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HERRING (CLUPEA HARENGUS) ISOENZYME STUDIES

by
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Abstract

The isoenzyme systems of Atlantic and Pacific herring have been compared. They appear to share 55% of the enzymic and non-enzymic protein loci.

A comparison of allele frequencies of Atlantic herring sampled in 1968-70 with Atlantic herring sampled in 1979-80 shows no change in the observed allele frequencies of phosphohexose isomerase, lactate dehydrogenase and aspartate amino transferase. Similarly, preliminary comparisons of Halifax and Bay of Fundy herring population samples have shown no significant allele frequency differences. Isoelectric focusing of eye lens proteins and hemoglobins of these two samples showed no variation. More enzymes are being tested and the results are being examined in the light of current concepts of population genetics.

Résumé

Les systèmes d'isoenzymes des harengs de l'Atlantique et du Pacifique ont été comparés. Ils ont l'air de portages 55% des loci protéiniques enzymatiques et non-enzymatiques.

Une comparaison de la fréquence d'allele des harengs de l'Atlantique échantillon née en 1968-1970 avec des harengs de l'Atlantique échantillon née en 1979-1980 ne démontre aucun changement dans les alleles observés de PHI, LDH et AAT. Similairement des comparaisons préliminaires d'echantillons de harengs d'Halifax et de la Baie de Fundy ne démontrent aucune différence significative d'allele. Le focus isoélectrique des lensilles protéiniques des yeux et de l'hemoglobine de deux échantillons ne varient pas. Nous testons encore d'autres enzymes et les résultats sont examinés en vue des concepts présents des populations génétiques.

Introduction

The allele frequencies of some isoenzyme systems of 58 Atlantic herring (Clupea harengus harengus L.) population samples of 100 fish each were compared in an earlier study (Odense et al., 1973). In the present work results of a comparison of the isoenzyme systems of Atlantic herring and Pacific herring (Clupea harengus pallasi V.) are reported, as well as initial results of a comparison with each of two recent Atlantic herring population samples, and a comparison of these results with the allele frequencies found 10-12 years earlier.

Fish Samples

Population samples of Pacific herring in lots of 100 fish each were obtained from Vancouver and Nanaimo through the kind assistance of Drs. H. Rosenthal, H. Tsuyuki, and R. Humphreys and W. Skinners. The fish were frozen and shipped by air to the Halifax Laboratory where they were kept at -40°C until needed for analysis. A sample of 100 Atlantic herring was obtained from a local fisherman at Eastern Passage near Halifax in May, 1980, and a second sample was obtained from Grand Manan in the Bay of Fundy in November, 1979. The latter fish were obtained with the assistance of personnel working with Dr. D. Iles of the St. Andrews's Biological Station.

Sampling procedures

Pacific herring samples were thawed; length, weight and sex of each fish were recorded. Tissue extracts were prepared for electrophoresis as described previously (Odense et al., 1973). The Atlantic herring were brought in alive and blood samples were collected in cooled heparinized containers (vacutainers). The blood was centrifuged, the serum removed and frozen, and the blood cells washed three times with 1% saline before the cells were lysed to obtain the hemoglobin solutions. Heart, muscle, liver and eye lens extracts were prepared and the same data (length, weight, sex, maturity) recorded. Isoenzyme stains used were those described by Shaw and Prasad (1970).

A. Comparison of Pacific and Atlantic herring proteins and enzymes.

The isoenzyme systems studied in the comparison of Atlantic and Pacific herring are listed in Table 1. The table lists the names and E.C. number of each enzyme, the number of subunits in the functioning enzyme, the buffer system used [one of the four described in the previous report (Odense et al., 1973) - Clayton and Tretiak's amine buffer pH 6.9 or 8.0, Lewis and Ridgeways tris-citrate-LiOH-borate buffer and a tris-EDTA-borate buffer], and the best tissue for typing was noted (muscle = M, heart = H, liver = L). Subunit molecular weights of the same enzyme in other species are listed if known, as well as an indication of whether or not the enzyme is a glycolytic one. Finally the electrophoretic results and the number of loci and mutant alleles are listed and the Atlantic and Pacific species are compared.

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A comparison of the starch gel electrophoresis total protein patterns of muscle extracts of the two species revealed similarities but each species had one clear-cut species characteristic band. The limited resolution of total protein bands on starch gel made it difficult to score the number of common and different total protein "loci". However, a comparison of the total protein pattern of eye lens proteins separated by isoelectric focusing on polyacrylamide slabs gave a sharper pattern for comparison (Fig. 2). Of some 40 bands in the Pacific and 41 in the Atlantic herring patterns, 23 corresponded. The proteins with isoelectric points in the higher pH range (near the cathode) are more identical than the proteins in the low pH section.

In the sample of 100 Pacific herring there was not a single mutant eye lens protein band and only 1 mutant in the 100 Halifax and 100 Bay of Fundy Atlantic herring samples. This would be in accord with the observation by Powell (1975) that non-enzymic proteins are less variable than enzymic proteins.

B. Comparison of Bay of Fundy and Halifax herring samples.

In an earlier study (Odense et al., 1973) the isoenzyme systems of 58 Atlantic herring population samples were compared. No suitable population markers were found even though samples were as geographically separated as England to Labrador, Canada, and to the coast of Virginia, U.S.A. It was suggested then that blood proteins might show more variation. Therefore, in the present study blood samples were taken and the hemoglobin extracts were run by isoelectric focusing on polyacrylamide gels. No hemoglobin variants were found in the two herring population samples. As noted in Section A (Fig. 2), only one variant was found in 200 eye lens extracts.

It was also of interest to re-run the phosphohexose isomerase, lactate dehydrogenase and aspartate amino transferase enzymes to see if the gene frequencies had shifted over the 10 year period. As shown in Table 2, no changes have occurred.

The mean values of the phosphohexose isomerase allele frequencies of the 25 population samples obtained in 1968-1970 are plotted in Figure 3, together with the allele frequencies of the two herring populations sampled recently. As Table 2 and Figure 3 show, there has been no significant drift or change in phosphohexose isomerase allele frequencies over the past 10 years. None of the isoenzymes or proteins examined have yet been found useful as a herring population marker. In a preliminary report from some Swedish workers they noted a similar lack of significant allele frequency differences among Baltic herring populations (N. Byman, personal communication). A similar lack of stock discriminants was found in a second European study (A. Jamieson, personal communication). Both these reports referred to are preliminary as is our current study, and it is possible that a suitable population marker may yet be found when we have completed comparing allele frequencies of the Halifax and Bay of Fundy samples, using all the systems mentioned in the Atlantic-Pacific herring comparison. It may also be that, as Avise (1974) noted "the electrophoretic and classical approaches should be used in concert. In some cases (local adaptions in morphological features), classical systematic criteria offer greater precision; and in some cases (e.g. sibling species), electrophoretic data are most discriminatory." Further studies are required to obtain discriminatory electrophoretic data for herring.

In the interspecies comparison some 20 enzymic loci of Atlantic and Pacific herring were compared. Of these, 13 appear identical on the basis of the electrophoretic mobility of the isoenzymes, 6 are different and 1 could not be compared. About half of the eye lens proteins appear similar. These species then share 55% of the loci examined.

There are 5 tetrameric enzymes listed in Table 1, 7 dimeric and 3 monomeric. Of these 4, 4 and 3 respectively are polymorphic. While the number of enzymes examined is not great, it does indicate that the tetrameric enzymes are as polymorphic as the monomeric, a result not in accord with the suggestion that heterozygosity is related to subunit number (Ward, 1977). Considered from the viewpoint that enzymes of glycolysis have more structural contraints and hence can tolerate fewer mutations than non-glycolytic enzymes (Gillespie and Kojima, 1968), it would appear that the herring enzymes examined do not show this relationship. Of 9 glycolytic enzymes, 6 were polymorphic, some at more than one locus or with many mutant alleles at 1 locus, while 5 of the 6 non-glycolytic enzymes were polymorphic and none of these had more than 1 mutant allele. In a study on plaice, Ward and Beardmore (1977) reported that glucose metabolizing enzymes were more variable than non-glucose metabolizing enzymes, a finding which would concur with the herring results.

In other studies subunit molecular weights of the same enzymes have been found not to vary widely with species and Koehn and Eanes (1977) have reported an increase in heterozygosity with an increase in molecular weights. The molecular weight estimates listed in Table 1 are not those of herring enzyme subunits but of those listed in the literature for other species but the same enzyme. In the case of herring, the superoxide dismutase enzyme does not exhibit heterozygosity. It has the lowest molecular weight. However, the next three in ascending order of subunit molecular weight, galactose dehydrogenase, peroxidase and malate dehydrogenase are all polymorphic, as are the enzymes at the top end of the scale, phosphohexase isomerase, lactate dehydrogenase, isocitrate dehydrogenase and glutamate dehydrogenase. Thus the herring enzymes again seem to fail to conform to the conventional pattern. However, it must be cautioned that both the sample sizes (100 fish per population) and the number of enzymes sampled is relatively small. Other enzymes were studied but failed to show activity or were too inconsistent (e.g. leucine aminopeptidase) to be run routinely. It is therefore necessary to find more suitable enzymes and continue to collect population data before some of the generalizations referred to above can be either established or disproven. In the meantime, more enzyme studies may yet find suitable population markers for herring as has been done with other fish species.

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Table 1

(1) E.C. 1.1.1.8 Glycerolphosphate dehydrogenase dimer glycolytic enzyme
Buffer - Amine pH 8.0, M.

Atlantic and Pacific herring have same pattern, one single main band, no mutants.

1 locus, no mutants - same locus in Atlantic and Pacific herring.

(2) E.C. 1.1.1.14 Sorbitol dehydrogenase tetramer non-glycolytic enzyme
Buffer - Amine pH 6.9, H.

Results were not consistant. Appears to be 1 locus and 1 mutant (5 band heterozygote found in Atlantic sample).

(3) E.C. 1.1.1.27 Lactate dehydrogenase tetramer glycolytic enzyme
Buffer - T.E.B., M,H m.w. - 140,000

Atlantic and Pacific herring have same 3 banded isoenzyme pattern but subunit mobilities and mutant allele frequencies differ (Fig. 1).

Atlantic Herring	A	A'	B	B'	B"
Halifax	.97	.03	.995	.045	0
Bay of Fundy	.97	.03	.94	.06	0

Pacific Herring	$\bar{A}$	$\bar{A}'$	$\bar{B}$
	.995	.005	1.00

The $\bar{A}$ and $\bar{B}$ subunits of the Pacific herring migrate faster respectively than the A and B subunits of the Atlantic herring. Nevertheless, the familiar 3 band pattern is found in both, suggesting initial preferential formation of homodimers followed by tetramer subunit fromation.

2 loci, A locus - 1 mutant in Atlantic and Pacific herring
B locus - 1 mutant in Atlantic herring only.

(4) E.C. 1.1.1.37 Malate dehydrogenase dimer glycolytic enzyme
Buffer - Amine pH 8.0, H,M m.w. - 60-70,000

Atlantic and Pacific herring have similar Malate dehydrogenase zymogram patterns. The patterns could not be scored. It appeared that 4 loci are involved (Odense et al., 1973), and variable subunit production could account for the many phenotypic patterns observed.

4 loci, same in Atlantic and Pacific

Table 1 (Continued)

(5) E.C. 1.1.1.42 — Isocitrate dehydrogenase — dimer — glycolytic enzyme
Buffer - Amine pH 6.9, L(H)

Atlantic herring showed one Isocitrate dehydrogenase band in liver with exception of one heterozygote pattern of 3 bands. Pacific herring showed presence of two mutant alleles and many heterozygotes.

Atlantic - 1 locus, 1 mutant allele (only 1 heterozygote observed)
Pacific - 1 locus (same as Atlantic), 2 mutant alleles, high frequency of heterozygotes

(6) E.C. 1.1.1.44 — Phosphogluconic dehydrogenase — dimer — glycolytic enzyme
Buffer - T.E.B., H — m.w. 79,000

Atlantic - 1 locus, single band, no mutants
Pacific - 1 locus (same as Atlantic), single band, no mutants

(7) E.C. 1.1.1.48 — Galactose dehydrogenase — tetramer — non-glycolytic enzyme
Buffer - T.E.B., H — m.w. - 40,000

Atlantic - Zymogram indicates 2 loci, 6 heterozygotes observed
Pacific - same pattern as Atlantic but no mutants and subunits had faster mobility than Atlantic types

Atlantic - 2 loci A,B mutants at faster B locus, A activity weak
Pacific - 2 loci, no mutants, A activity weak

(8) E.C. 1.2.1.12 — Glyceraldehyde 3 phosphate dehydrogenase — tetramer — glycolytic enzyme
Buffer - T.E.B., H — m.w. 78,000

Atlantic and Pacific patterns identical, single main band.

(9) E.C. 1.4.1.2 — Glutamate dehydrogenase — tetramer — non-glycolytic enzyme
Buffer - Ridgeway's buffer, H — m.w. - 250,000

Results were variable. 1 mutant heterozygote tetramer patter was observed.

Atlantic - 1 locus, 1 mutant
Pacific - 1 locus, not the same as Atlantic, no mutant observed

(10) E.C. 1.11.1.7 — Peroxidase — monomer — non-glycolytic enzyme
Buffer - T.E.B., Ridgeway's both used, H,L — m.w. - 40,000

Table 1 (Continued)

(10) Cont.

Atlantic - 17 heterozygotes (2 bands) in 50 fish
Pacific - single band, same place as mutant Atlantic band

1 locus, 2 mutant alleles in Atlantic sample
no mutant alleles in Pacific sample but common allele has same mobility as mutant Atlantic allele.

(11) E.C. 1.15.1.1

Superoxide dismutase (tetrazolium oxidase) dimer non-glycolytic enzyme
Buffer - T.E.B., M m.w. - 32,000

1 band in same position in all Atlantic and Pacific samples

1 locus, no mutants

(12) E.C. 2.6.1.1

Aspartate aminotransferase dimer glycolytic enzyme
Buffer - T.E.B., M m.w. - 90,000

The Aspartate aminotransferase pattern consisted of a slow moving MM (mitochondrial) dimer band and a faster SS band.

Atlantic and Pacific Aspartate aminotransferase patterns were identical, including the frequency and occurrence of S allele mutants.

2 loci, mutant S allele

(13) E.C. 2.7.5.1

Phosphoglucomutase monomer glycolytic enzyme
Buffer - Ridgeway's, H,M m.w. - 62,000

Atlantic and Pacific patterns identical.
2 alleles at same locus A and A' with different frequencies in Atlantic and Pacific samples.

(14) E.C. 3.1.1.1

Esterase monomer non-glycolytic enzyme
Buffer - T.E.B., M

In previous study (Odense et al., 1973) esterases were scored as phenotypes but clear-cut genotypic composition could not be determined. Pacific herring have similar multiplicity of muscle esterases but until system is resolved, identity of loci cannot be made.

(15) E.C. 5.3.1.9

Phosphohexase isomerase dimer glycolytic enzyme
Buffer - Ridgeway's, H and M m.w. - 132,000

Atlantic - 1 locus with 7 alleles (discussion in Section B)
Pacific - 1 locus with 2 alleles, subunits migrate more anodally than Atlantic, although alleles do not correspond, the familiar herring phosphohexose isomerase pattern is seen with many subbands or sattelite bands, unlike phosphohexose isomerase patterns of other fish species.

Table 2

Lactate dehydrogenase	Alleles A	A'	B	B'	B''
$\bar{x}$ or mean of 58 samples (1968-1970)	0.985	0.0139	0.951	0.0484	0.00025
Standard deviation	0.0135	0.00837	0.0160	0.01563	0.00
Halifax sample 1979	0.97	0.03	0.955	0.045	
Bay of Fundy 1979	0.97	0.03	0.94	0.060	

Aspartate amino transferase	S	S'	S''	S'''
$\bar{x}$ of 58 samples (1968-70)	0.982	0.0170	0.0019	0.00009
Standard deviation	0.0119	0.015	0.0039	0
Halifax 1979	0.99	0.01	0	0
Bay of Fundy 1979	0.985	0.015	0	0

Phosphohexose isomerase	A	B	C	D	E	F	G
$\bar{x}$ of 58 samples (1968-70)	0.00144	0.0974	0.661	0.0995	0.1248	0.0141	0.00188
Standard deviation	0.00514	0.02915	0.2851	0.02357	0.0240	0.0094	0.00259
Halifax 1979	0	0.095	0.63	0.095	0.155	0.025	0
Bay of Fundy	0	0.110	0.705	0.050	0.125	0.005	0

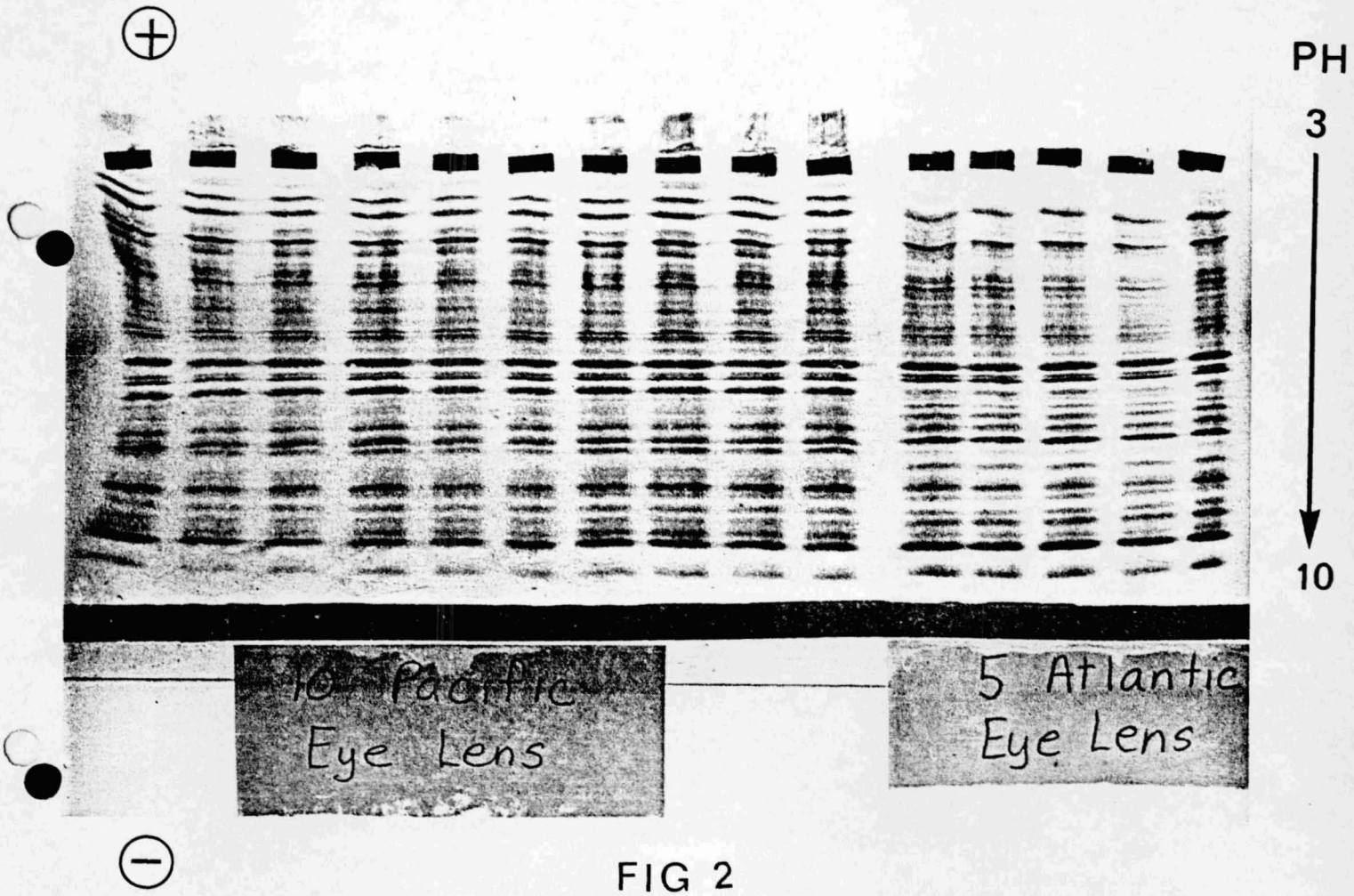
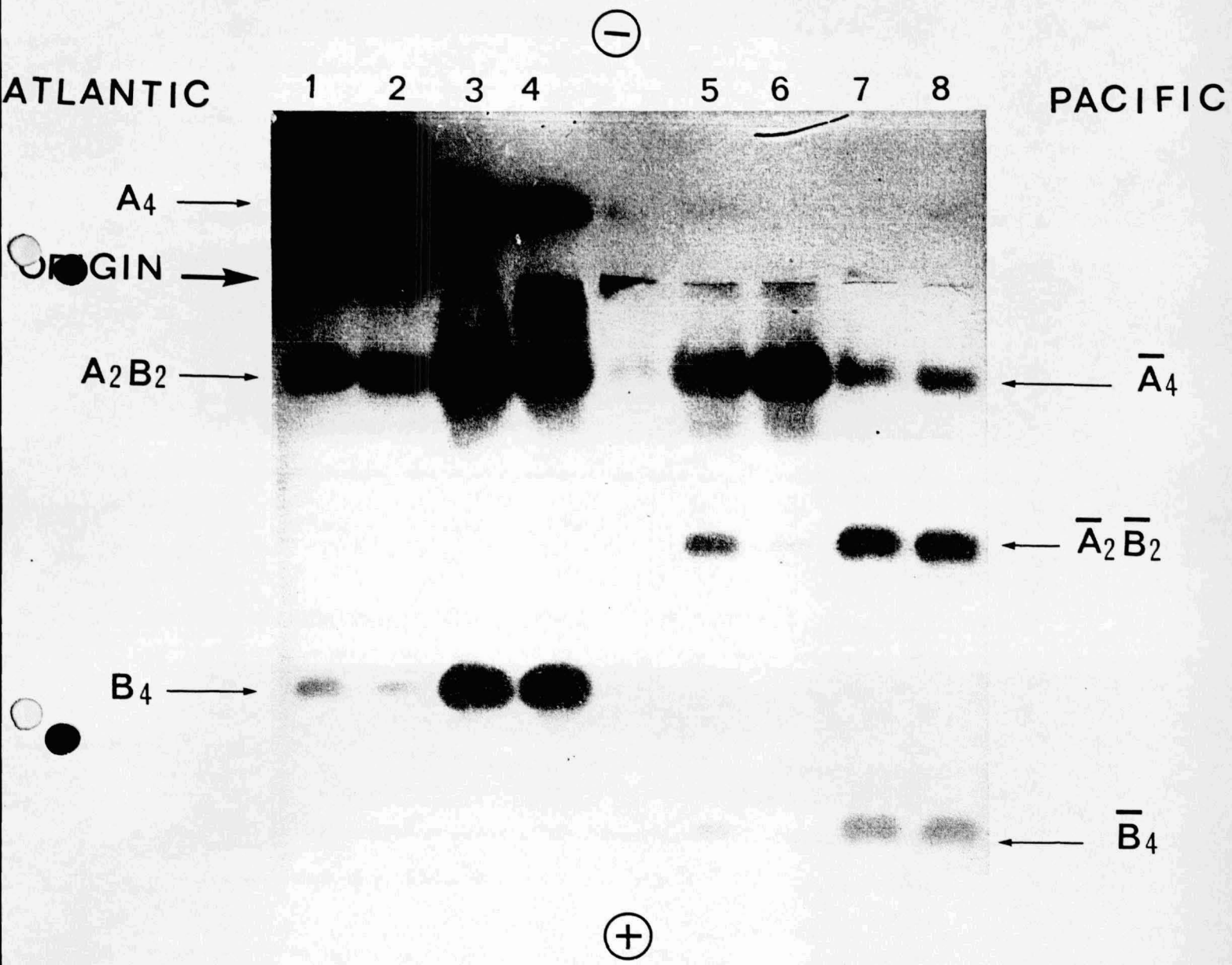


FIG 2

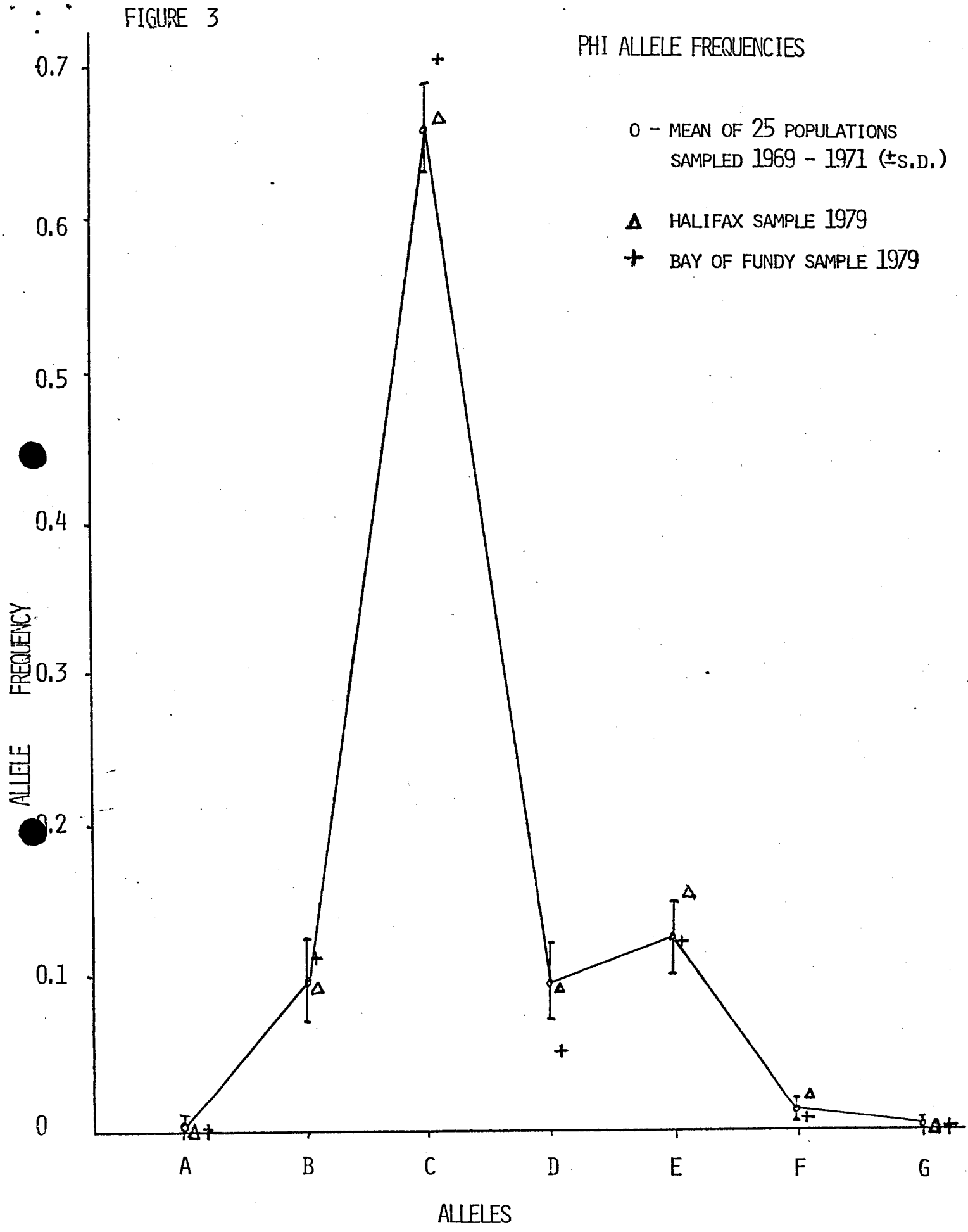
FIG 1



LDH ZYMOGRAM

1,2	ATLANTIC HERRING MUSCLE EXTRACTS
3,4	ATLANTIC HERRING HEART EXTRACTS
5,6	PACIFIC HERRING MUSCLE EXTRACTS
7,8	PACIFIC HERRING HEART EXTRACTS

FIGURE 3


PHI ALLELE FREQUENCIES
O - MEAN OF 25 POPULATIONS SAMPLED 1969 - 1971 (±S.D.)
HALIFAX SAMPLE 1979
BAY OF FUNDY SAMPLE 1979
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ALLELE FREQUENCY
A
B
C
D
E
F
G
ALLELES