, collected at different times of the year, may differ significantly. Thus the samples nos. 37, 38, and 39, which were collected at the same position in August, October, and November 1961 respectively, vary from 4 to 17 per cent.

The pronounced heterogeneity of the cod population in the central Baltic makes itself evident also in the genotypic composition of the individual samples. With the exception of the four samples (nos. 17, 18, 19, and 23) showing the highest q^1 values for the area, and eight samples (nos. 34, 36, 38, 40, 44, 46, 47, and 48) with very low values ranging from 2 to 6 per cent, all the samples deviate significantly from the Hardy-Weinberg distribution in a unidirectional way, namely by possessing an excess of homozygotes.

An excess of homozygotes is expected if the samples represent mixtures of two or more populations, which differ with respect to their haemoglobin gene frequencies. It therefore seemed obvious to test whether simple mechanical mixing of cod from the two adjacent populations in west and east with q^1 values of 61 and 3 per cent respectively, could account for these results. In Table 6 the observed genotypic composition of these samples is compared with the compositions expected according to this hypothesis.

The calculations were made in the following way: The proportions of the two populations that are supposed to enter the mixture are esti-

TABLE 6. *The genotypic composition of the 36 samples (Nos. 15—50) collected in area 3, compared to the distribution expected according to the hypothesis, that they represent mixtures of two discrete cod populations possessing different values of q^1, namely 0.03 and 0.61.*

Sample No.	1–1 homozygotes		1–2 heterozygotes		2–2 homozygotes		Total	χ^2	Probability of worse fit	Percentage in the sample of specimens belonging to the eastern Baltic population
	obs.	exp.	obs.	exp.	obs.	exp.				
15	20	19.1	25	26.8	48	47.1	93	0.1813	0.50—0.70	45
16	29	31.0	48	43.9	80	82.0	157	0.5677	0.30—0.50	47
17	17	20.6	35	27.7	27	30.6	79	2.9764	0.05—0.10	30
18	14	16.7	28	22.6	26	28.7	68	1.9433	0.10—0.20	34
19	36	38.5	55	50.1	28	30.5	119	0.8410	0.30—0.50	13
20	64	71.0	113	98.9	156	163.0	333	2.9935	0.05—0.10	43
21	20	22.0	40	36.0	138	140.0	198	0.6392	0.30—0.50	70
22	17	21.7	41	31.5	65	69.7	123	4.2242	0.02—0.05	53
23	15	16.3	26	22.7	29	30.6	70	0.7330	0.30—0.50	36
24	16	17.2	26	23.7	35	36.2	77	0.3374	0.50—0.70	40
25	7	7.9	14	12.2	38	38.9	59	0.3715	0.50—0.70	64
26	14	17.6	35	27.7	89	92.6	138	2.7972	0.05—0.10	66
27	14	13.8	20	20.4	52	51.8	86	0.0131	0.90—0.95	57
28	12	11.6	16	16.7	36	35.6	64	0.0486	0.80—0.90	51
29	13	12.2	17	18.5	53	52.2	83	0.1825	0.50—0.70	61
30	2	0.5	2	5.0	74	72.5	78	6.4566	0.01—0.02	99
31	6	5.2	8	9.6	51	50.2	65	0.3923	0.50—0.70	79
32	4	5.3	13	10.5	62	63.3	79	0.9550	0.30—0.50	82
33	4	4.6	9	7.7	31	31.6	44	0.3082	0.50—0.70	72
34	0	—	3	—	78	—	81	—	—	100
35	12	10.1	12	15.8	54	52.1	78	1.3505	0.20—0.30	65
36	0	—	5	—	112	—	117	—	—	100
37	5	6.0	13	11.1	59	60.0	77	0.4929	0.30—0.50	79
38	0	0.3	5	4.4	65	65.3	70	0.4139	0.50—0.70	99
39	10	9.6	16	16.7	77	76.6	103	0.0474	0.70—0.80	75
40	0	1.5	9	6.0	66	67.5	75	3.0600	0.05—0.10	95
41	14	12.3	15	18.4	51	49.3	80	0.9260	0.30—0.50	59
42	6	7.2	15	12.6	57	58.2	78	0.6969	0.30—0.50	75
43	26	23.3	31	36.5	123	120.3	180	1.2033	0.20—0.30	65
44	0	1.4	9	6.2	71	72.4	80	2.7440	0.05—0.10	95
45	17	15.9	20	22.3	40	38.9	77	0.3422	0.50—0.70	45
46	1	1.2	5	4.5	49	49.2	55	0.0934	0.70—0.80	94
47	0	0.9	7	5.2	65	65.9	72	1.5955	0.20—0.30	97
48	0	—	4	—	81	—	85	—	—	100
49	2	1.2	4	5.5	66	65.2	72	0.8870	0.30—0.50	96
50	1	0.8	5	5.4	74	73.8	80	0.1022	0.30—0.50	98

mated for each sample. Thus for sample no. 24 which has a gene frequency of 38 per cent, the percentage of specimens from the eastern population is inferred to be $(61-38):(61-3)=40$ per cent. This percentage of the specimens in the sample is then distributed according to the Hardy-Weinberg law with 3 per cent for the parameter q^1. The remaining 60 per cent of the specimens are likewise distributed according to this law, but with 61 per cent as the frequency of the *Hb* I^1 allele. The resulting two sets of distributions are then added to yield the "expected" numbers presented in the table. When, as in samples nos. 34, 36, and 48, the gene frequency calculated on the basis of the sample, falls outside the range from 3 to 61 per cent, such calculations become meaningless by giving negative values. These samples contain no Hb I-1 homozygotes and may therefore be considered of purely eastern Baltic origin.

In the table the χ^2 values for goodness of fit and the corresponding probability limits for one degree of freedom are given. The χ^2 test is not reliable when expected numbers are smaller than 5 (FISCHER, 1954). The test will, however, tend to overestimate the significance of the deviations, and it may therefore safely be concluded that the vast majority of the samples agree well with the mixture hypothesis. In only two cases (nos. 22—30) does the deviation from that expected exceed the significance level of 5 per cent, and in no case is the 1 per cent level exceeded. The deviations of these two samples can hardly be considered significant, since they occur among 33 such comparisons. One would be inclined to accept the hypothesis, that the samples consist of mixtures of the two populations, to account very well for all these observations.

If, however, the direction of the deviations, i.e. whether too many or too few heterozygotes, is observed, is also taken into consideration, then a conspicuous unidirectional trend is revealed for the westernmost samples. All the samples from no. 16 through no. 26 possess a larger number of type Hb I-1-2 specimens than expected. This observation should certainly not be neglected, and the hypothesis of simple mixing may need modification to account for the genotypic composition of this westernmost group of samples. There are at least three possible explanations for the peculiar genotypic compositions of these samples:

a) There are only two populations. They mix mechanically but do not interbreed. The excess of type Hb I-1-2 specimens is due to heterosis. In this particular hydrographical area the heterozygotes are endowed with fitness superiority.

b) One or more small self-contained population units with q^1 values

intermediate between 3 per cent and 61 per cent, are intercalated between the large eastern and western populations. Pure samples from these populations are rarely obtained because of swamping by migrants from the adjacent large populations. However, sample nos. 17, 18, and 19 could actually represent such intermediate populations in a relatively clean state, since they conform well with the distributions expected in homogeneous populations (Table 5).

In this connection it is worth-while to mention that most of our samples came from trawl hawls of one hour's duration, and thus cover rather long stretches. A number of different shoals may have been included in each sample. The possibility that each of these shoals was homogeneous with respect to the haemoglobin genes cannot be excluded. The mixed nature of samples from this area need not reflect complete mixing of the popualtions in the sea, but could also result from our method of sampling.

c) The eastern and western populations do interbreed to a limited extent in a rather narrow hybrid zone, but the gene flow from one population to the other is restricted for instance by hybrid sterility or by very different selective forces prevailing in the two regions.

It is not possible on the basis of our material to choose between the different situations suggested above. It should also be stressed that these situations are not true alternatives; combinations between them could exist.

Length measurements were taken of the majority of the samples from this area. In some cases there was a pronounced difference between the genotypic compositions of different size groups within the same sample. The results for sample no. 24, which was rather extreme in this respect, are given in Table 7. The q^1 value for the part of the sample measuring from 14 to 25 cm was 58 per cent, while the specimens measuring more than 25 cm had only 16 per cent of the *Hb* I^1 allele. Thus in this sample all the smaller specimens could be of western origin and the majority of the large specimens of eastern origin.

Summarizing the results for area 3, it may be said that the cod population from the central Baltic is marked by heterogeneity caused by the transition from the Belt Sea population to the eastern Baltic population. Most of the samples reveal genotypic compositions that could result from simple mechanical (non-reproductive) mixing of these two populations. For the westernmost samples, mechanical mixing, although probably occurring to a large extent, is hardly a completely adequate explanation for the observed genotypic distributions. For this region the

TABLE 7. *The genotypic composition of different size groups in sample no. 24.*

Length in cm	Number of specimens	Haemoglobin type			q^1
		Hb I—1	Hb I—1—2	Hb I—2	
14—20	33	12	13	8	
21—25	7	2	5		
Total 14—25	40	14	18	8	0.58
26—30	6		4	2	
31—35	5	1	2	2	
36—40	3			3	
41—45	2			2	
46—50	4	1	1	2	
51—55	2			2	
56—60	2			2	
61—65	7		1	6	
66—70	4			4	
71—75	1			1	
76—80					
81—85	1			1	
Total 26—85	37	2	8	27	0.16
Total 14—85	77	16	26	35	0.38

occurrence of heterosis, intermediate populations, or limited hybridization is suggested. Reasonably enough, the inferred percentage in the samples of fish from the Belt Sea population, decreases from west towards east. It will be remembered that rare stray specimens from the western population were postulated to occur as far east as Gotland. A similar small number of migrants from the eastern population could very well live in the Belt Sea without being detected, because of the relatively large percentage of Hb I-2 specimens inherent to the western population.

IV. DISCUSSION

The haemoglobin studies presented here have shown beyond any reasonable doubt, that the cod in the Belt Sea and the western Baltic is genetically different from the cod that lives in the eastern part of the

Baltic. Both populations are polymorphic for the haemoglobin alleles *Hb* I^1 and *Hb* I^2, but with radically different gene frequencies. The western population has 61 per cent of the *Hb* I^1 allele and 39 per cent of the *Hb* I^2 allele, while the corresponding figures for the eastern population are 3 per cent and 97 per cent respectively.

FORD (1953) has defined polymorphism as "the occurrence together in the same environment of two or more discontinuous forms of a species in such proportions as the rarest of them cannot be maintained be recurrent mutation". The haemoglobin variation of the western cod population clearly conforms to this definition. The rôle of mutation in maintaining the low frequency of *Hb* I^1 alleles in the Baltic population can, on the other hand, not be excluded. If mutation was important one would, however, expect also to encounter alleles that would be electrophoretically distinguishable from the two actually observed. Of the 5439 cod specimens from the Belt Sea and the Baltic that have been analysed not one single individual had a haemoglobin pattern deviating from the three common ones. This suggests that mutations affecting the electrical properties of these haemoglobins are rare, or that individuals carrying haemoglobin components with electrophoretic mobilities different from Hb I-1 and Hb I-2 are more or less inviable. In humans a large number of electrophoretically different rare haemoglobins have been found, most of which do not seem to be harmful in heterozygous or even in homozygous condition (INGRAM, 1963). In human populations outside the polymorphic regions, for instance in England (HUNTSMAN *et al.*, 1963), heterozygotes for haemoglobin variants constitute only about one per mille of the individuals, and are thus much rarer than the Hb I-1-2 type of cod in the eastern Baltic Sea. If these comparisons between the haemoglobin variation of man and cod are valid, one would conclude that the *Hb* I^1 alleles met with in the eastern Baltic are there for other reasons than mutation pressure.

In the cod populations of the northern Atlantic Ocean, a small percentage of haemoglobin patterns different from the three described here are usually found (FRYDENBERG *et al.*, 1965; SICK, 1965). It is remarkable that not one single aberrant haemoglobin type occurred among the 5439 specimens collected in the Baltic and the Belt Sea. If the occurrence of these "rare types" in the northern cod populations is due to recurrent mutations, one would have expected to find them also in the present material. The seemingly complete absence of "rare types" from the Baltic and the Belt Sea makes it unlikely that their presence in other areas is due to mutation pressure alone, since the mutational rate of the

haemoglobin loci could hardly differ very much from population to population. If the "rare types" are subject to very different selection pressures in different geographical areas, this could explain their differential distribution. Another possible explanation is that the "rare types" are common in a cod population, of which the haemoglobins have not yet been investigated, and that stray specimens from this population visit other areas in the northern Atlantic but never reach the Belt Sea or the Baltic.

Balanced polymorphic systems based upon fitness superiority of heterozygotes can be stabilized at any gene frequency level; this level being determined by the relative fitness of the two types of homozygotes (FISHER, 1930). If the haemoglobin polymorphism of cod in the Belt Sea and the Baltic Sea is stabilized by heterosis, then the relative coefficients of selection of the two types of homozygotes must be very different in the two areas. The relationship between these coefficients of selection and the gene frequencies attained at equilibrium is given by the formula:

$$\frac{q}{1-q} = \frac{s_2}{s_1}$$

where s_1 is the coefficient of selection of the type which is homozygous for the allele that attains the frequency level q, and s_2 is the coefficient of selection of the other homozygotes. Substituting in this formula gives for the Belt Sea:

$$\frac{0.61}{0.39} = \frac{s_2}{s_1} \text{ or } s_1 = 0.6\, s_2$$

For the Baltic we obtain:

$$\frac{0.03}{0.97} = \frac{s_2}{s_1} \text{ or } s_1 = 32\, s_2$$

The hydrographical conditions in the areas inhabited by these two populations are indeed very different both with respect to temperature and salinity (GESSNER, 1958), and different selection pressures could prevail in the two regions. The two hereditary haemoglobin variants found in sheep have been shown to possess different oxygen dissociation curves (HUISMAN *et al.*, 1959). Similar differences of the cod haemoglobins could convey different adaptive values to the genotypes, depending upon environmental factors.

The geographical and hydrographical development of the Baltic region since the last glaciation is rather well known (MAGNUSSON *et al.*, 1949), it is therefore possible to put a reasonable time limit on the occurrence of cod in the Baltic Sea. Although the species can live in rather brackish water, it is hardly conceivable that it could have survived here during the Ancylus period, when the Baltic was a fresh-water lake. This means that in all probability the cod has not lived continuously in the Baltic for more than approx. 7000 years, i.e. since the beginning of the Litorina period. Since that time the Baltic has always been connected with the ocean through the Danish Belts and its water has remained salt or brackish. If, after the Ancylus time, one homogeneous cod population gained entrance to the Baltic from west, then the actual difference in haemoglobin gene frequencies between present eastern and western population must have developed during 7000 years or less, in the absence of geographical isolation. In that case the transition observed in the central Baltic would be an example of a primary intergradation zone, i.e. a steep slope developed gradually while the populations involved were in continuous contact (MAYR, 1942). As long as nothing is known about the influence of environmental factors on the adaptive value of the cod haemoglobin types, it is impossible to say whether the change in hydrographical conditions from west to east could account for the abrupt shift from a gene frequency of 61 per cent to one of only 3 per cent. The change from oceanic hydrographical conditions in the Skagerrak to the almost fresh waters of the eastern Baltic takes place gradually all the way from the Kattegat to the bottom of the Gulf Bothnia (GESSNER, 1958), and unless decisive threshold values exist, it is difficult to understand why the transition from the western cod population to the eastern should make itself marked in such a narrow zone only.

The narrow width of this transition zone is more easily understood if it is a case of secondary intergradation, which according to MAYR (1942) "refers to cases in which the two units now connected by a steeply sloping character gradient were separated completely at one time and have now come into contact again, after a number of differences had evolved".

If the present contact between two formerly allopatric populations is of very recent date, this would in itself explain the narrowness of a hybrid belt, but also if the contact has been of long duration, a number of biological mechanisms such as selective mating or hybrid inviability or infertility could prohibit or strongly delay the widening of a hybrid

belt, even if the environmental conditions on the two sides of the zone did not differ.

Zones of secondary intergradation are especially common in areas where major geographic or climatic changes have recently taken place. The Baltic Sea is well-known for the number of more or less dramatic upheavels it has experienced since the end of the last glaciation. Even if we exclude the possibility that a cod population survived in the Baltic during the Ancylus period, these post-glacial changes still offer ample possibilities for the formation of an allopatric hybridization zone in the central Baltic.

In the period preceding the Ancylus stage, the Baltic had wide connections with the oceans through the Lake District of Sweden and probably also through Ladoga and Onega to the White Sea (SAURAMO, 1958). At that time the Baltic could have been colonized by cod from the Barents Sea. This cod population could have survived the Ancylus period outside the Baltic itself, namely in the deep "Kattegat Fjord", as suggested by SPÄRCK (1942) for a number of marine arctic invertebrate species found in the Baltic today. This cod population which originally came from the Arctic Sea, could very well have shown some sort of reproductive isolation towards the population it met in the west, and therefore have conserved its integrity as a distinct population. When, at the beginning of the Litorina period, the Danish Belts were opened again, this population could leave its temporary refuge and be the first cod to recolonize the Baltic. The border between the eastern and western populations would later have been pushed towards south and east to its present location in the central Baltic. There is also the possibility that the influx of oceanic water to the Baltic after the Ancylus period did not take place exclusively through the Danish Belts but also came from the Barents Sea. This could lead to a similar situation as that described above, without the need of a sojourn of Baltic cod on Kattegat.

Investigation of the haemoglobin types of Norwegian cod populations has revealed a steady decrease of the frequency of the *Hb* I^1 allele all along the coast from 60 per cent in the Oslo Fjord to only 10 per cent in the Barents Sea (FRYDENBERG *et al.*, 1965). Arctic cod populations such as those of Greenland and Iceland have q^1 values of only 2 per cent (SICK, 1965), so with respect to the haemoglobins the Baltic cod actually resembles the Arctic cod much closer than its immediate neighbour in the Belt Sea. This resemblance suggests, but does not prove a true relationship between Arctic and Baltic cod populations.

If our speculations about the evolutionary history of the Baltic cod

are correct, then the case of cod is comparable to a "ring of races" such as that described by RENCH (1933) for the titmouse (*Parus major*) and its relatives. A number of closely related subspecies of this bird grade into each other all around the central mountain ranges of Asia. In the Amur region, however, two forms meet which do not intergrade. If we imagine a marine connection between the Barents Sea and the Baltic, so that Fenno-Scandia was an island, the cod could be said to form a ring of races round this island, with the central Baltic as an area of no or very limited intergradation.

The above discussion of the evolutionary history of cod in the Baltic region is admittedly highly speculative. The concept of the Baltic cod as a sort of arctic relict does, however, seem to the author more probable than the occurrence of sympatric differentiation in a species, which is highly mobile at all stages of its life cycle.

Whatever the true history of the haemoglobin difference between the Baltic and the Belt Sea cod may be, we are undoubtedly today dealing with two distinct and to a large extent also separate populations.

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SUMMARY

A technique for electrophoresis of fish haemoglobins is described. By means of this technique three haemoglobin phenotypes are demonstrated in the cod, and it is inferred that this variation is due to the segregation of two allelic genes.

The haemoglobin type of a large number of cod specimens from the Kattegat, the Belt Sea, and the Baltic have been described. Very different gene frequencies were found to prevail in the eastern Baltic as opposed to the western Baltic, the Belt Sea and the Kattegat, while the central Baltic showed the characteristics of a transition area. The evolutionary aspects of the observed difference between Baltic and Belt Sea cod are discussed.

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