RESTRICTED

INTERNATIONAL COMMISSION FOR THE NORTHWEST ATLANTIC FISHERIES

Serial No.1840
(D.c.5)

ICNAF Res.Doc.67/52

ANNUAL MEETING - JUNE 1967

A study of blood serum protein composition by agar gel electrophoresis in marinus and mentella types of redfish

by Ju.P.Altukhov and G.N.Nefyodov
Moscow University

ABSTRACT

By the method of agar gel electrophoresis the protein pattern of blood serum was studied in 49 specimens of 'marinus', 53 of 'mentella' and in 43 of the 'giant' types of redfish from the Iceland - Greenland Area of the North-West Atlantic. It was found that the studied forms of redfish differ from each other statistically significant in α_1- globulins and 'A' and 'B' albumins, being probably, under a genetic control of a pair of autosomal alleles. A general evaluation of the biochemical position of the 'giants' leads to the conclusion on their hybrid origin.

The 'marinus' and 'mentella' types of redfish from the North-West Atlantic differ in thermostability of isolated muscles (Altukhov et al., 1967). Inasmuch as this feature had been established as the species-specific criterion for poikilothermal animals (Ushakov, 1959a; 1959b; 1964) we surmised the intraspecific level of divergence in the studied forms of redfish.

On the other hand, it was discovered that the 'marinus' West Greenland population appeared to be characterised by a certain level of thermostability occupying an intermediate position between the 'marinus' and 'mentella' from the Iceland area. The circumstances of such kind as well as an examination of number of biological peculiarities of the West Greenland 'marinus' type, including a relatively high frequency of so called 'giants' in this region gave us grounds to regard the West Greenland 'marinus' as 'marinus' type only by its external outlook, but actually being hybrids F_I between 'marinus' and 'mentella' forms partially interbreeding in Iceland waters. Apparently, both for a solution

and for a fuller understanding of this problem, closely connected with the taxonomical relations of the redfishes, it is advisable to analyse their differentiation not only in any single diagnostical characteristic but by a complex of features. In addition to the work done in this direction from the position of molecular taxonomy by other authors (O'Rourke, I96I; Schaeffer, I96I) we represent in this report the data of our investigations of the blood serum proteins of 'marinus' and 'mentella' and also the 'giant' form of the redfishes by the method of agar gel electrophoresis.

Materials and Methods.

The work took place on board the r/v 'Sevastopol' of the Polar Research Institute of Marine Fisheries and Oceanography (Murmansk) and in the laboratory of Moscow University in autumn I965- winter I966. During the period of investigations I42 samples of serum from 'marinus', 'mentella' and the 'giant' forms of redfish were put to electrophoresis in agar gel. To 'giants' we refered specimens of more than 60 cm. in length. The places of trawling this fish are shown in the map (fig.I). Below we describe the main methodical procedures.

Blood sampling and preparation of serum. The blood was taken with the help of sterile Paster's pipette by cardiae puncture. and put into test-tubes. As is known, blood of many and especially of fresh-water fishes coagulates in a very short time, measured in dozens of seconds or even in seconds (Puchkov, I954). But in redfish, due to yet unknown reasons, the process of blood clotting is extreemly delayed. That is why we centrifuged the fresh-sampled blood for I0-I5 min. at 6000-7000 revolutions per minute without waiting for coagulation, put it in glass ampullas, conserved with mertiolate (I:I0000) and kept it frozen. At further unfreezing colourless clots appeared in some of them, in the others clots were absent for they, probably, had been removed after centrifugation together with blood cells.

Electrophoresis was conducted in a handmade plastiglass, apparatus (Zilber and Abelev, I962) in I per cent 'Difco' agar gel spread over 9xI2 cm photographic glassplate with emulsion washed off.

Agar was preparated in medinal-veronal buffer with pH- 8,6 (fluctuation 8,4-8,8). This buffer was also in the vessel sections of the camera. Samples diluted with the same buffer in I to 2 proportion were placed in standard trenches and were put to electrophoresis for I,5 hours at a voltage of 60 v and a current of I2-I5 ma. After this procedure the plates were put in a 5 per cent acetic acid for fixation, dried and coloured during an hour with 'Amido Black IOB'.

The results were read visually and with a help of a microphotometer, taking into account only qualitative characterestics of protein spectrum-the absence or presence of distinct components differing in mobility.

Results

The results are represented in photographs of phoregrams (fig.2,a) and by three densitometric curves (fig.2b). Photographs NN I-7 show the phoregrams of blood serum from individual specimens of redfish and N 8 represents normal human serum given here for comparison. It's protein composition has been well studied (Grabar et Burtin, I960) and is designated by us according to these data (fig.2, below).

The examination of the material obtained showed following differences between the redfish and human blood serum proteins to be the most conspecious: I) contrary to the redfish albumin, human albumin is characterised by a much lesser mobility; 2) two distinct fractions are visible in the cathode half of the human serum phoregram (β - and γ - globulins), whereas in the appropriate part of redfish protein spectrum the separation of the proteins is weak and their small concentration allows us to consider them to be identical with γ - globulins. Hence, from this point of view in the anode part of the redfish blood phoregrams β, α - globulins and albumins are situated - the most mobile fractions (fig.2, above).

The data received also establish a wide individual variability of the samples expressed by the presence or absence of certain proteins, differing from each other by their electrophoretic mobility. Such pattern is characteristic both for albumin and to a great extent for globulin parts of the phoregram. As has been already mentioned, the differentiation of proteins is not clear enough in the γ - globulin zone only; that's why we do not discuss this zone of spectrum now. On the contrary, in the zone of α - β - globulins it is possible to reveal four fractions (phoregrams NN I,4), and in some cases a division of α_2 - globulins into two subfractions may be seen.

The variability of albumins is also typical. Thus, sera from the redfish NN I-3 possess slow albumin (it may be marked as 'B' type), the sera of redfish NN 6,7 have a more rapid 'A' type, and in fish NN 4,5 both types ('AB') are found. The mobility of albumins varies from test to test depending on several circumstances (Grabar et Burtin, I960), but if in the samples studied there is even a single serum containing both albumins it is relatively simple to understand to which type, A or B, these variations refer.

Table I.

Frequency of the protein fractions in redfish blood sera

Forms of redfish	Number of tested fish	Protein fractions							
		Albumins			Globulins				
		A	AB	B	α_1	α_2	β_1	β_2	γ
'marinus'	46 x/	8,7	8,7	82,6	42.5	I00	I00	2,5	I00
'giants'	43	I8,6	I6,4	65,0	I6,3	I00	I00	7,0	I00
'mentella'	53	22,6	37,8	39,6	I7,0	I00	I00	9,4	I00

Note: x/ As in 6 cases the protein pattern in this part of the spectrum appeared to be unsatisfactory, the globulins were studied only in 40 specimens.

The individual variability of blood protein composition in redfishes takes place independently from their taxonomical relations, viz., examining the samples of 'mentella', 'marinus' or 'giants' it is possible to find fish of any of the discovered

types. At the same time, there is a difference in frequency of these proteins between the forms of redfish (table I). According to this feature the most significant is the difference between the 'marinus' and 'mentella' redfish, substantially differing from each other in frequency of the specimens with albumin B, AB and α_1 - globulins ($P < 0.05$). By albumin A and β_2 - globulins the difference is not statistically significant. However, the likeness is probably determined not by a real absence of differences, but rather by an insufficient values of compared samples.

As to the protein composition of the 'giants' sera - they do not show biochemical identity with 'marinus', in spite of their striking external likeness, though these differences are statistically significant not by all variable protein fractions, except albumin - B and α_1 - globulin ($P \leq 0,05$). On the other hand, the 'giants' are very close, if not identical to S.marinus by fuequency of albumin A and α_1 - and β_2 - globulins, but frequency of albumin B and AB significantly differ from 'marinus' ($P \leq 0,05$).

It is known, that the individual variability of serum proteins connected with their presence or absence in some specimens, is often genetically controlled. It is usually especially well noticeable in albumins, where two types of the protein show three distinct phenogroups, viz., A, AB and B. Such interdependency suggests a genetic control by a pair of autosomal codominant alleles. This hypothesis is verified below by the Hardy-Weinberg distribution on an example of frequencies in a sample of 'mentella' (0,41pA and 0,59qB).

Table 2.

Verification of genetic equilibrium and control by a two-allelic gene of 'mentella' albumins.

Phenotype	Genotype	Number of phenotypes observed (f)	Number of phenotypes expected (F)	$\chi^2 = \frac{(f-F)^2}{F}$
A	AA	I2	8,90	I,08
AB	AB	20	25,60	I,22
B	BB	2I	I8,50	0,33
		N = 53	N = 53	χ^2= 2,63

One can see from the table, that there is no significant difference between the factual and expected numbers of the phenotypes both in any of the three phenotypical classes and summarilly. This means the system of a genetic control of the albumins is really two-allelic, and the 'mentella' population from which the given sample is taken is in genetic equilibium. Making the same calculations for 'marinus' and the 'giants' the Hardy-Weinberg law does not realize and χ^2 comes to I7,33 and I4,64 respectively.

Discussion

Examination of experimental data shows significant differences in protein composition of blood among the investigated forms of redfish. Especially interesting is the biochemical position of the 'giants'. In albumin A they approach the 'marinus' form, in globulin - α_1 - the 'mentella' and occupy an intermediate position in albumin B frequency, clearly indicating the hybrid origin of the 'giant' forms. This explains the reason why in applying a mathematical analysis of the 'marinus' samples and of the 'giants' the Hardy-Weinberg law does not realize - the violation of panmixion is the result of a sterilization of the majority of specimens. Of course, it is possible to mention some other circumstances preventing the establishment of a genetic equilibrium (e.g., Neel & Schull, I954; Li, I955, Stern,I960), but in this situation we actually pay attention to sterilization. This line of research has been discussed in our previous report.

The accomplished study reveals the differentiation of the

'marinus' and 'mentella' redfish in one more interested feature - the protein composition of the blood serum. The fact agrees with the results obtained by Schuffer and O'Rourke which demonstrate the possibility to distinguish both forms in the antigene composition of blood and aminoacid composition of gill mucus and muscles. Together with our data on electrophresis this again raises the question about the taxonomical range of divergency of the 'marinus' and 'mentella' redfish from the North Atlantic.

Conclusions

I) By the method of agar gel electrophoresis statistically significant differences are discovered in protein composition of blood serum of the 'marinus' and 'mentella' redfish from the North-West Atlantic. These differences refer both to α_1 - globulins and to albumins B and AB, evidently being under direct genetic control.

2) The 'giant' redfish despite of their striking external likeness to 'marinus' show no biochemical identity with them differing sufficiently from the latter in frequency of albumin B and α_1 - globulins. The 'giants' also differ significantly from the 'mentella' in frequency of albumin phenotypes B and AB. A general evaluation of the biochemical position of the 'giants' leads to the conclusion on their hybrid nature.

3) A summarization of data recieved by us and by other investigators enable us to raise a question about the taxonomical range of divergence between the 'marinus' and 'mentella' redfish.

Refferences

Altukhov J.F., Nefyodov G.N. & Fajusova A.N. (1967) Cytophysiological Analysis of Divergence in S.marinus and S.mentella from the North-West Atlantic. In: Teploustojchivost Kletok Zhivotnykh v onto- i filogeneze. Moscow-Leningrad: 82-95.

Grdbar P. et Burtin P. (1960). Analyse immuno-électrophoretique. Mdsson et C^ie Editeurs.

Li C.C. (1955). Population Genetics. Chicago Univ.Press.Chicago.

Neel J.V. & Schull W.J. (1954). Human Heredity. Chicago Univ.Press. Chicago.

O'Rourke F.J.(1961). An Immunological and Chromotographic Study of Sebastes marinus (L.) and S.mentella Travin. Cons.Perm.Intern.Expl.Mer.Rapp.Proc.-Verb., 150:100-103.

Puchkov N.V. (1954). Fish Physiology. Moscow.

Schaeffer H.(1961). A Biochemical Contribution to the Redfish Problem.Cons.Perm.Intern.Expl.Mer.Rapp.Proc.-Verb., I50: I04-110.

Stern C. (1960). Principles of Human Genetics. S.-Francisco & London.

Ushakov B.P.(1959a). Physiology of Cell and the Problem of Species in Zoology.Cytology, USSR, I,5:541-565.

Ushakov B.P. (1959b). Thermostability of Tissues-a Specific Criterion in Poikilothermal Animals. Zoology, USSR, 38, 9:1292-302.

Ushakov B.P. (1964). Thermostability of Cells and Proteins of Poikilotherms and its significance in Speciation. Physiol.Rev.,44,3:518-560.

Zilber L.A. & Abelev G.I. (1962). Virusology and Cancer Immunology. Moscow.

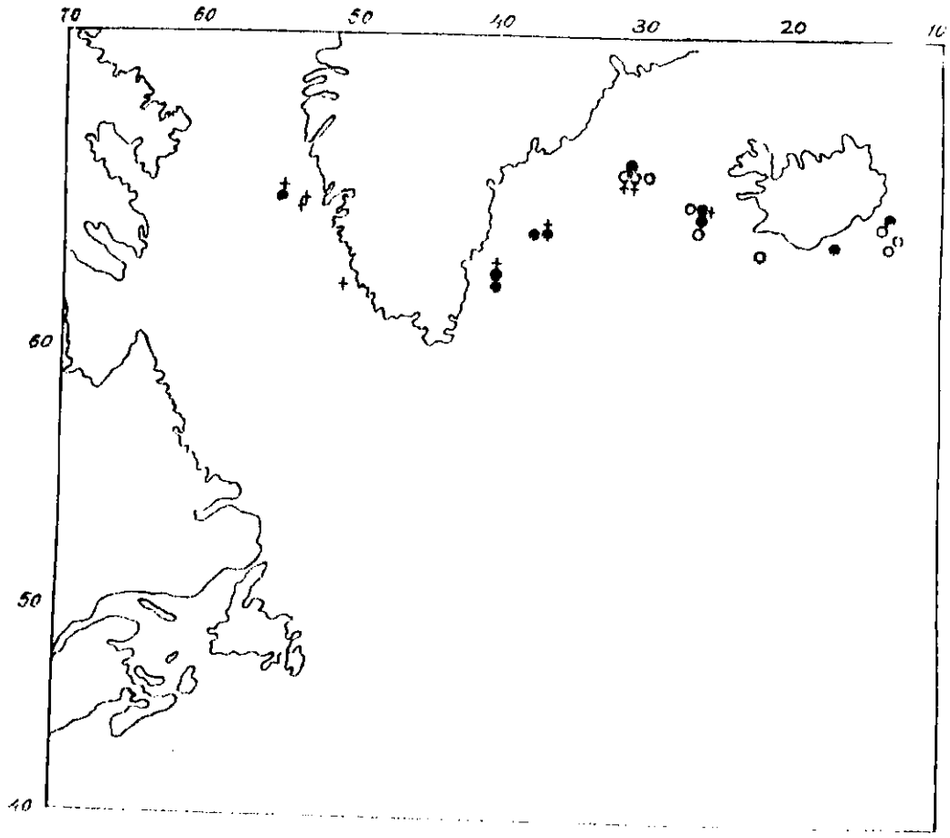

Fig. 1. Places of sampling redfish blood. Light circles = mentella; dark circles - marinus; crosses = the 'giants'.

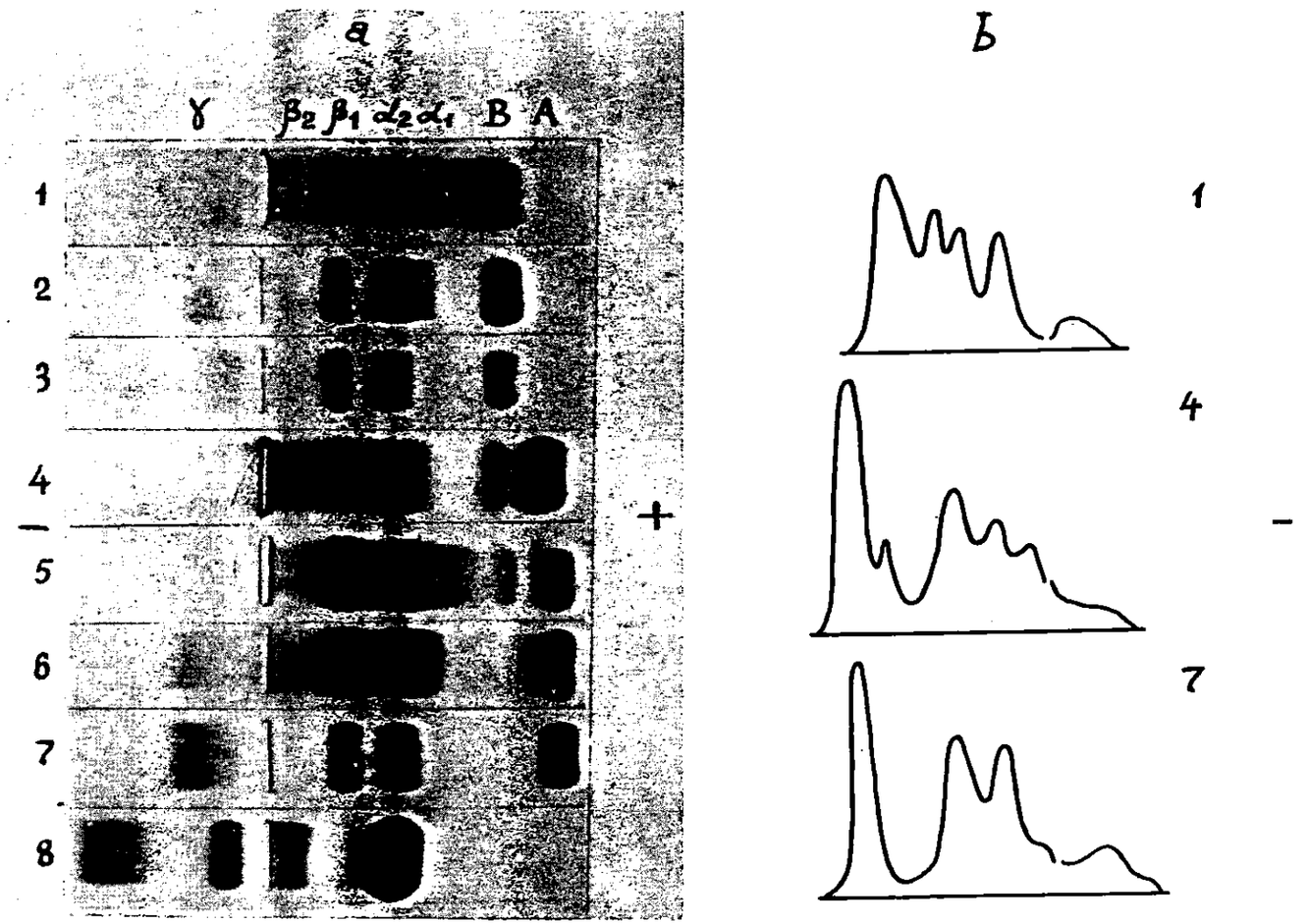

Fig. 2. (a) Phoregrams of the redfish (NN 1-7) and human (N 8) blood sera:
NN 1, 4-6 = mentella; N 3 = marinus; NN 2,7 = 'giants'; N 8 = human serum
(b) Densitometric curves of redfish blood phoregrams. Numeration as in (a).