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Liver aspartate aminotransferase polymorphism: a new tool for estimating proportions of European and North American salmon at West Greenland

by

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INTRODUCTION

The discovery that Atlantic salmon (Salmo salar L.) from rivers on both sides of the Atlantic Ocean migrate to common feeding grounds off Greenland (Pyefinch 1958; Allan and Bulleid 1963; Hansen 1965; Saunders, Kerswill and Elson 1965) and the subsequent establishment of a major high-seas drift net fishery in the Davis Strait between Greenland and Canada has necessitated the development of methods to assess the relative contributions of Atlantic salmon stocks from Europe and North America to this fishery.

Payne (1973) has developed a method based on starch gel electrophoresis of serum transferrin variants to estimate the relative contribution from each continent. Another approach to the problem (Lear and Sandeman 1974) is discriminant function analysis of scale growth characteristics. While preliminary results (Lear and Payne 1975; Payne, Lear and Møller-Jensen 1976) indicate that both independent approaches give similar estimates of relative continental contribution to the fishery, it seems important to develop additional independent approaches to the problem to reinforce the veracity of the two published techniques and to permit assignment of confidence limits to our estimates of year-to-year fluctuations in stock composition. To further refine

the discriminatory power of electrophoretic analysis, a number of enzyme loci have been screened for polymorphism: this paper reports a liver aspartate aminotransferase polymorphism that can be used to provide an estimate of stock composition at West Greenland.

MATERIALS AND METHODS

Blood, scale and liver samples were collected from 364 salmon taken by drift net off West Greenland near Godthaab during early August 1976 by the Danish research vessel Adolf Jensen. Blood samples were obtained by direct cardiac puncture into 5 ml disposable plastic hypodermic syringes without anticoagulant. Serum was obtained by centrifugation and immediately deep-frozen. Liver samples were also frozen. An additional set of 100 liver and heart specimens was obtained from the commercial drift net fishery outside the estuary of the River Blackwater in southern Ireland during June 1975.

Tissue specimens were homogenized mechanically in equal volumes of cold 30% dimethyl sulphoxide, 70 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol, pH 7.5 (Dando 1974). Homogenates were centrifuged for 15 m at 4000 X G. Portions of the supernatants were then sonicated briefly with equal volumes of a saturated solution of pyridoxal-5'-phosphate in buffered 30% DMSO. The supernatants were then used for electrophoresis.

The buffer systems for electrophoresis were 135 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol, 45 mM citric acid, pH 7.0, which was used full strength in the electrode vessels and diluted 1 in 15 for gel preparation (Ayala et al. 1972); or 50 mM sodium citrate, pH 5.0, used full strength in the electrode vessels and diluted 1 in 10 for gels. Starch gels (15% starch; 10 mg/gel pyridoxal-5'-phosphate) were prepared in 180 mm x 180 mm x 6 mm plastic moulds. Samples were applied to the gels on 5 mm squares of Whatman No. 1 filter paper and horizontal electrophoresis was conducted at 150 v and room temperature with forced air cooling for 5 hr. Serum samples were saturated with iron and analysed electrophoretically for transferrin by the method described previously (Payne 1973).

Gel slices were stained for aspartate aminotransferase activity by incubating in the dark at room temperature in 100 ml of 100 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol containing 300 mg DL-aspartic acid, 80 mg α-ketoglutaric acid, 25 mg pyridoxal-5'-phosphate and 50 mg polyvinylpyrrolidone (44,000 daltons). After 10 minutes, a freshly-prepared solution of 100 mg Fast Blue BB salt (Sigma Chemical Co., F 0250) in 25 ml water was added and incubation continued for a further 20 minutes.

RESULTS AND DISCUSSION

ASPARTATE AMINOTRANSFERASE (aspartate transaminase; glutamate-oxaloacetate transaminase; E.C.2.6.1.1) is a dimeric enzyme (Polyanovsky 1965) which occurs as two electrophoretically-distinct forms; a soluble cytoplasmic (supernatant) form, s-AAT, and a membrane-bound mitochondrial form, m-AAT (Decker and Rau 1963; Bertland and Kaplan 1968). Studies of genetic polymorphism in vertebrates indicate that both forms vary independently and are most likely coded by different loci (Odense et al. 1966; DeLorenzo and Ruddle 1970; Davidson et al. 1970; Chen and Giblett 1971).

Two regions of AAT activity were resolved upon electrophoresis at pH 7.0 of liver extracts from Irish salmon (Fig. 1). The least anodal fraction, which is probably the mitochondrial enzyme (Schmidtke and Engel 1972) was invariant. The three distinct s-AAT phenotypes are interpreted as two homozygotes and a heterozygote respectively for a diallelic polymorphism. A similar polymorphism in the Atlantic herring, Clupea harengus, consists of a single band in homozygotes and three bands in the heterozygote (Odense et al. 1966). This contrasts with the situation in Atlantic salmon liver extracts where the homozygotes exhibit a 3-banded pattern and the heterozygote is 5-banded; and suggests that either there is a simple diallelic polymorphism at a single locus (AAT-A) with secondary polypeptide modification to yield a series of isozymes (Model I), or that the products of the polymorphic AAT-A locus are interacting with the products of an additional, invariant AAT-B locus (Model II). Both these models (Fig. 1) are consistent with the observed phenotype frequencies (Table 1), which are in close agreement with Hardy-Weinberg-Castle equilibrium expectations for a frequency of 0.68 for the AAT-A^1 allele.

Electrophoresis of heart extracts from Irish salmon at pH 7.0 yields a single fraction similar in mobility to the central band of the liver heterozygote pattern. However, at pH 5.0 it is of lesser electrophoretic mobility than any of the liver s-AAT isozymes. The heart pattern was invariant regardless of liver s-AAT phenotype which could indicate that it is the product of another s-AAT locus (AAT-C).

Analysis for AAT polymorphism in liver extracts of Atlantic salmon taken at West Greenland demonstrates a significant deviation from Hardy-Weinberg-Castle expectations (Table 1). This situation contrasts strongly with the Irish sample. As salmon at West Greenland are a mixture of North American and European fish, the most likely explanation of the observed genetic disequilibrium is that there is a pronounced difference in the frequency of the AAT-A^1 allele between populations from each continent. Confirmation is provided from a consideration of definite North American salmon in the West Greenland sample (i.e. only those specimens with Tf4 and Tf1/Tf4 transferrin phenotypes: vide, Payne 1973, 1974; Payne et al. 1971a and b): in this subset the AAT-A polymorphism gives a good fit to H-W-C equilibrium expectations with a frequency of 0.09 for the AAT-A^1 allele.

Since the frequency of the AAT-A^1 allele is very different in European and North American salmon (0.68 and 0.09) respectively, it is possible to estimate the proportion of fish from each continent in a mixture such as at West Greenland from the observed frequency of the AAT-A^1 allele. This proportion is conveniently estimated from the relationship:

$$Y = 115.124 - 168X$$

which is the equation of the line through (0.09, 100) and (0.68, 0), where Y is the percentage of North American fish in the mixture and X is the frequency of the AAT-A^1 allele. Application of this relationship to our data yields an estimate of 36.15% North American salmon in the 1976 West Greenland sample. This estimate agrees well with those obtained independently from transferrin phenotype frequencies (34.50%) and discriminant function analysis of scale characteristics (41.19%; Lear, private communication).

It is clear that the AAT polymorphism provides a valuable third approach to monitoring the stock composition of the West Greenland salmon fishery. It may also be of value with IDH-A (Payne and Cross 1976) and Tf in studying the evolution of the two races of Atlantic salmon in Europe (Payne et al. 1971; Wilkins 1972a and b; Child et al. 1976) and in serving as a linkage marker for experimental breeding and stock improvement programs.

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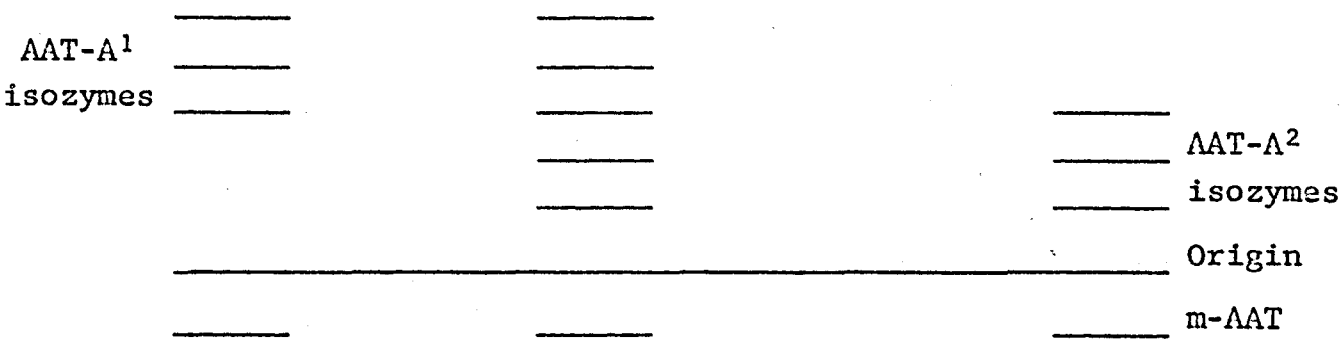
Table 1. AAT-A phenotype frequencies in the Irish and West Greenland samples. The expected frequencies for a H-W-C equilibrium are given in parentheses.

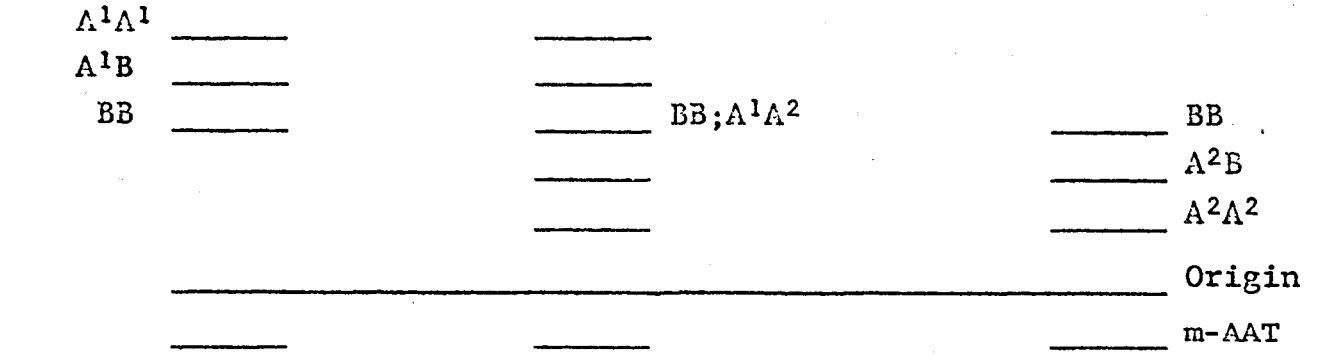
Sample	AAT-A phenotype						P
	1		1/2		2		
Ireland	44	(43.4)	39	(40.3)	10	(9.4)	> 0.7
West Greenland (all specimens)	124	(79.9)	93	(181.3)	147	(102.9)	< 0.001
West Greenland (Tf1/Tf4 and Tf4 specimens)	0	(0.6)	14	(12.7)	64	(64.6)	> 0.3
West Greenland (Tf1 specimens)	124	(93.5)	79	(140.1)	83	(52.5)	< 0.001

Fig. 1. Diagrams of the three common liver aspartate aminotransferase isozyme patterns illustrating the two interpretative models we have developed.

Model I. A single AAT-A locus with two alleles, AAT-A^1 and AAT-A^2, and secondary modification of polypeptides to yield 3-banded homozygote patterns.



Model II. Two loci, AAT-A and AAT-B; AAT-A is polymorphic with two alleles, AAT-A^1 and AAT-A^2; AAT-B is invariant. Interactive association of the polypeptides as dimers yields 3-banded homozygotes and a 5-banded heterozygote upon electrophoresis.



While both models are consistent with our observations, we prefer Model II. This model is closer to the properties of s-AAT in other vertebrate species where polymorphism has been described and it yields a satisfactory structural interpretation for every isozyme.