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An Analysis of Genetic Differentiation in Greenland Halibut (Reinhardtius hippoglossoides W.) in the Northwest Atlantic

by

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ABSTRACT

As part of a stock identification study of Greenland halibut frequencies of electroforetically detectable protein loci were analyzed from six areas in the western North Atlantic in order to elucidate the genetic differentiation. Generally, differences in allele frequencies are small between the samples. Concerning four polymorphic loci the phenotypic distribution for all six samples was in accordance with the expectations from the Hardy-Weinberg proportions. A trend of deficiency of heterozygotes indicates mixed populations. Differences in homogeneity of the allele frequencies between samples are interpreted as that the populations studied do not belong to the same breeding group. However, the genetic distances between the samples are too small to be interpreted as differentiation in local populations. Furthermore, the method of electroforesis is sensitive to intermingling, which is expected to occur between the areas studied.

1. INTRODUCTION.

Greenland halibut (Reinhardtius hippoglossoides Walb.) is widely distributed in the Northwest Atlantic. Spawning is supposed to take place in the deeper waters of the Davis Strait south of 67° N (Jensen, 1935 and Smidt, 1969). The larvae are dispersed by the currents both to the west coast of Greenland and to the eastern Canadian coast (Templeman, 1973). While growing up Greenland halibut in West Greenland migrate to the deeper parts of the fjords. When reaching maturity they are assumed to migrate to the spawning area in the Davis Strait (Smidt, 1969).

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Similarly, in the East Greenland/Iceland area, spawning seems to occur on the continental slopes west of Iceland. Eggs and larvae are supposed to be carried either north-eastward along the northern Icelandic coast or more possibly first north-west ward later south-westward toward East Greenland by the Irminger Current (Sigurdsson, 1980). Fish growing up in the northern area at Iceland are assumed to migrate to the spawning area when reaching maturity. It therefore seems that Greenland halibut in the western North Atlantic (i.e. including Icelandic waters) forms two spawning stocks, although migrations between the areas have been observed (Riget & Boje 1989).

Several studies on stock identification of Greenland halibut in delimited areas of the Northwest Atlantic have been carried out. Templeman (1970) and Misra & Bowering (1984) have analysed meristic characters, Bowering (1988) analysed morphometric characters, Fairbairn (1981) investigated frequencies of electrophoretically detectable protein loci, and Khan et al. (1982) dealt with blood protozoa used as biological tags. All authors suggest that Greenland halibut form a single interbreeding stock throughout the Northwest Atlantic (NAFO Convention Area) although there is evidence that those in the Gulf of St. Lawrence and in Fortune Bay constitute two separate stocks (Bowering, 1982).

Recently, Riget and Boje (1989) summarized the present knowledge of the biology of Greenland halibut in West Greenland waters. They hypothesize that the stocks in the southernmost fjords of West Greenland can be recruited from the spawning grounds west of Iceland and further that stocks in the West Greenland fjords may be regarded as mainly stationary. Furthermore they point out, that adults from the West Greenland fjords have never been included in former stock identification studies. From these points of view a stock identification study of Greenland halibut in the Northwest Atlantic was initiated in 1987.

The study covers samples from the Denmark Strait, the Davis Strait, the inshore areas of West Greenland and an area off Newfoundland north of Grand Bank. The study includes methods analysing genetic variation, meristic characters and the natural parasite fauna.

This paper presents the results of analyses of the genetic differentiation. The results of the analysis of the meristic characters are also presented at this meeting (NAFO SCR Doc. 89/25), while the analyses of the parasite fauna are in progress for later presentation.

An a priori hypothesis predicts little differentiation in the area investigated because of assumed migrations between the areas. The Denmark Strait sample is expected to differ from the other samples apart from the Julianehaab sample (Div.1F), as drift of larvae from Iceland may affect this area. Little differentiation is expected between samples from the Davis Strait, from Newfoundland and from the inshore areas in West Greenland (apart from Julianehaab), although the latter may differ from the offshore samples if it is assumed that spawning occurs in the fjords.

2. MATERIALS AND METHODS.

Sampling.

Samples for the study were collected at six sampling localities as shown at Fig.1. The sampling at Newfoundland (NAFO Div.3K) was done in

December 1987 by staff from Northwest Atlantic Fisheries Centre, St.John's. The sample from the Davis Strait (NAFO Div.1C) and from the Denmark Strait (ICES Subarea XIVb) were taken in September 1988 and November 1987, respectively, by staff of the Greenland Fisheries Research Institute on board the Japanese research vessel SHINKAI MARU. Samples from the inshore areas in West Greenland, Julianehaab (NAFO Div.1F), Godthaab (Div. 1D) and Umanak (Div.1A) were taken in January 1988, January 1987 and August 1987, respectively, by staff of the Greenland Fisheries Research Institute on board research vessels of the institute.

Around 100 specimens of Greenland halibut in the length range 50-70 cm were sampled from each locality. Separate tissue samples from each fish were taken immediately after capture. Sex of all specimens was determined and length and weight measured. Otoliths were taken for age determination. From each specimen eye, heart, liver and muscle were removed, with exception of the Godthaab sample where no eyes were taken. The tissue samples were stored quickly in plastic bags at -18°C until use for electrophoresis. Furthermore, samples for the meristic and parasitic studies were taken from the same fish.

Biochemical techniques.

The electrophoresis was ordinary starch gel electrophoresis. The following enzymes were tested : adenosine deaminase (ADA), creatine phosphate kinase, esterase, glucosephosphate isomerase (GPI), glutamic oxaloacetic transaminase, a-glycerophosphate dehydrogenase, isocitric acid dehydrogenase (ICD), lactate dehydrogenase, malate dehydrogenase (MDH), mannosephosphate isomerase, phosphoglucomutase (PGM) and superoxid dismutase. Only ADA, GPI, ICD ,MDH and PGM expressed various zymograms. The six other enzymes did not reveal more than one zymogram, and hence were not useful for the study of divergence among populations. The buffer system used for GPI was the one described by Clayton and Tretiak (1972). The buffer system used for the remaining four enzymes was described by Ayala et al. (1972). Heart tissue was used for GPI, MDH and PGM, liver for ICD and muscle for ADA. The staining procedures used for the five enzymes were described by Frydenberg and Simonsen (1973).

Genotypic frequencies were tested for fitting Hardy-Weinberg expectations, using t=F sqroot(N), F being the interbreeding coefficient and N the sample size (Brown, 1970). Allele frequency differences between populations were tested for homegeneity using replicated G-test (likelihood-ratio test, see Sokal and Rohlf 1981). Estimates of genetic identity (I) and genetic distance (D) was performed as described by Nei (1972) and an estimation of a dendrogram based on an UPGMA is proceeded as described by Sneath and Sokal (1973).

3. RESULTS.

The zymograms for the isozymes GPI, MDH,and PGM are in accordance with the description given by Fairbairn (1981). In the present study the locus Mdh-1 and the locus Gpi-2 are polymorphic at the 99% level. The used nomenclature of the loci is the same as that used by Fairbairn (1981) except that Fairbairn uses the abbreviation PHI for the GPI enzyme. The zymogram for ICD is most likely determined by one locus with four codominant alleles. The ADA enzyme is determined by one locus with three common alleles and four rare alleles all expressing codominance.

The frequency of the most common allele at each locus is listed in Table 1. The number of specimens scored at each locus for each locality are given in the table. The value for the testator F(sqroot(N)) is given in the same table. No test for the loci Gpi-2 and Mdh-1 are done as the frequency of the most common allele is very close to 1.000. The tests are based on the assumption, that each locus has two codominant alleles, the common and all the others taken together. Only 1 out of 24 test values is significant. This is the order of magnitude, which would be excepted and hence acceptable. The conclusion is, that the phenotypic distribution for all six populations for each of the four polymorphic loci are in accordance with the expectations from the Hardy-Weinberg proportions.

A negative test value means an excess of heterozygotes and a positive test value means a deficit. Among 24 test values 7 show an excess and 17 a deficit of heterozygotes. The expectation is that the samples with deficit and those with excess of heterozygotes are randomly distributed e.g. 1:1. A chi square test gives significance at the 5% level, thereby showing a trend of deficiency of heterozygotes. This is an indication of mixed populations which deviate from having random mating or selection against the heterozygotes.

Tests for homogeneity of the allele frequencies among the samples are shown in Table 2. Again, each locus is assumed to have one common allele and the rest of the alleles are treated as one allele. The distribution of the test values are the same as for chi square test. The sample from Newfoundland reveals significant test values in 4 out of 5 comparisons with the other sampling localities. The sample from Julianehaab shows significant test values in 3 out of 5 comparisons. The sample from the Denmark Strait expresses significance in two cases and the samples from Godthaab, Umanak and Davis Strait all shows significance in one case. With these differences in homogeneity between the samples, it does not seem that the populations studied are belonging to the same breeding stock.

The genetic distance was calculated according to Nei (1972) and listed in Table 3. Closest connection exists between Denmark Strait and Godthaab (D=0.0019), while Denmark Strait and Julianehaab are having the greatest distance (D=0.0071) in the material.

4. DISCUSSION.

Previous investigations on isozymes in Greenland halibut have revealed that the population in the Gulf of St. Lawrence differs from the populations off Newfoundland and Labrador (Fairbairn 1981). The divergence between the populations is based on allelic frequencies of the allozymes phosphoglucomutase (PGM) and phosphohexose isomerase (PHI) and on enzymatic properties of the PGM enzyme (Dey 1982). In the present study, which covers a wider geographical area, additional enzymes are included in the analyses. Among those, besides the allozymes mentioned, adenosine deaminase (ADA), isocitric acid dehydrogenase (ICD) and malate dehydrogenase (MDH) showed polymorphism.

Generally, it seems as there are weak differences in allele frequencies between the populations tested. This fits well with the results obtained by Fairbairn (1981), who found a rather low degree of divergence between samples from Labrador, Newfoundland and Gulf of St. Lawrence. From Table 1 it is obvious that frequencies are very similar between the samples. This confirms the a priori hypothesis that genetic divergence is expected to be low in the area, which might be

caused by intermingling. That intermingling occurs is supported by the fact of a trend of deficiency of heterozygotes, indicating mixed populations. Further, differences in homogeneity indicates that populations represented by the samples do not belong to the same breeding group. Therefore, the area studied may cover more than one breeding population in accordance with present knowledge of at least two spawning areas.

An UPGMA analysis on the basis of genetic distances resulting in a dendrogram do not give any explainable result in the cluster grouping. With the achieved genetic distances one can hardly conclude that any clusters are mutually different. According to Ayala (1975) the present genetic distances although based on only six loci are well below the level for a 'local population' stage of evolutionary divergence for fish as a group, which is given at the level D=0.02. According to Riget and Boje (1989), tagging experiments show that long distance migrations do occur in the investigated area, although to a minor extent. However, the method of electrophoresis is very sensitive to such intermingling, and the weak differences in genetic differentiation between the populations tested can be due to this fact.

In conclusion, the method of electroforesis do not seem usefull for elucidating the detailed stock connections between stocks of Greenland halibut in the Northwest Atlantic. However, results indicate that Greenland halibut in the Northwest Atlantic consist of several spawning stocks, which however are not mutually isolated.

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6. REFERENCES.

Ayala,F.J., J.R.Powell, M.L.Tracey, C.A.Mourro and S.Perez-Salas. (1972). Enzyme variability in the Drosophila willistoni group. IV. Genetic variation in natural populations of Drosophila willistoni. Genetics 70: 113-139.

Ayala,F.J. (1975). Genetic differentiation during the speciation process. Evol. Biol. 8: 1-78.

Bowering,W.R. (1982). Stock identificationstudies of Greenland halibut (Reinhardtius hippoglossoides) in the Northwest Atlantic from tagging experiments. NAFO SCR Doc. 82/IX/78 (mimeo).

Bowering, W.R. (1988). An Analysis of Morphometric Characters of Greenland halibut (Reinhardtius hippoglossoides) in the Northwest Atlantic Using a Multivariate Analysis of Covariance. Can.J.Fish. Aquat. Sci. Vol.45:580-585.

Brown,A.D.H. (1970). The estimation of Wright's fixation index from genotype frequencies. Genetica 41: 399-406.

Clayton,J.W. and D.N.Tretiak. (1972). Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Canada 29: 1169-1172.

Fairbairn,D.J. (1981). Biochemical genetic analysis of population differentiation in Greenland halibut (Reinhardtius hippoglossoides) from the Northwest Atlantic, Gulf of St. Lawrence, and Bering Sea. Can. J. Fish. Aquat. Sci. 38: 669-677.

Frydenberg,O. and V.Simonsen. (1973). Genetics of Zoarces populations. V. Amount of protein polymorphism and degree of genetic heterozygosity. Hereditas 75: 221-232.

Jensen,Ad.S. (1935). The Greenland halibut (Reinhardtius hippoglossoides) its development and migrations. K. Danske Vidensk. Selsk. Skr. 9 Rk. 6: 1-32.

Khan,R.A., M.Dawe, R.Bowering and R.K.Misra. (1982). Blood protozoa as an aid for separating stocks of Greenland halibut, Reinhardtius hippoglossoides in the northwestern Atlantic. Can. J. Fish. Aquat. Sci. 39: 1317-1322.

Misra,R.K., and W.R.Bowering. (1984). Stock delineation of Greenland halibut in the Northwest Atlantic using a recently developed, multivariate statistical analysis based on meristic charaters. N. Am.J.Fish. Manage. 4A: 390-398.

Nei,M. (1972). Genetic distance between populations. Amer. Nat. 106: 283-292.

Riget,F. and J.Boje (1989). Fishery and some biological aspects of Greenland halibut at West Greenland. NAFO Sci.Coun.Studies (in press).

Sigurdsson, A. (1980). On the nursery grounds of the Greenland halibut spawning in Icelandic waters. I.C.E.S. Coun. Meet. Doc. 1980/G:45 (8 pp)(mimeo).

Smidt, E. (1969). The Greenland halibut, Reinhardtius hippoglossoides (Walb.) Biologi and Exploitation in Greenland Waters. Medel. Danm. Fisk.- og Havunders. N.S., 6:79-148.

Sneath,P.H.A. and R.R.Sokal (1973). Numerical taxonomi. San Francisco, W.H.Freeman and Co.

Sokal,R.R. and F.J.Rohlf (1981). Biometry (2. ed.). San Fransisco, W.H.Freeman and Co.

Templeman, W. (1970). Vertebral and other meristic characteristics of Greenland halibut, Reinhardtius hippoglossoides, from the Northwest Atlantic. J.Fish. Res. Bd. Canada., 27: 1549-1562.

Templeman, W. (1973). Distribution and abundance of Greenland halibut, Reinhardtius hippoglossoides (Walbaum), in the Northwest Atlantic. ICNAF Res. Bull., 10:83-98.

Table 1. List of allele fequencies of the most common alleles (p(C)) at six loci in six samples of Greenland halibut, the number of specimens scored (N) and the test values (F(sqroot(N))) for testing the accordance with the Hardy-Weinberg proportions.

Locality / Locus		Newfoundland	Davis Strait	Umanak	Godthaab	Julianehaab	Denmark-Strait
Ada	p(C)	0.580	0.384	0.420	0.459	0.539	0.429
	N	87	99	106	98	102	98
	F sqroot(N)	0.74	-0.25	-1.07	0.14	0.54	2.06*
Gpi-1	p(C)	0.742	0.725	0.712	0.680	0.683	0.677
	N	93	100	104	86	104	96
	F sqroot(N)	-0.65	0.22	1.60	0.60	-1.12	0.46
Gpi-2	p(C)	0.995	0.985	0.995	0.995	1.000	1.000
	N	92	100	106	100	103	89
Icd	p(C)	0.860	0.865	0.836	0.879	0.840	0.865
	N	89	100	106	99	100	89
	F sqroot(N)	1.09	1.01	0.08	0.52	-0.42	-0.56
Mdh-1	p(C)	0.954	0.975	0.991	0.995	0.990	0.990
	N	98	99	107	101	104	100
Pgm	p(C)	0.802	0.809	0.825	0.776	0.890	0.740
	N	91	99	106	96	104	96
	F sqroot(N)	0.29	0.23	0.52	0.70	-0.27	1.85

*significant deviation from the expected values for the Hardy-Weinberg proportions.

Table 2. Test values (G-tests) for homogeneity of the allelic distribution between the samples.

Locality	Newfoundland	Davis Strait	Umanak	Godthaab	Julianehaab	Denmark-Strait
Newfoundland		14.63*	11.17*	7.79	13.12*	12.57*
Davis Strait			1.58	3.96	9.11	4.51
Umanak				4.28	4.68	5.69
Godthaab					10.65*	1.23
Julianehaab						16.17*

*significant, no homogeneity between the two samples

Table 3. Genetic identities (I) above the diagonal and genetic distances (D) under the diagonal.

Locality	Newfoundland	Davis Strait	Umanak	Godthaab	Julianehaab	Denmark-Strait
Newfoundland	-	0.9940	0.9944	0.9959	0.9972	0.9940
Davis Strait	0.0060	-	0.9976	0.9973	0.9943	0.9976
Umanak	0.0056	0.0024	-	0.9972	0.9965	0.9947
Godthaab	0.0041	0.0026	0.0028	-	0.9961	0.9981
Julianehaab	0.0028	0.0056	0.0035	0.0039	-	0.9930
Denmark Strait	0.0060	0.0024	0.0053	0.0019	0.0071	-

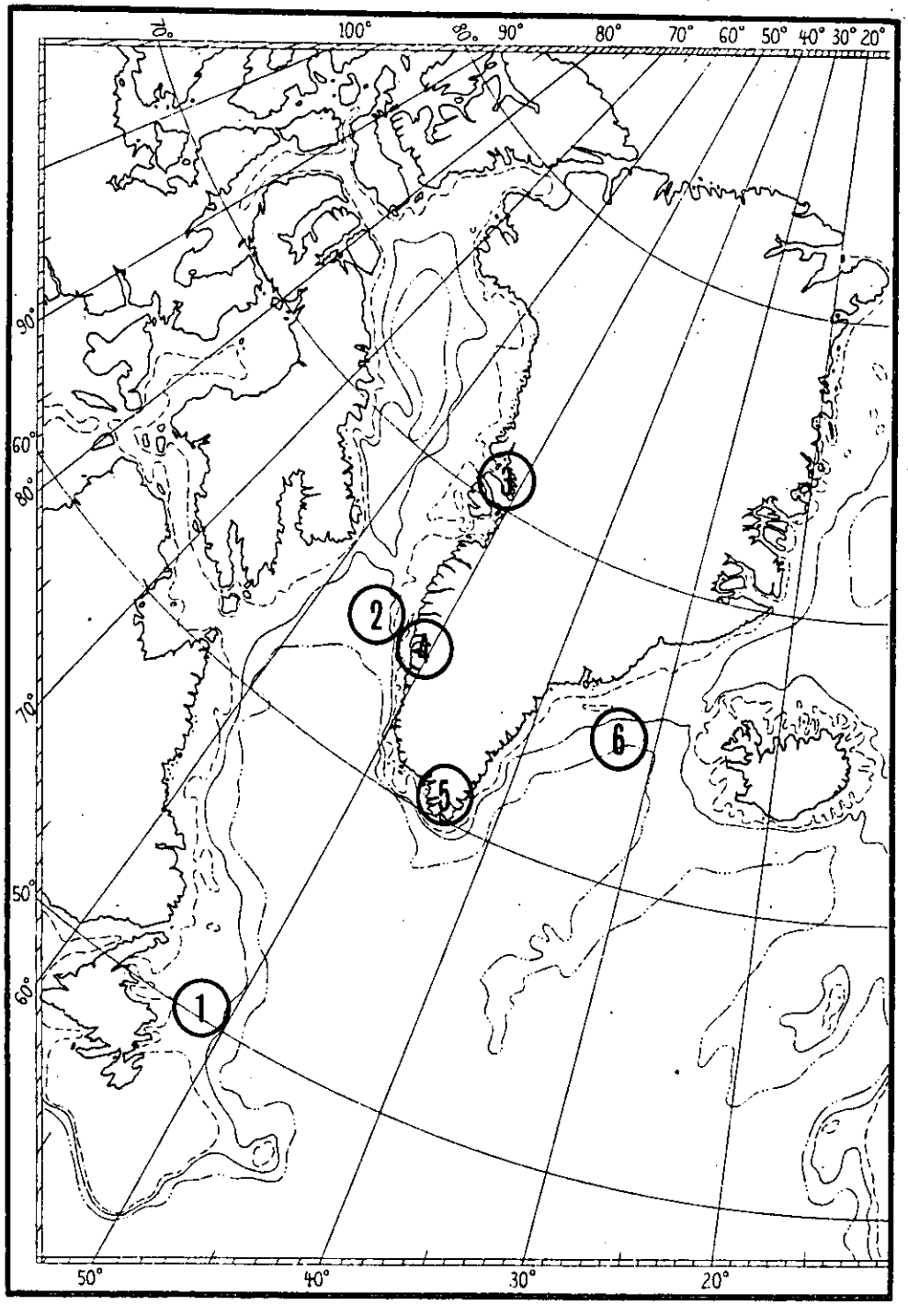

Fig. 1. Distribution of Greenland halibut samples in the Northwest Atlantic. 1: Newfoundland, 2: Davis Strait, 3: Umanak, 4: Godthaab, 5: Julianehaab, 6: Denmark Strait.