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Esterase polymorphism in the capelin, Mallotus villosus: Preliminary evidence for geographic variation in allele frequencies at three loci

by

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SUMMARY

1. Liver and skeletal muscle esterases of the capelin are determined by three genetic loci: Est-A, Est-B and Est-C.
2. All three esterase loci are polymorphic: Est-A has two alleles, Est-B and Est-C have eight alleles each.
3. Allele frequencies are very similar in all samples from south-eastern Canada but a single collection from Melville Sound, N.W.T., is very different from the southeastern Canadian populations sampled. Also there are four alleles at the Est-C locus which have a joint frequency of 0.26 in Melville Sound capelin but seem to be absent from the other populations examined.
4. It is suggested that these relationships may be a first indication that the capelin of the North American arctic shelf should be treated as taxonomically distinct from those of the western Atlantic, and possibly the eastern Pacific as well. An alternative possibility is that there is a strong geographic cline in genetic composition.

INTRODUCTION

The capelin, Mallotus villosus (Müller), is a smelt which is widely distributed along continental margins in the northern Atlantic and Pacific Oceans. Off North America the species has a distribution from Juan de Fuca Strait in the Pacific through Alaska and arctic Canada to the coast of Maine in the Atlantic (Leim and Scott 1966; Hart 1973). Capelin is an important member of the food chain off eastern Canada, particularly

for cod, Atlantic salmon and marine mammals (Templeman 1965; Sergeant 1963, 1973; Lear 1972), it is a major item in the diet of native peoples, and is increasingly exploited by modern fishing methods (Jangaard 1974).

It seems likely that a species with such a wide geographic range in an area subjected to major climatic perturbations in the recent geological past should have been subjected to divergent selection pressures in different sectors of its range (McPhail and Lindsay 1970; Payne 1974; Payne et al 1971a,b). While the classical techniques of meristic and morphometric analysis do detect geographic variation (Winters 1969), they have been singularly unsuccessful in distinguishing intraspecific groups.

In view of the success obtained with electrophoretic analysis of protein polymorphism in distinguishing stocks of other fish species (de Ligny 1969; Payne 1973; Lear and Payne 1975), a program was initiated to investigate the geographic distribution of esterase variants in the capelin. Nyman (1971) reported serum esterase polymorphism in capelin and suggested there were at least 10 alleles at a single locus but he gave no population data to support this interpretation and did not report comparisons of populations from different areas.

MATERIALS AND METHODS

Whole specimens of capelin were collected from Torbay, Newfoundland, (47°35'N, 52° 40'W;N=95); Bonavista, Newfoundland, (48°40'N, 53°10'W;N=96); off St. Anthony, Newfoundland, (51°13'N, 54°42'W;N=150); the lower St. Lawrence Estuary, (48°55'N, 67° 32'W;N=197); and Melville Sound, N.W.T., (68°20'N, 107°41'W;N=60). The specimens from Torbay, Bonavista and Melville Sound were taken during the spawning period. The specimens from off St. Anthony and the lower St. Lawrence Estuary were taken from over-wintering populations. Specimens were frozen immediately after collection.

In the laboratory, specimens were thawed in cold water, livers and skeletal muscle were removed and homogenized with an equal volume of 30% dimethyl sulphoxide, 0.7 M 'tris'-HCl, pH 7.5, and homogenates were centrifuged at 4000 X G for 15 min.

Starch gels (15%) were prepared in 180 mm X 180 mm X 6 mm moulds with a buffer containing 1.4 g/l citric acid, 5.58 g/l 'tris', 1.18 g/l boric acid and 0.12 g/l lithium hydroxide. The vessel buffer contained 11.8 g/l boric acid 1.2 g/l lithium hydroxide. This system was described by Ashton and Bradon (1961).

Electrophoresis was conducted at 250 v until the 'front' had migrated 100 mm past the sample origin. Gel slices were stained for esterase with 1-naphthyl propionate and Fast Red TR salt: 10 mg 1-naphthyl propionate was dissolved in 5 ml 2-ethoxyethanol, diluted to 200 ml with water, and mixed with another 200 ml of water containing 100 mg Fast Red TR salt. Stained gels were rinsed with cold water, blotted dry, and stored in 50:50 glycerol/methanol under refrigeration.

RESULTS AND DISCUSSION

Electropherograms of skeletal muscle extracts demonstrate two distinct regions of esterase activity; Est-B which stains rapidly with 1-naphthyl propionate substrate, and Est-A which only becomes visible after prolonged staining. The Est-A region is

the more anodal. In addition to these two regions of esterase activity, liver extracts demonstrate the existence of a third region, Est-C (Fig. 1). The Est-C region stains very rapidly.

The liver and skeletal muscle esterases of capelin are interpreted as the products of three independent loci: Est-A, Est-B and Est-C. All three esterase loci are polymorphic and generate patterns of single or double bands (Fig. 1) which are considered as the products of homozygous or heterozygous genotypes respectively. The Est-A locus has two alleles and the Est-B and Est-C loci have eight alleles each. In all, there are 3888 theoretically-possible esterase phenotypes.

The frequencies of the observed phenotypes in each of the five samples (Table 1) have been used to estimate allele frequencies in the capelin population at each provenance (Table 2). The genetic distance between populations was computed as the Euclidean interval in n-dimensional space between samples (Rogers 1972) with respect to allele frequencies at the three esterase loci (Table 3). The resulting phenogram (Fig. 2) was produced by UPGMA clustering (Sneath and Sokal 1973).

The magnitude of the genetic distance between Melville Sound and Atlantic coast capelin is striking. The frequencies of all alleles in common except Est-B^4 and Est-B^6 differed significantly between the two regions; and four alleles, Est-C^5 Est-C^6, Est-C^7 and Est-C^8, which occur in the Melville Sound sample (N=60) with a joint frequency of 0.26 were not found in the sample of 536 Atlantic coast specimens. These relationships are demonstrated visually in the phenogram (Fig. 2). The importance of the genetic dichotomy between Atlantic coast and Melville Sound capelin is that this may be a first indication that capelin of the North American arctic shelf should best be considered as taxonomically-distinct from those of the Atlantic region, and possibly from the Pacific Ocean as well. An alternative possibility is that there is a strong geographic cline in genetic composition.

Further work is currently in progress to collect and analyse capelin samples from the region between Melville Sound and the Atlantic Ocean to determine which of these possibilities is true. It is also hoped that material can be obtained from Greenland and British Columbia. A small heterozygote deficit in all samples, which is apparently not the result of stock mixing, is also being investigated.

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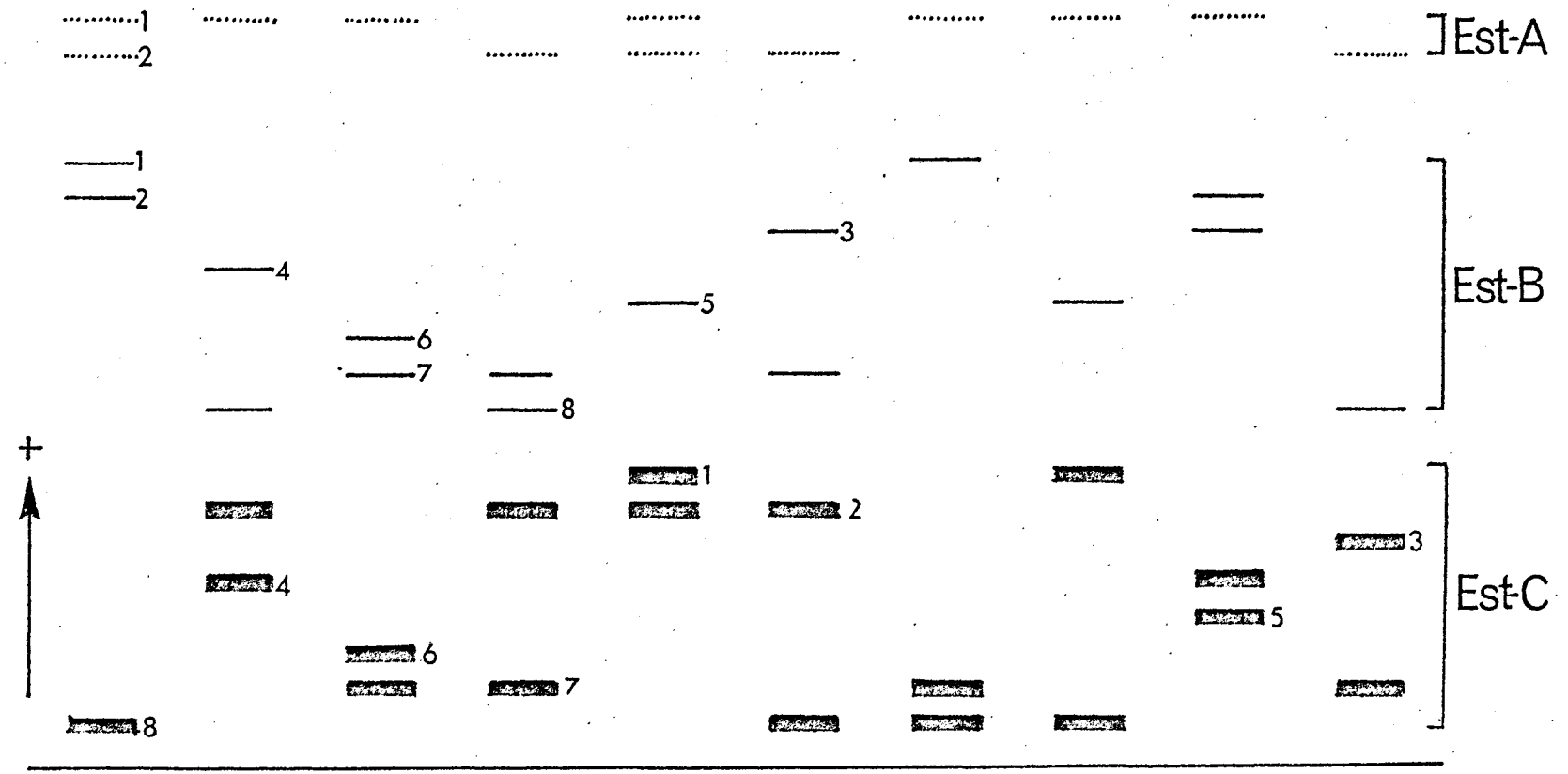

Fig. 1. Diagram of capelin liver esterase patterns showing three regions of esterase activity; Est-A, Est-B and Est-C. These are interpreted as the products of three independent loci. All three loci are polymorphic; Est-A has two alleles (three phenotypes), Est-B and Est-C have eight alleles each (36 phenotypes each). Only ten of the 3888 theoretically-possible liver esterase patterns are shown here.

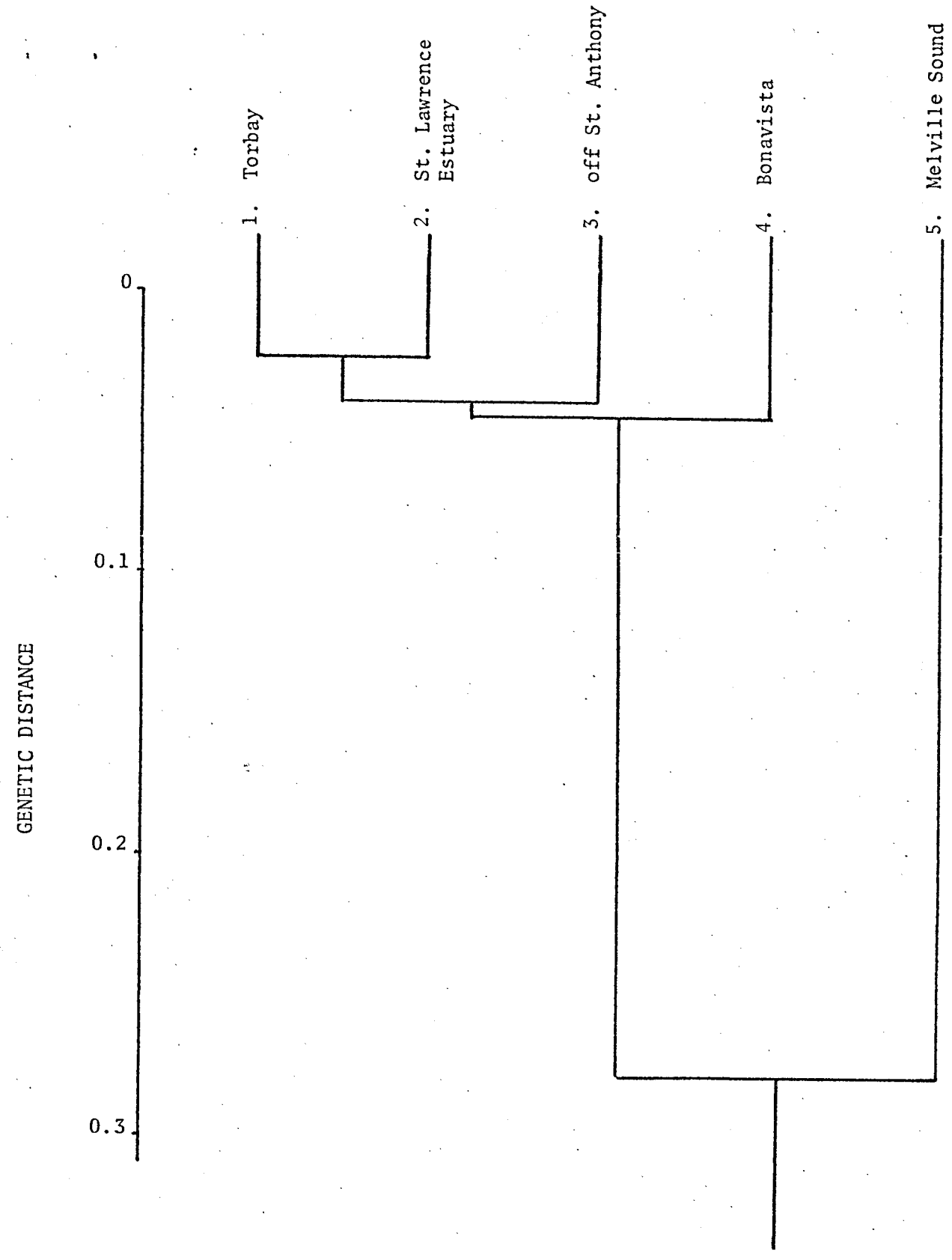

Fig. 2. UPGMA phenogram of estimated genetic distance between the capelin populations sampled.

Table 1. Observed esterase phenotype frequencies in each of the five samples. (Each locus is treated independently.)

Locus	Phenotype	Sample No. 1	2	3	4	5
Est-A	1	5	13	2	4	5
	1-2	28	49	25	28	23
	2	62	135	69	64	32
Est-B	1	0	0	0	0	0
	1-2	1	0	0	0	0
	1-3	1	0	1	1	0
	1-4	1	1	0	0	0
	1-5	0	0	3	1	0
	1-6	2	3	1	2	0
	1-7	0	0	1	0	0
	1-8	0	0	0	0	0
	2	2	2	2	2	0
	2-3	2	6	1	0	0
	2-4	6	4	4	5	0
	2-5	5	14	6	5	0
	2-6	2	6	2	1	1
	2-7	2	6	1	0	0
	2-8	0	0	0	2	0
	3	0	6	6	1	1
	3-4	2	3	2	2	1
	3-5	4	10	8	5	1
	3-6	6	11	7	2	0
	3-7	4	6	7	2	0
	3-8	0	1	1	0	0
	4	2	3	7	2	3
	4-5	2	12	14	8	7
	4-6	9	24	10	7	4
	4-7	3	5	6	2	0
	4-8	0	0	1	0	0
	5	11	16	7	15	23
	5-6	8	21	19	11	10
	5-7	7	5	10	2	3
	5-8	1	4	1	2	0
	6	5	17	16	9	6
	6-7	2	5	2	3	0
	6-8	3	0	1	1	0
	7	1	4	3	1	0
	7-8	0	0	0	1	0
	8	1	0	0	1	0
Est-C	1	0	1	1	1	0
	1-2	2	11	7	2	0
	1-3	2	0	0	5	0
	1-4	0	0	0	0	0
	1-5	0	0	0	0	0
	1-6	0	0	0	0	0
	1-7	0	0	0	0	0

Table 1. Continued

1-8	0	0	0	0	0
2	46	92	67	41	0
2-3	30	64	47	28	4
2-4	2	0	3	5	0
2-5	0	0	0	0	0
2-6	0	0	0	0	0
2-7	0	0	0	0	0
2-8	0	0	0	0	0
3	11	27	24	13	29
3-4	2	0	1	0	0
3-5	0	0	0	0	9
3-6	0	0	0	0	8
3-7	0	0	0	0	2
3-8	0	0	0	0	2
4	0	0	0	1	0
4-5	0	0	0	0	0
4-6	0	0	0	0	0
4-7	0	0	0	0	0
4-8	0	0	0	0	0
5	0	0	0	0	2
5-6	0	0	0	0	1
5-7	0	0	0	0	0
5-8	0	0	0	0	0
6	0	0	0	0	1
6-7	0	0	0	0	0
6-8	0	0	0	0	0
7	0	0	0	0	1
7-8	0	0	0	0	0
8	0	0	0	0	0

Sample No. 1 : Torbay, Nfld.
Sample No. 2 : St. Lawrence Estuary.
Sample No. 3 : off St. Anthony, Nfld.
Sample No. 4 : Bonavista, Nfld.
Sample No. 5 : Melville Sound, N.W.T.

Table 2. Estimated allele frequencies at the three esterase loci, Est-A, Est-B and Est-C, in the capelin populations sampled.

Locus	Allele	Allele Frequency (95% confidence interval)				
		Pop.1	Pop.2	Pop.3	Pop.4	Pop.5
Est-A	1	0.20(0.06)	0.19(0.04)	0.15(0.05)	0.19(0.05)	0.27(0.08)
	2	0.80(0.06)	0.81(0.04)	0.85(0.05)	0.81(0.05)	0.73(0.08)
Est-B	1	0.03(0.02)	0.01(0.01)	0.02(0.02)	0.02(0.02)	0
	2	0.12(0.05)	0.10(0.03)	0.06(0.03)	0.09(0.04)	0.01(0.02)
	3	0.10(0.04)	0.13(0.03)	0.13(0.04)	0.07(0.04)	0.03(0.03)
	4	0.14(0.05)	0.14(0.03)	0.17(0.04)	0.15(0.05)	0.15(0.07)
	5	0.26(0.06)	0.25(0.04)	0.25(0.05)	0.33(0.07)	0.56(0.09)
	6	0.22(0.06)	0.27(0.04)	0.25(0.05)	0.23(0.06)	0.22(0.07)
	7	0.11(0.04)	0.09(0.03	0.11(0.03)	0.06(0.03)	0.03(0.03)
	8	0.03(0.02)	0.01(0.01	0.01(0.01)	0.04(0.03)	0
Est-C	1	0.02(0.02)	0.03(0.02)	0.03(0.02)	0.05(0.03)	0
	2	0.66(0.07)	0.66(0.05)	0.64(0.05)	0.61(0.07)	0.03(0.03)
	3	0.29(0.06)	0.30(0.05)	0.32(0.05)	0.31(0.07)	0.70(0.08)
	4	0.02(0.02)	0	0.01(0.01)	0.04(0.03)	0
	5	0	0		0	0.12(0.06)
	6	0	0		0	0.09(0.05)
	7	0	0		0	0.03(0.03)
	8	0	0		0	0.02(0.02)

Pop.1 : Torbay, Nfld.
Pop.2 : St. Lawrence Estuary
Pop.3 : off St. Anthony, Nfld.
Pop.4 : Bonavista, Nfld.
Pop.5 : Melville Sound, N.W.T.

Table 3. Euclidean interval in n-dimensional space between samples with respect to differences in allele frequencies at the three esterase loci.

	Capelin population				
	1	2	3	4	5
1	.	.	.	.	
2	0.0238	.	.	.	
3	0.0435	0.0355	.	.	
4	0.0417	0.0431	0.0507	.	
5	0.2855	0.2891	0.2940	0.2573	.

Pop.1 : Torbay, Nfld.
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