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SOME RESULTS OF INVESTIGATION ON ERYTHROCYTE ANTIGENS OF BALTIC HERRING

A herring subspecies inhabiting the Baltic Sea — the Baltic herring (*Clupea harengus membras* L.) — consists of many infraspecific groups being at various taxonomic levels. For both theoretical and practical purposes it is extraordinarily important to reveal which of them intermix reproductively and which are isolated in this sense. The Baltic herring groups are differentiated mainly on the basis of morphological and physiological characters. Undoubtedly, these features are hereditary, but they can greatly vary under the influence of various environmental conditions. Therefore, in many cases it is not possible to determine by these features whether between the groups under consideration genetic differences exist or not. Investigations on characters determined genetically and being independent of external conditions are therefore of great importance. It is shown on cartilaginous fish *Squalus acanthias* (Sindermann and Mairs, 1961) as well as on bony fish *Salmo gairdneri* (Sanders and Wright, 1962) that one of such characters is the composition of erythrocyte antigens.

We have shown (Оявеер, 1962) that individuals of the Baltic herring related to the spring or autumn race as well as originating from various parts of the sea, can be discerned on the basis of their otolith structure. In this paper some data on geographical and racial differences in the frequency of certain erythrocyte antigens of the Baltic herring are presented, permitting to draw preliminary conclusions as to reproductive relations of the groups classified by this handy character.

Material and methods

The material was collected in 1965—1967 in the Gulf of Finland, the Gulf of Riga and off the east coast of the open Baltic, from the Gulf of Finland to the Klaipeda region. One small sample was caught south of the Isle of Öland. The erythrocyte antigens of about 1400 individual herrings have been analyzed. Antibodies for erythrocyte antigens of the Baltic herring were found in normal sera of *A*, *B* and 0 group of human blood as well as in normal sera of pig, sheep, cattle and perch. Weak antigens for erythrocytes of some herrings were found in normal serum of flounder. In normal sera of pike-perch, *Myoxocephalus quadricornis* (L.) and *Lampetra fluviatilis* (L.) no antigens for herring erythrocytes were discovered. From these sera the normal serum of sheep was commonly used at the differentiation of two main types of herring erythrocytes. Five immune sera were used: 1) The serum of a rabbit immunized with erythrocytes of the Gulf of Riga spring herring (the serum Spr. *R.*). 2) In 1967, two rabbits of one

clone were immunized, one with erythrocytes of the spring herring (the serum Spr. *B.*) and the other with the autumn herring erythrocytes (the serum Aut. *B.*). Both rabbits were immunized by exactly the same method and amount of erythrocytes (0.5 ml packed erythrocytes in 0.65 per cent NaCl and 1 per cent citrate solution at a time) five times at three-day intervals. Erythrocytes for the immunization were taken from the spring and autumn herring caught in the Gulf of Finland, in the Gulf of Riga, west of the Soela and Irben Sounds and in the Klaipeda region. 3) Two antisera used — the rabbit anti-herring serum Spr. *V.* (immunized with erythrocytes of spring herring of the Vistula Bay) and the rabbit anti-herring serum Aut. *N. S.* (immunized with erythrocytes of the North Sea autumn herring) — were kindly placed at the author's disposal by V. Zenkin. Agglutination reactions were made by the methods indicated by Altukhov, Apekin and Limanski (Алтухов, Апекин и Лиманский, 1964). Otoliths were taken from all the individuals analyzed. From the otoliths, the race and age of the individual as well as the otolith type was determined. Later the data derived from otolith investigation were connected with correspondent data of erythrocyte antigen study.

Results and discussion

Cross reactions between erythrocytes and sera of individual spring and autumn herrings originating from various regions of the Baltic Sea showed that there are no isoagglutinins in the Baltic herring sera.

Investigations were carried out mainly in two directions: 1) it was tried to find reagents for a differentiation of erythrocytes of herring seasonal races and geographical groups by reaction titer, 2) attempts were made at revealing the types of erythrocyte antigens for studying their frequency in various groups.

To achieve the first aim, two sets of experiments were carried out. In 1965—1966 reactions of erythrocytes of various herring groups with normal human sera of *A*, *B* and 0 blood groups, normal sheep serum as well as immune serum Spr. *R.* were tested. Although the immune serum originated from the rabbit immunized with erythrocytes of the Gulf of Riga spring herring, generally it reacted better with the erythrocytes of the Southern Baltic herring than with the erythrocytes of the Gulf of Riga herring. Moreover, in comparison with erythrocytes of the Gulf of Riga herring, the reactions of the normal sera used were stronger with the erythrocytes of the Southern Baltic herring.

In 1967, reactions of four immune sera (Spr. *B.*, Aut. *B.*, Spr. *V.* and Aut. *N. S.*) with the erythrocytes of three groups of herring (spring and autumn herring of the Gulf of Riga, spring herring of the Klaipeda region) were carried out. From Table 1 it can be seen that: 1) generally, from the sera tested, the serum Spr. *B.* (especially in the Gulf of Riga) and the serum Spr. *V.* (especially in the Southern Baltic) reacted with the erythrocytes of a given individual herring better than the others; 2) from the sera Spr. *B.* and Aut. *B.* the first reacted with the erythrocytes of a Gulf of Riga herring at lower concentration than the second; 3) the serum Aut. *N. S.* reacted with the erythrocytes used at a considerably weaker rate than the other sera.

The above comparison allows to conclude that some differences occur in reactions of different immune sera with erythrocytes of the same herring group as well as in reactions of the same serum with erythrocytes of various herring groups. This indicates that there are some general differences between the erythrocytes of the herring groups investigated as well as between the four tested antisera. A remarkable feature about the

Table 1

Reactions of immune sera with erythrocytes of the Gulf of Riga spring and autumn herring, spring herring of Southern Baltic, sprat and smelt

Region	No.	Species	Race	Age	Serum dilutions																									
					Spr. *B.*							Aut. *B.*							Spr. *V.*							Aut. *N.S.*					
					1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Gulf of Riga	1	C. harengus membras	autumn	3	+	+	+	+	+	+		+	+	+	+	+			+	+	+	+	+			+	+	+	+			
	3	„	„	3	+	+	+	+	+			+	+	+					+	+	+	+				+	+	+				
	7	„	„	3	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+			+	+	+	+	+		
	24	„	„	2	+	+	+	+	+	+		+	+	+	+				+	+	+	+	+	+		+	+	+				
	25	„	„	3	+	+	+	+				+	+						+	+						+						
	6	„	spring	3	+	+	+	+	+	+		+	+	+	+	+			+	+	+	+	+			+	+	+	+			
	8	„	„	4	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+		+	+	+				
	12	„	„	4	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+			+	+	+	+			
	13	„	„	4	+	+	+					+							+	+	+					+						
Southern Baltic	1	„	„	3	+	+	+	+				+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	
	2	„	„	2	+	+	+	+				+	+	+	+	+			+	+	+	+	+			+	+	+	+			
	5	„	„	3	+	+	+	+	+			+	+	+					+	+	+	+	+			+						
	6	„	„	2	+	+	+	+	+	+		+	+	+	+				+	+	+	+	+	+		+	+	+	+			
	8	„	„	2	+							+							+	+	+	+				−						
Gulf of Riga	1	S. sprattus balticus		4	+	+	+	+	+	+		+	+	+	+	+			+	+	+	+				+	+					
	2	„		2	+	+	+	+	+			+	+	+					+	+	+					+						
	3	„		4	+	+	+	+	+			+	+	+					+	+	+	+	+	+		+	+	+	+			
	1	O. eparlanus eparlanus		4	−							−							−							−						
	2	„		4	−							−							−							−						
	3	„		4	−							−							−							−						

reactions was that considerable individual variation of agglutination titers occurred in the erythrocytes of all the herring groups studied. It seems that because of this, on the basis of titer of reaction with the reagents used, we cannot satisfactorily differentiate the erythrocytes belonging to individuals of various herring groups or make conclusions about reproductive relations of the herring groups studied.

The failure in finding reagents allowing for a qualitative discerning of herring groups by agglutination titers of their erythrocytes shows a possible qualitative similarity of the erythrocyte antigens of the herring groups. This is quite natural within the same subspecies. Moreover, from Table 1 it can be seen that even in different species of clupeoids (herring and sprat) at least a part of erythrocyte antigens is quite similar and gives much the same reactions with the immune rabbit sera. Specifity of the reaction for the erythrocytes of clupeoids is maintained by an absence of reaction between the erythrocytes of smelt (*Osmerus eperlanus*) and the immune rabbit sera at a dilution 1:8 already.

Investigations on erythrocyte antigens showed a distinct difference between two kinds of Baltic herring erythrocytes. Erythrocytes of one type react strongly at least with some of the normal sera used and with the immune sera to dilutions 1:64—1:512. Erythrocytes of the other type give no reactions with the normal sera and react with the immune sera only at rather high concentrations (chiefly at dilutions 1:2—1:64). Occurrence of similar strongly and weakly agglutinating cells in the Gulf of Maine herring was detected by Sindermann and Mairs (1959). These authors classified individual herrings possessing or lacking corresponding erythrocytes as *C*-positive or *C*-negative ones. We assume that the strongly and weakly agglutinating cells of the Baltic herring correspond virtually with the *C*-positive and *C*-negative erythrocytes of the Gulf of Maine herring. Therefore, according to these authors, we regard this system of Baltic herring erythrocyte antigens as the *C*-system.

Preliminary investigations in the frequencies of individuals possessing *C*-positive or *C*-negative types of erythrocytes showed that, same as in the Gulf of Maine herring (Sindermann and Mairs, 1959) and Pacific sardine (Sprague and Vrooman, 1962), there are some differences in the geographical distribution of different erythrocyte antigens in the Baltic herring (Table 2).

Table 2

Frequency of *C*-negative individuals in herring in various parts of the Baltic

Region	Spring herring			Autumn herring		
	Number of fish tested	Number of *C*-negative fish	Percentage of *C*-negative fish	Number of fish tested	Number of *C*-negative fish	Percentage of *C*-negative fish
Gulf of Finland	249	17	6.8	12	2	
Gulf of Riga	205	15	7.3	109	8	7.3
West of Soela Sound	197	10	5.1	34	3	
West of Irben Sound	123	5	4.1	41	2	
Ventspils	83	1	1.2	13	1	
Liepaja	32	2		9	0	
Klaipeda	144	2	1.4	67	2	3.0
South of Öland	32	0		24	1	

It is known that because of feeding and spawning migrations (Раннак, 1954; Popiel, 1958) the herring caught in a certain area may originate from several parts of the Baltic. Therefore we suppose that it is more correct to determine the frequency of erythrocyte types on the basis of otolith types since the otolith type indicates the origin of the individual from one or another part of the Baltic. In Table 3 it is seen that in both the spring and autumn race the individuals of otolith type I differ considerably from the individuals of type II and III by the frequency of the *C*-negative erythrocytes. Herring of the latter two otolith types has practically no differences in this respect. Also, in both races it can be noticed that individuals of the southern otolith type I differ from the herrings of the northern otolith type I by a lower percentage of *C*-negative individuals. And, finally, Table 3 shows a rather noticeable difference of the herring seasonal races by the frequency of *C*-negative individuals — in all otolith types the percentage of *C*-negative individuals among the autumn herring is higher as compared to the spring race.

Table 3

Frequency of *C*-negative individuals in different otolith types of Baltic herring

Otolith type	Spring herring			Autumn herring		
	Number of fish tested	Number of *C*-negative fish	Percentage of *C*-negative fish	Number of fish tested	Number of *C*-negative fish	Percentage of *C*-negative fish
I southern	191	2	1.0	98	3	3.1
I northern	171	6	3.5	62	3	4.8
I total	362	8	2.2	160	6	3.8
II	159	12	7.5	53	5	9.4
III	455	32	7.0	88	8	9.1

Our investigations revealed heterogeneity of the *C*-positive type of erythrocyte antigens. On the basis of the character of reactions of the erythrocyte antigens with the absorbed immune sera we succeeded in discerning at least three types of herring erythrocytes (Table 4). Zenkin (in press) suggests that the Baltic herring erythrocyte antigens are under the control of a three-allele genetic system. According to this hypothesis and on the basis of data in Table 4 one can suppose that individuals with erythrocytes of type I (strongly *C*-positive ones) are heterozygotes possessing two different *C*-positive antigens (C_1C_2) and individuals with erythrocytes of type III (*C*-negative ones) are homozygotes lacking both these antigens (C_0C_0). Reactions of herring erythrocyte antigens with the cattle and human *A*-group normal sera show a further differentiation of the *C*-positive erythrocyte antigens. The types of *C*-positive herring erythrocytes discerned by these normal sera are as follows:

Table 4

Reactions of types of Baltic herring erythrocytes differentiated by the absorbed immune sera

Type	Immune rabbit serum absorbed by erythrocytes of type			Sheep normal serum
	I	II	III	
I	−	+	+	+
II	−	−	+	+
III	−	−	−	−

1) erythrocytes giving positive reaction with both the human *A*-group and cattle sera;

2) erythrocytes reacting positively with the cattle serum, but giving no reaction with the human *A*-group serum;

3) erythrocytes reacting positively with the human *A*-group serum, but giving no reaction with the cattle serum;

4) erythrocytes giving no reaction either with the cattle or human *A*-group sera.

Relations between the two systems of erythrocyte antigens presented are not yet sufficiently clear.

The investigations on Baltic herring erythrocyte antigens carried out hitherto show that similar studies give valuable information on genetical relations of various infraspecific groups. Further detailed investigations are needed to reveal the relations and true number of types of erythrocyte antigens and to determine their distribution in various herring groups.

Conclusions

1. Erythrocyte antigens in various groups of the Baltic herring are probably qualitatively similar.

2. At least a part of erythrocyte antigens is quite similar in the Baltic clupeoids (herring and sprat).

3. Similarly to the Gulf of Maine herring, individuals with strongly and weakly agglutinating (*C*-positive and *C*-negative) erythrocytes occur in the Baltic herring as well.

4. In frequency of the *C*-negative individuals the sea herring (otoliths of type I) differs from the gulfs herring (otoliths of type III) of the same seasonal race. In this feature some differences are also found between the seasonal herring races.

5. On the basis of reactions with absorbed immune serum, preliminarily three types of erythrocytes are differentiated in the Baltic herring, whereas by the human *A*-group and cattle normal sera four types are discerned in the *C*-positive erythrocytes.

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MÕNINGAID RÄIME ERÜTROTSÜTAARSETE ANTIGEENIDE UURIMISE TULEMUSI

Resümee

Umbes 1400 mitmesugusesse liigisisesesse rühmitusse kuuluva räime erütrotsüütide ja mitmete normaalsete ning immuunsete seerumite vaheliste reaktsioonide uurimise tulemusena järeldatakse:

1. Räime erinevate liigisiseste rühmituste erütrotsütaarsete antigeenide kvalitatiivne koosseis on tõenäoliselt ühesugune.

2. Heeringlaste (räime ja kilu) erütrotsütaarsed antigeenid on vähemalt osaliselt sarnased.

3. Sarnaselt Maine'i lahe heeringale võib ka räimi nende erütrotsüütide ja kasutatud normaalsete ning immuunsete seerumite vahel tekkivate reaktsioonide alusel jagada nõrgalt ja tugevalt reageerivate erütrotsüütidega isendeiks (*C*-negatiivsed ja *C*-positiivsed kalad; Sindermann, Mairs, 1959).

4. *C*-negatiivsete isendite protsendi poolest erineb mereräim (I otoliiditüüp) laheräimest (III otoliiditüüp). Sügisräime kõigis otoliiditüüpides on *C*-negatiivsete kalade osatähtsus suurem kui kevadräimel.

5. Absorbeeritud immuunsete seerumitega reageerimise alusel on räimel eristatud kolm erütrotsüütide tüüpi, *C*-positiivsed erütrotsüüdid on aga veise ja inimese *A* vererühma seerumiga reageerimise põhjal jaotatud veel neljaks tüübiks.

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НЕКОТОРЫЕ РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЯ ЭРИТРОЦИТАРНЫХ АНТИГЕНОВ САЛАКИ

Резюме

Исследовались эритроцитарные антигены примерно 1400 экземпляров салаки разных группировок. Реагентами служили сыворотки крови человека (группы *A*, *B* и *AB*) и некоторых видов млекопитающих и рыб, а также сыворотки крови кроликов, проиммунизированных эритроцитами салаки разных группировок и североморской сельди.

Изоагглютининов в крови салаки не обнаружено.

Удалось установить некоторые заметные различия в реакциях разных иммунных сывороток с эритроцитами салаки одной и той же группировки или одной и той же сыворотки с эритроцитами салаки разных группировок (табл. 1). Но ввиду значительной индивидуальной вариации титров реакций во всех группировках, использование этих различий для качественного различения группировок салаки или определения их репродуктивных связей проблематично.

Кроме качественного сходства эритроцитарных антигенов у изученных группировок подвида *Clupea harengus membras*, выявлено сходство между собой по меньшей мере части эритроцитарных антигенов балтийских сельдевых (салаки и кильки).

Предполагается, что антигены слабо- и сильнореагирующих эритроцитов салаки сходны с антигенами *C*-отрицательных и *C*-положительных эритроцитов сельди залива Мэйн (Sindermann, Mairs, 1959).

По значению особей с эритроцитами *C*-отрицательного типа, салака, имеющая отолиты I типа, в обеих сезонных расах значительно отличается от салаки с отолитами II и III типов (табл. 3). По сравнению с весенней салакой у осенней расы во всех типах отолитов процент *C*-отрицательных особей больше.

На основе реакций с абсорбированными иммунными сыворотками до сих пор нам удалось различить у салаки три типа эритроцитов, а характер реакции с нормальными сыворотками крови человека группы *A* и крупного рогатого скота позволяет у особей с *C*-положительными эритроцитами выделить четыре типа.

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