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DIFFERENTIATION OF SPAWNING POPULATIONS OF THE SURF SMELT *HYPOMESUS PRETIOSUS* (GIRARD) BY SEROLOGICAL METHODS[1]

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INTRODUCTION

THE use of blood group systems in population studies has been on the increase in recent years. Widespread use of polymorphic erythrocyte antigens is based on several factors. The characters studied, usually reflect simple genetic variations and are generally independent of environmental influences. Collection and processing of the materials are relatively easy. Only small volumes of material are required and the techniques used are not complicated.

Since the pioneering work of Landsteiner (1901) on the blood types of man, blood groups have found increasing use as population or breed markers in warm blooded animals. From about 1950 accelerating attention has been focused on the blood groups of poikilothermic animals (Cushing, 1964).

The main objective of this study is to evaluate the usefulness of serological techniques in separating spawning populations of the surf smelt, *Hypomesus pretiosus* (Girard).

MATERIALS AND METHODS

The materials used in this study were obtained from the surf smelt spawning at Lapush, on the Pacific coast, and Utsaladdy and Hood Canal in Puget Sound, in the State of Washington (Fig. 1). Lapush and Utsaladdy smelt spawn during summer (May-October) and the Hood Canal smelt in fall (August-December).

Collection and Preparation of Samples

Blood samples were collected by caudal bleeding into open mouth screw-cap vials containing 2 cc. of modified Alsever's solution (Bukantz *et al.*, 1946) having 0.5 gm. of antibiotics (Aureomycin) in 100 cc. of solution. The samples were brought to the laboratory on ice and tested within twelve hours. They remained refrigerated at all times prior to testing. The samples were washed three times in Alsever's solution and suspended in approximately two per cent Alsever's solution for testing.

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Choice of Reagent(Serum)

Usefulness of a reagent in serological studies depends on its ability to detect the individual variability of erythrocyte antigens. The blood samples of smelt collected

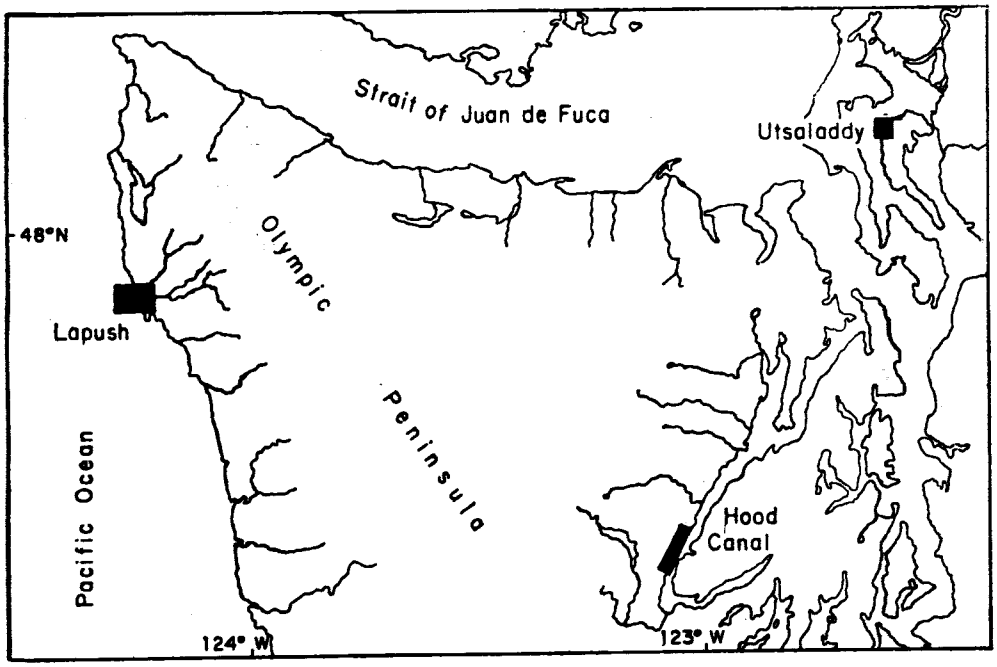

Figure 1. Surf smelt spawning localities at Hood Canal and Utsaladdy in Puget Sound and Lapush on the coast of Washington.

during 1962 from Hood Canal, Utsaladdy and Lapush were tested with a variety of plant extracts and animal normal and immune sera. Only two reagents consistently detected individual variations. These were a pooled sand sole (*Psettichthys melanostictus*) normal serum and a rabbit anti-smelt erythrocyte serum. Results based on the use of these two sera were derived from 1963 samples only.

Procedure of Agglutination Tests

Aliquots of the sera used in these tests were thawed in a 45° C. water bath. The required dilutions of the sera were then made with one per cent saline solution. Agglutination tests were conducted by mixing one drop of specified dilution of serum with one drop of 2% erythrocyte suspension in agglutination tiles. The tiles were then incubated at 4° C. for one to two hours and the degree of agglutination was recorded. The degree of agglutination was expressed as follows :

4, Cells agglutinated in one complete clump

3, Cells agglutinated in several large clumps

2, Cells agglutinated in moderate clumps and visibility was clear

1, Cells agglutinated in small clumps and hazy

0.5, Cells agglutinated in barely discernible clumps

0, No visible agglutination

For each sample the score was obtained by adding the degree of agglutination for all the dilutions (Ridgway, Cushing and Durall, 1958).

RESULTS

Testing with Pooled Sand Sole Normal Serum

The sand sole normal serum was tested at 1 : 8, 1 : 16 and 1 : 32 dilutions. Agglutination scores obtained are given in Table I. Test for differences in the mean scores between localities showed significance at less than the 0.001 level of probability (Table II). Mean scores for the Hood Canal and Utsaladdy fish were not significantly different ($F_{1,172}=0.59$) but each of these localities differed significantly from Lapush at less than the 0.001 level ($F_{1,138}=31.67$, and $F_{1,112}=21.58$).

TABLE I

Agglutination scores of smelt erythrocyte antigens from Hood Canal, Utsaladdy and Lapush tested with pooled sand sole normal serum

Scores	Number of fish		
	Hood Canal	Utsaladdy	Lapush
0	0	1	0
0.5	0	1	0
1.0	0	3	0
1.5	1	2	0
2.0	2	2	1
2.5	2	3	0
3.0	6	3	0
3.5	1	0	0
4.0	4	11	0
4.5	2	1	0
5.0	12	10	2
5.5	2	1	0
6.0	18	14	10
6.5	2	0	1
7.0	21	11	5
8.0	12	7	9
9.0	1	5	11
10.0	3	3	10
11.0	0	3	1
12.0	1	3	0
Total	90	84	50
Mean score	6.03	5.86	7.91

TABLE II

Analysis of variance on agglutination scores tested with pooled sand sole normal serum

Source	d.f.	Mean square	F
Between localities ..	2	75.40819	14.61
Within localities ..	221	5.16077	

Testing with Immune Serum

The immune serum was tested at 1 : 4, 1 : 8 and 1 : 16 dilutions (Table III). Analysis to test the differences in the mean agglutination strengths between the three localities showed significance at the 0.001 level (Table IV) and from further analysis it was found that the Lapush fish differed significantly at less than the 0.001 level from the Hood Canal and the Utsaladdy fish ($F_{1,148}$=17.78 and $F_{1,144}$=23.84 respectively). Differences between the Hood Canal and Utsaladdy fish were not significant ($F_{1,172}$=0.41).

TABLE III

Agglutination scores of smelt erythrocyte antigens from Hood Canal, Utsaladdy and Lapush tested with rabbit anti-smelt erythrocyte serum

	Number of fish		
Scores	Hood Canal	Utsaladdy	Lapush
0	1	1	0
0.5	0	1	1
1.5	1	0	0
2.0	0	1	1
2.5	3	1	2
3.0	5	3	1
3.5	3	0	0
4.0	1	5	2
4.5	3	0	0
5.0	12	9	4
6.0	6	20	0
7.0	9	10	0
7.5	0	0	1
8.0	9	7	5
8.5	0	1	0
9.0	12	11	2
10.0	12	4	7
11.0	5	5	21
12.0	7	6	14
Total	89	85	61
Mean score	7.30	7.05	9.29

TABLE IV

Analysis of variance on agglutination scores tested with rabbit anti-smelt erythrocyte serum

Source	d.f.	Mean square	F
Between localities ..	2	112.15093	13.52
Within localities ..	232	8.29689	

DISCUSSION

Choice of characters that are relatively free from environmental influences is desirable when evaluating the genetic differences. Blood from three spawning populations of the surf smelt was studied because the blood factors are generally believed to be under genetic control. Stormont (1961) stated '. . . . It is axiomatic in immunogenetics that all blood factors are inherited traits which are subject to

little or no influence by the environment. Consequently, any differences which two or more subpopulations exhibit with respect to the incidence of common blood factors are fully meaningful even in the absence of information concerning the inheritance of blood factors . . . .'

Based on these quantitative serological tests, it is concluded that the Hood Canal and Utsaladdy populations have similar erythrocyte antigen composition but the smelt of Puget Sound differ from those of Lapush. On the basis of the blood study, it is also tentatively concluded that the smelt populations of Hood Canal and Utsaladdy have similar genetic composition, assuming that the frequencies of agglutination strengths reflect relative gene frequencies in these two populations. It is also evident, on the same assumption, that the ocean population spawning at Lapush possesses significantly different genetic composition than that of the Puget Sound populations. The fact that the tests with the normal sand sole serum and the immune serum lead to the same conclusions strengthens this assumption.

Further work along lines of isoimmunization and absorption tests is desirable to identify qualitative differences and to pin point the blood types. However, the conclusions drawn here on the closer affinity of the Puget Sound smelt populations and diversity of these populations from that of Lapush are substantiated from the analysis of meristic characters, morphometric characters and parasite incidence (Kilambi, 1965).

The following hypothesis is consistent with the above evidence and assumptions : Similarity of the Hood Canal and Utsaladdy populations in their antigenic properties is the result of their sharing a common gene pool for some time after their entry into Puget Sound whereas the differences between the Puget Sound and Lapush populations is attributed to total isolation of these populations since about 13,000 years ago (Kilambi, 1965).

SUMMARY

Pooled sand sole normal serum and rabbit antismelt immune serum were useful in detecting the individual variability of erythrocyte antigens of the surf smelt. There were no statistically significant differences in the mean agglutination scores between the two spawning populations of Puget Sound but significant differences were found between Puget Sound and Ocean (Lapush) populations. This situation was deduced to be the result of degree of isolation of these populations.

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