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Protein variation and duplicate loci in the Atlantic salmon, *Salmo salar* L.

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SUMMARY

A natural population of Atlantic salmon, *Salmo salar* L. was screened for genetic variation at 59 protein loci, using a sample of parr, the juvenile freshwater stage. The mean heterozygosity per locus, estimated at $0{\cdot}033 \pm 0{\cdot}014$, is similar to that described in other salmonid species but low for fish species in general. Variation was observed at AAT, IDH, MD–ME, GLO, ADA, and SDH loci. The methods described should prove useful in stock discrimination and in producing salmon for restocking and sea-rearing. The observed extent of gene duplication is discussed in relation to the evolution and systematics of the salmonid fishes.

1. INTRODUCTION

The Atlantic salmon, *Salmo salar* L. is an anadromous species which occurs in rivers on both sides of the North Atlantic. Young *Salmo salar*, which are referred to as 'parr', spend one or more years in freshwater prior to undergoing a process termed smoltification and migrating to the sea. The period spent at sea is characterized by rapid growth and attainment of sexual maturity. A proportion of the salmon from a river spend only one winter at sea and their exact location during this time is unknown. These fish return to the natal river during the later summer and are referred to as 'grilse'. The rest of the salmon from both sides of the North Atlantic spend two or occasionally three or four winters at sea and migrate to the rich feeding grounds off West Greenland. These fish usually return to the river in the spring or early summer and are referred to as 'salmon'. Spawning takes place in late autumn and early winter and most Atlantic salmon die after spawning.

The Atlantic salmon is a species of considerable commercial importance and traditionally was fished by rod and line in freshwater and by nets in the estuaries. During the 1960s the Danish drift-net fishery off West Greenland led to serious depletions of salmon stocks (Hansen, 1965), and the use of offshore drift-nets off southern Ireland and also off the Northumberland coast, N.E. England is currently causing concern. To counteract the depletion of stocks, national laws limit the

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uses of certain fishing gears while hatcheries have been established on many northwestern European rivers, particularly those where hydro-electric schemes have disrupted natural spawning. Recently, operations have been established along the west coasts of Scandinavia and the British Isles where hatchery-produced salmon are reared to marketable size in cages in the sea.

Because of its unique life-cycle and commercial importance, the Atlantic salmon has been the subject of a number of genetic studies. Payne, Child & Forrest (1971) showed that while all *Salmo salar* populations examined shared a common allele (Tf_1) at the transferrin locus, two alleles (Tf_3 and Tf_4) occurred only in salmon of North American origin, while another rare allele (Tf_2) was specific to European salmon. Variation in the frequency of Tf_2 indicated the presence of two races of *Salmo salar* in the British Isles, a north-western and western Boreal race and a south-western and southern 'Celtic' race (Payne *et al.* 1971; Child, Burnell & Wilkins, 1976). The transferrin polymorphism has also proved useful in demonstrating differences between populations of salmon from different rivers in eastern North America (Møller, 1970; Payne, 1974). Unfortunately, the variant transferrin allele (Tf_2) in European salmon is too rare to be useful for inter-river comparison. Up to 1972, the transferrin locus and an esterase locus, were the only polymorphic loci described in *Salmo salar* (Wilkins, 1972*a*, *b*). Since that time, polymorphism has been reported at a number of esterase loci and a lactate dehydrogenase locus in *Salmo salar* from Sweden (Khanna *et al.* 1975*a*, *b*) whereas isocitrate dehydrogenase, aspartate aminotransferase and malic enzyme loci have been shown to be variable in samples from the British Isles (Cross & Payne, 1977; Payne & Cross, 1977; Cross, Ward & Abreu-Grobois, 1979).

The present paper describes electrophoretic analysis of 59 loci in a sample of *Salmo salar* from the River Blackwater in southern Ireland and the results are compared with those obtained by Khanna *et al.* (1975*a*, *b*) who investigated a smaller number of loci in Swedish salmon. Our investigation aimed at describing mean heterozygosity in a natural population of *Salmo salar* and at finding polymorphic gene markers for use in population studies. We also aimed to assess the number of duplicate loci in a large sample of structural gene loci since the salmonid fishes are thought to be of tetraploid origin (Ohno, 1970) and the rate of rediploidization of tetraploid loci has been shown to be useful in evolutionary studies in fishes (Ferris & Whitt, 1977, 1978; Allendorf, 1978).

2. MATERIALS AND METHODS

(i) *General*

Parr were collected from the River Funshion, a tributary of the River Blackwater in southern Ireland during August 1977 ($N = 119$). The fish were stored intact at -20 °C until required for electrophoresis. All enzymes except sorbitol dehydrogenase were typed in the 1977 sample. An additional sample of 101 parr, collected in the River Funshion in June 1978 was typed for SDH, The majority of these parr was one-summer old, although a small number of two-summer-old

fish was included. Most of the fish were analysed within six months of collection, since the lability of certain enzymes necessitated such assay. Two adult salmon from the Llandyssyl hatchery were donated by the Welsh Water Authority.

(ii) *Sample preparation*

Muscle, liver, eye or heart samples were homogenized in an equal volume of water or 0·05 M-tris HCl buffer pH 7·8, using an electrically-driven glass rod. The homogenates were centrifuged for 15 min at 5000 *g* and the supernatants used for electrophoresis. An alternative method of preparing muscle samples was placing an appropriately-sized piece of muscle in a 0·5 cm well in a perspex block, adding a few drops of distilled water and grinding manually with a glass rod. The crude homogenate was then absorbed directly on to squares of filter paper. All preparative procedures were carried out at 0–4 °C. The subcellular location of certain enzymes was investigated using mitochondrial and cytoplasmic fractions prepared as described by Cross *et al.* (1979).

(iii) *Electrophoresis*

Horizontal starch gel electrophoresis was carried out in 12·5–14 % starch gels (Connaught Laboratories, Canada) measuring 184 × 152 × 6 mm. Six buffer systems were utilized:

A. Electrode: 0·3 M-boric acid adjusted to pH 8·2 with sodium hydroxide. Gel: 0·076 M-tris, 0·005 M-citric acid, pH 8·7. Potential: 300 V for 3 h.

B. Electrode: 0·2 M-boric acid adjusted to pH 8·0 with lithium hydroxide. Gel: Stock solution 0·23 M-tris, 0·034 M-citric acid, 0·095 M-boric acid, pH 8·0, diluted 1 in 5 for use. Potential: 150–300 V for 4–6 h.

C. Electrode: 0·25 M-tris, 0·057 M-citric acid, pH 8·0. Gel: 1 in 25 dilution of electrode buffer. Potential: 200 V for 4 h. For glyceraldehyde-3-phosphate dehydrogenase staining, 0·01 % v/v mercaptoethanol and 10–15 mg NAD were added to the heated starch prior to degassing.

D. Electrode: 0·135 M-tris, 0·045 M-citric acid, pH 7·2. Gel: 1 in 15 dilution of electrode buffer. Potential: 150 V for 5–6 h.

E. Electrode: 0·41 M-trisodium citrate, adjusted to pH 7·0 with 0·5 M-citric acid. Gel: 0·005 M-histidine HCl adjusted to pH 7·0 with 0·1 M-sodium hydroxide. Potential: 150 V for 4 h.

F. Stock solution: 0·5 M-citric acid adjusted to pH 5·0 with sodium hydroxide. Electrode: 1 in 10 dilution of stock solution. Gel: 1 in 100 dilution of stock solution. Potential: 150 V for 5–6 h.

After electrophoresis, gels were sliced horizontally once or twice to provide two or three slices, each of which could be stained for a different enzyme. Runs were carried out in a refrigerator or cold room at 4 °C. Most enzyme stains were modified from those listed in Shaw & Prasad (1970) and Harris & Hopkinson (1976).

3. RESULTS

The proteins assayed, together with buffers, locus designations and tissues screened, are given in Table 1. The tissues listed are those in which each protein was routinely scored. The most common allele at any locus was given the superscript 100. This represents a distance of 100 units from the original insertion point of each sample. Other alleles were given numbers that indicate the mobility of their products relative to that of the common allele. In the case of multiple loci, we have designated them in ascending order with increasing anodal mobility of their products, to facilitate comparison with other salmonid species (Allendorf *et al.* 1977; May, Wright & Stoneking, 1979). The previously published nomenclature of multiple *Idh*, *Aat* and *Me* loci in *Salmo salar* (Cross & Payne, 1977; Payne & Cross, 1977; Cross *et al.* 1979) has been revised accordingly.

Alcohol and Octanol dehydrogenases: Both enzymes occurred as single invariant fractions. ADH migrates cathodally in the buffer systems used.

α-Glycerophosphate dehydrogenase: A total of four invariant loci with electrophoretically distinct polypeptide products appear to code for this enzyme in the Atlantic salmon. These subunits combine to form all possible homodimeric and heterodimeric isozymes. Diagrammatic interpretation of the observed patterns is given in Fig. 1. There are developmental changes in the expression of *α-Gpdh-3* and *-4*, with these loci being more active in parr muscle than in adult muscle. Three loci have been shown to determine this enzyme in *Salmo trutta* and other members of the Isospondyli: families Salmonidae, Clupeidae (Engel Schmidtke & Wolf, 1971) and Coregonidae (Clayton, Franzin & Tretiak, 1973). Two *α-Gpdh* loci have been described in members of the family Pleuronectidae, order Heterosomata, (Ward & Beardmore, 1977; Ward & Galleguillos, 1978), and similar developmental changes in the expression of the more anodal *α-Gpdh* in the muscle samples of the plaice, *Pleuronectes platessa*, have been recorded (Ward, unpublished).

Sorbitol dehydrogenase: Two loci, one of which is polymorphic, code for products of differing electrophoretic mobility in brown trout, *S. trutta* (Engel, Op't Hof & Wolf, 1970; Allendorf *et al.* 1977). On the basis of observed phenotypes, it is apparent that either two disomic loci or one tetrasomic locus determine this tetrameric enzyme in *S. salar*, and that a total of three alleles are segregating in the River Blackwater sample (Fig. 2). Since the common allele at each of the two loci has the same electrophoretic mobility, and we cannot distinguish the products of *Sdh-1* from *Sdh-2*, uniquivocal assignment of genotypes is impossible. Furthermore, in certain cases, assignment of allelic combinations is difficult, depending, as it does, upon the relative intensities of different bands. The genotypes given in Fig. 2 are based on certain assumptions: One, that two disomic loci code for this enzyme rather than a single tetrasomic locus (this has been shown for a number of loci in salmonids, including *Sdh* in *S. trutta*, see Engel *et al.* 1970; Allendorf, Utter & May, 1975; Allendorf & Utter, 1976); two, that the allele Sdh^{-72} is associated with *Sdh-1*, and the allele Sdh^{28} associated with *Sdh-2*. The assumed genotype distributions and derived gene frequencies are given in Table 2.

It will be observed that there is close agreement between the observed values and expected values, assuming a Hardy-Weinberg equilibrium.

Lactate dehydrogenase: Wright, Heckman & Atherton (1975) and Bailey, Tsuyuki & Wilson (1976) showed that this tetrameric enzyme is coded for by five loci in many salmonids. No variants for the products of these five loci were observed in the sample analysed, in contrast to the situation in salmon from Sweden, where the eye-specific *Ldh-5* is polymorphic (Khanna *et al.* 1975*a*).

Malate dehydrogenase: Bailey *et al.* (1970) showed that both the predominant muscle supernatant MDH (form B) and the liver supernatant MDH (form A) are each represented by two loci in salmonids and that all possible heterodimeric isozymes are observed. The two B loci, designated Mdh_s-*3* and *4* here, have common alleles of identical mobility in the population of *S. salar* sampled, and one (or both) of these loci is occasionally heterozygous. The asymmetric staining distributions of such heterozygotes (Fig. 3) reflect the dosage effects of the expression of three common alleles and one variant allele for a dimeric enzyme. So far, no individual carrying two doses of the variant allele has been detected. When calculating allele frequencies (Table 3), it has been assumed that only the Mdh_s-*3* locus is variable. The two loci (designated Mdh_s-*1* and Mdh_s-*2*) have become fixed for different alleles, giving three invariant bands in this region. These patterns appear to be similar to those found in *S. trutta* (Allendorf *et al.* 1977). That these four loci code for supernatant enzymes was confirmed by subcellular fractionations of adult muscle and liver tissues. Electrophoresis of the muscle mitochondrial preparations from the two adults gave three strong bands in the same positions as those specified by Mdh_s-*1* and Mdh_s-*2*, the two supernatant loci predominating in the liver (Fig. 3). Thus it appears possible that salmon mitochondrial MDH is specified by two loci with products similar in mobility, under our electrophoretic conditions, to those of the two A loci, Mdh_s-*1* and Mdh_s-*2*.

The two loci Mdh_s-*1* and *2* are scored as monomorphic because: (*a*) the bands assumed to represent the interlocus dimers between the two A loci and B loci are invariant, except when Mdh_s-*3* is heterozygous, and (*b*) in liver extracts mitochondrial enzyme activity is low yet bands in the position of MDH_s-1 and 2 stain well and are monomorphic. In adult fish Mdh_s-*1* and *2* may best be scored from electrophoresis of liver extracts and Mdh_m-*1* and *2* from muscle extracts. It is possible that either or both of the isozymes MDH_m-1 and 2 are in fact determined by two loci but in the absence of variants we have scored them as two loci only. At least three loci determine mitochondrial MDH in *Salmo gairdneri*, two of which have common alleles with electrophoretically indistinguishable products (Clayton *et al.* 1975). The mitochondrial MDH isozymes in *S. gairdneri* ran cathodally to the supernatant isozymes.

We are not as yet certain whether the three slower bands observed from parr muscle extracts stored at -20 °C represent the products of Mdh_s-*1* and *2* or of two mitochondrial loci. On the basis of the low activity of Mdh_s-*1* and *2* in supernatant extracts of adults, we suspect the latter possibility. If this is the case, then two mitochondrial loci (Mdh_m-*1* and Mdh_m-*2*) are invariant in the Blackwater population.

Table 1. *Proteins studied in the River Blackwater* Salmo salar *population and designation of loci determining them*

Protein	EC no.	Buffer	Locus	Tissue*	Number of fish sampled	Frequency of common allele	Quaternary structure‖
Alcohol dehydrogenase	1.1.1.1	A	*Adh*	L	76	1·000	Dimer
Octanol dehydrogenase	1.1.1.73	E	*Odh*	L	46	1·000	Dimer
α-Glycerophosphate dehydrogenase	1.1.1.8	D	*α-Gpdh-1*	M	98	1·000	Dimer
			α-Gpdh-2	M	98	1·000	Dimer
			α-Gpdh-3	L	42	1·000	Dimer
			α-Gpdh-4	L	42	1·000	Dimer
Sorbitol dehydrogenase†	1.1.1.14	A	*Sdh-1*	L	73	0·582†	Tetramer
			Sdh-2	L	73	0·952†	Tetramer
Lactate dehydrogenase	1.1.1.27	E	*Ldh-1*	M	53	1·000	Tetramer
			Ldh-2	M, E	72	1·000	Tetramer
			Ldh-3	M, E	58	1·000	Tetramer
			Ldh-4	L, M, E	59	1·000	Tetramer
			Ldh-5	E	84	1·000	Tetramer
Malate dehydrogenase†	1.1.1.37	D, E	Mdh_s-*1*	L	119	1·000	Dimer
			Mdh_s-*2*	L	119	1·000	Dimer
			Mdh_s-*3*	M	111	0·986†	Dimer
			Mdh_s-*4*	M	111	1·000	Dimer
			Mdh_m-*1*	M	111	1·000	Dimer
			Mdh_m-*2*	M	111	1·000	Dimer
Malic enzyme	1.1.1.40	D, E	Me_m-*1*	H	118	1·000	Tetramer
			Me_m-*2*	M, H	118	0·547	Tetramer
			Me_s	M, L	78	1·000	Tetramer
Isocitrate dehydrogenase	1.1.1.42	D	*Idh-1*	M	60	1·000	Dimer
			Idh-2	L	119	1·000	Dimer
			Idh-3	L	119	0·832	Dimer
6-Phosphogluconate dehydrogenase	1.1.1.44	E(C)	*6-Pgdh*	M, L	83	1·000	Dimer
Glyceraldehyde 3-phosphate dehydrogenase	1.2.1.12	C‡	*G-3pdh-1*	M	70	1·000	Tetramer
			G-3pdh-2	E	83	1·000	Tetramer
Superoxide dismutase	1.15.1.1	E(C)	Sod_m	L	67	1·000	Tetramer
			Sod_s	L, M	138	1·000	Dimer

Table 1 (cont.)

Aspartate aminotransferase	2.6.1.1	D, F	*Aat_m-1*	M	50	1·000	Dimer
			Aat_m-2	L	101	1·000	Dimer
			Aat_s-1	M	76	1·000	Dimer
			Aat_s-2	L	119	0·672	Dimer
Creatine kinase	2.7.3.2	A, C	*Ck-1*	M	46	1·000	Dimer
			Ck-2	M	46	1·000	Dimer
			Ck-3	M	51	1·000	Dimer
Adenylate kinase	2.7.4.3	C	*Ak-1*	M	92	1·000	Monomer
			Ak-2	M	92	1·000	Monomer
Phosphoglucomutase	2.7.5.1	B	*Pgm*	M, L	117	1·000	Monomer
Esterase	3.1.1.1	B	*Est-1*	M	94	1·000	Monomer
			Est-2	L	119	1·000	Monomer
			Est-3	M, L	99	1·000	Monomer
			Est-4	L	76	1·000	Monomer
Esterase-D	3.1.1.1	B	*Est-D*	M, L	66	1·000	Dimer
Acid phosphatase	3.1.3.2	E	*Acp*	L	54	1·000	Monomer
Aminopeptidase	—	A	*Ap-1*	M, L	72	1·000	Dimer
			Ap-2	M, L	72	1·000	Dimer
Adenosine deaminase	3.5.4.4	E	*Ada-1*	M	35	1·000	Monomer
			Ada-2	L	18	0·972	Monomer
			Ada-3	M, L	48	1·000	Monomer
Glyoxalase	4.4.1.5	A	*Glo*	M	78	0·994	Dimer
Phosphomannose isomerase	5.3.1.8	C	*Pmi*	M	26	1·000	Monomer
Phosphoglucose isomerase	5.3.1.9	B	*Pgi-1*	M	116	1·000	Dimer
			Pgi-2	M, L	116	1·000	Dimer
Muscle protein	—	A	*Mp-1*	M	75	1·000	—
			Mp-2	M	35	1·000	—
			Mp-3	M	37	1·000	—
Transferrin§	—	—	*Tf*	S	187	0·981	Monomer

* Tissue: L (Liver), M (Muscle), E (Eye), H (Heart), S (Serum).
† These estimates require certain assumptions. See text.
‡ Modified buffer system. See text.
§ Data from Child *et al.* (1976).
‖ From Hopkinson, Edwards & Harris (1976) and Ward (1977).

Malic enzyme: A full account of variation for this enzyme is given by Cross *et al.* (1979). The fastest band of activity represents the supernatant enzyme and is invariant. The mitochondrial form of the enzyme is encoded by two loci, one of which is polymorphic with two alleles and the other fixed for an allele producing a slow-migrating enzyme. These loci were previously designated Me_m-*1* and Me_m-*2* respectively (Cross *et al.* 1979). Here, the designation is reversed to conform

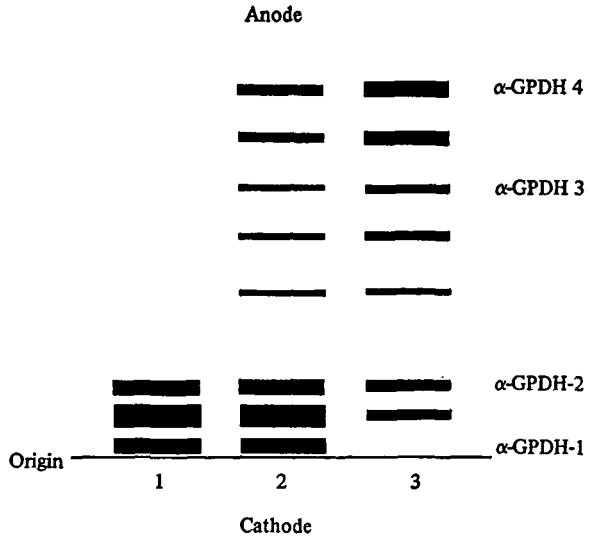

Fig. 1. α-glycerophosphate dehydrogenase patterns and the proposed localization of the four homodimers. (1) adult muscle; (2) parr muscle; (3) parr liver.

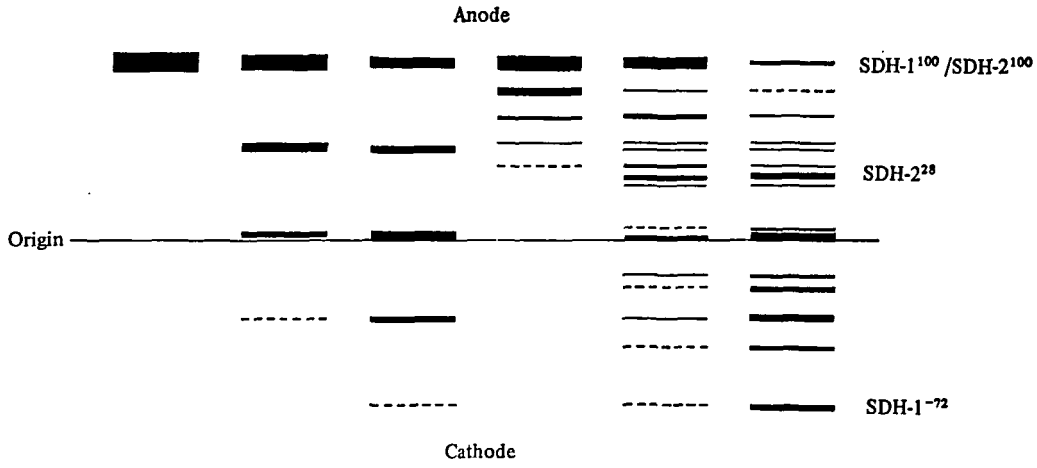

Fig. 2. Sorbitol dehydrogenase patterns observed from liver extracts. Presumed genotypes are as follows: (1) *Sdh-1*^{100}/*Sdh-1*^{100}; *Sdh-2*^{100}/*Sdh-2*^{100}. (2) *Sdh-1*$^{-72}$/*Sdh-1*^{100}; *Sdh-2*^{100}/*Sdh-2*^{100}. (3) *Sdh-1*$^{-72}$/*Sdh-1*$^{-72}$; *Sdh-2*^{100}/*Sdh-2*^{100}. (4) *Sdh-1*^{100}/*Sdh-1*^{100}; *Sdh-2*^{28}/*Sdh-2*^{100}. (5) *Sdh-1*$^{-72}$/*Sdh-1*^{100}; *Sdh-2*^{28}/*Sdh-2*^{100}. (6) *Sdh-1*$^{-72}$/*Sdh-1*$^{-72}$; *Sdh-2*^{28}/*Sdh-2*^{100}.

Note: In the case of multiple heterozygotes, closely-spaced isozymes cannot always be distinguished.

with the practice used throughout this report. The polymorphic locus, Me_m-*2*, is best scored from muscle tissue, while the invariant locus, Me_m-*1*, is expressed most strongly in heart tissue. All possible heterotetramers between the products of Me_m-*1* and Me_m-*2* are found. Allele frequencies for Me_m-*2* are given in Table 3.

Isocitrate dehydrogenase: A description of variation for this enzyme is given by Cross & Payne (1977). Two loci were designated *IDH-A* and *B* by Cross & Payne (1977) but here we designate them *Idh-3* and *2*. A third locus, lacking variation, is expressed in muscle or heart tissue. It is possible that the muscle enzyme is encoded by two loci (as it is in *S. gairdneri*; Allendorf *et al.* 1975), but since we have no direct evidence for this we have designated the single isozyme observed as the product of *Idh-1*. Frequencies at *Idh-3* for parr collected in the River Blackwater in 1977 and for adults collected in the sea close to the estuary of the Blackwater in 1975 are given in Table 4; both samples fit Hardy-Weinberg expectations and do not differ significantly from one another.

Table 2. *Observed and expected genotype frequencies at the two loci encoding sorbitol dehydrogenase in River Blackwater salmon*

		Genotype distributions				
	N	*100/100*	*100/−72*	*−72/−72*	*100/28*	*28/28*
Sdh-1	73	24(24·8)	37(35·5)	12(12·7)	—	—
Sdh-2	73	66(66·2)	—	—	7(6·7)	0(0·2)

Hardy-Weinberg expectations are given in parentheses, and are based on the following allele frequencies: $Sdh\text{-}1^{100} = 0{\cdot}582$; $Sdh\text{-}1^{-72} = 0{\cdot}418$; $Sdh\text{-}2^{100} = 0{\cdot}952$; $Sdh\text{-}2^{28} = 0{\cdot}048$. For further details see text and Fig. 2.

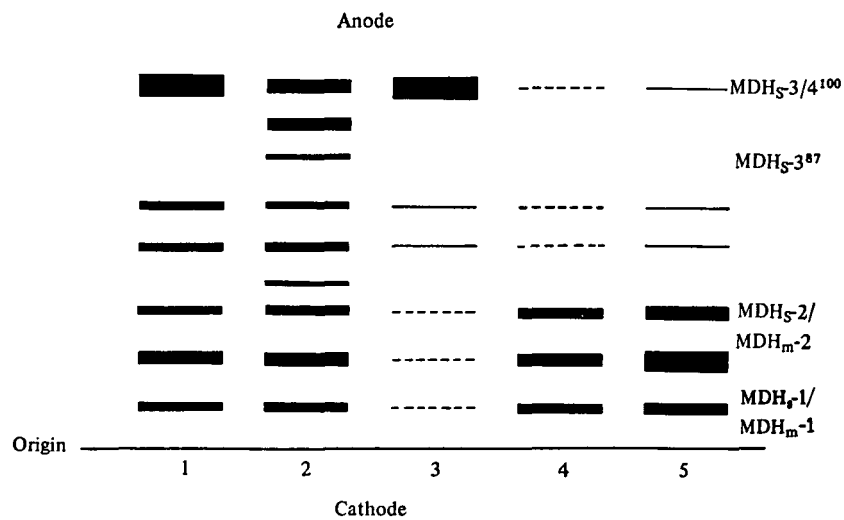

Fig. 3. Malate dehydrogenase patterns. (1) and (2): par muscle extracts; (1) Mdh_s-$3^{100}/Mdh_s$-3^{100}; (2) Mdh_s-$3^{100}/Mdh_s$-3^{87}. (3) and (4); adult muscle extracts from Mdh_s-$3^{100}/Mdh_s$-3^{100}; (3) supernatant preparation; (4) mitochondrial preparation; (5) supernatant extract from adult liver. For further details see text.

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6-Phosphogluconate dehydrogenase: A single invariant band was observed in muscle and liver samples. We did not observe the less anodal faint band reported by Khanna *et al.* (1975*a*).

Glyceraldehyde-3-phosphate dehydrogenase: The observed isozymes were interpreted as the products of two invariant loci. A fast migrating isozyme stained strongly in eye tissue, but weakly in muscle tissue, while an isozyme migrating just cathodally stained strongly in muscle tissue.

Table 3. *Frequencies and heterozygosities of those loci shown to be variable in the River Blackwater salmon population*

No genotype distributions differ significantly from Hardy-Weinberg expectations. Data on transferrin locus only from Child *et al.* (1976).

Locus	Number of fish sampled	Allele designations and frequencies		Heterozygosities observed	expected
		100	*87*		
Mdh_S-3	111	0·986	0·014	0·027	0·028
		115	*100*		
Me_m-2	118	0·453	0·547	0·449	0·495
		116	*100*		
Idh-3	119	0·168	0·832	0·286	0·280
		100	*74*		
Aat_S-2	119	0·672	0·328	0·487	0·441
		100	*90*		
Ada-2	18	0·972	0·028	0·056	0·054
		115	*100*		
Glo	78	0·006	0·994	0·013	0·012
		100	*−72*		
Sdh-1	73	0·582	0·418	0·510	0·490
		100	*28*		
Sdh-2	73	0·952	0·048	0·100	0·090
		1	*2*		
Tf	187	0·981	0·019	0·037	0·037

Table 4. *Comparison of* Idh-3 *allele frequencies encoding isocitrate dehydrogenase variants in parr and adult salmon from the River Blackwater*

		Genotype distributions			Allele frequencies	
Sample	*N*	*116/116*	*116/100*	*100/100*	*Idh-3^{116}*	*Idh-3^{100}*
Parr	119	3(3·36)	34(33·27)	82(82·37)	0·168	0·832
Adults	100	6(3·24)	24(29·52)	70(67·24)	0·180	0·820

Figures in parentheses give Hardy-Weinberg expectations. Adult data are from Cross & Payne (1977).

Superoxide dismutase: We assume two invariant loci to determine this enzyme in *S. salar*. Subcellular fractionation revealed that the faster and more active isozyme is the supernatant form, the slower isozyme being mitochondrial in origin.

Aspartate aminotransferase: At least two supernatant loci determine this enzyme in *S. salar* (Payne & Cross, 1977), while the observed patterns also suggest that

two mitochondrial loci are active (Fig. 4), one predominating in liver, the other in muscle. The supernatant locus with the most negatively charged products (*Aat*$_s$-*2*) is active in liver and segregates for two alleles (Table 3) while the other three loci are invariant. The locus designated *AAT-A* (or *AAT-A* and *B*) is redesignated here *Aat*$_s$-*2*. Inspection of a large number of gels using different buffers has convinced us that only a single locus determines the liver specific supernatant enzyme, but secondary modification causes the appearance of three bands in homozygous individuals. The other loci are designated *Aat*$_s$-*1* and *Aat*$_m$-*1* and *2* in accordance with the mobility of the isozymes.

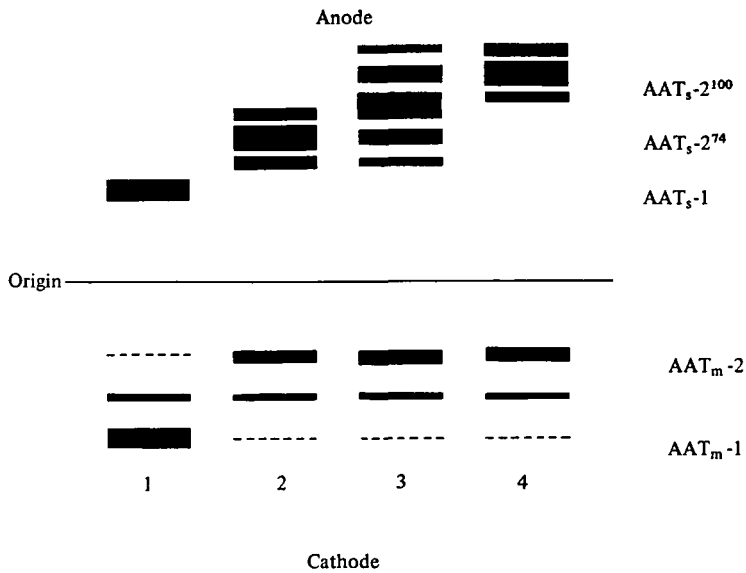

Fig. 4. Aspartate aminotransferase patterns. (1) muscle extract; (2) liver extract; *Aat*$_s$-2^{74}/*Aat*$_s$-2^{74}; (3) liver extract; *Aat*$_s$-2^{74}/*Aat*$_s$-2^{100}; (4) liver extract; *Aat*$_s$-2^{100}/*Aat*$_s$-2^{100}.

Adenylate and Creatine kinase: AK can be stained for specificially, but also appears on gels stained for CK. The fastest AK band was too diffuse too score, but each of the other bands, with the exception of the two slowest CK bands, (Fig. 5) is assumed to represent the products of a separate locus. The two slower CK isozymes observed in muscle extracts are assumed to be conformational isomers of the same primary protein product which is coded by the same common alleles shared by duplicate loci (*Ck-1/2*). Similar patterns of muscle CK expression have been observed in *Salmo trutta* (Allendorf *et al.* 1976), *Salvelinus alpinus* and *S. fontinalis* (May *et al.* 1979) and have been postulated for salmonid fishes in general (Utter, Allendorf & May, 1979). No variation could be detected for either the two AK loci or the three CK loci, although variants for the two slow CK could be masked by AK activity.

Phosphoglucomutase: Two closely spaced invariant isozymes were observed in all fishes tested. The relative staining intensities of these zones varied in different

tissues. We provisionally and conservatively interpret these patterns as the product of a single invariant locus. The rainbow trout, *Salmo gairdneri*, has also been assigned a single PGM locus (Utter & Hodgins, 1972), whereas two loci have been assigned to the brown trout, *Salmo trutta* (Allendorf *et al.* 1977).

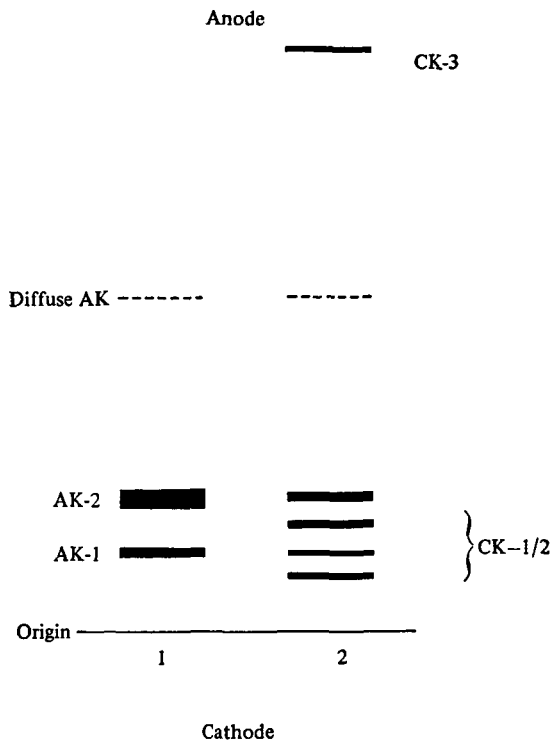

Fig. 5. (1) Adenylate kinase and (2) creatine kinase patterns from muscle extracts.

Esterases: Four zones resolved sufficiently well to be scored using α-naphthyl propionate as substrate. Each is invariant and is assumed to be coded by a single locus. There was no evidence of the variability described by Khanna *et al.* (1975*b*).

Esterase-D: Using methylumbelliferyl acetate, a single anodally-migrating invariant fluorescent zone was observed in all muscle and liver samples tested and this was presumed to be the product of a single locus.

Aminopeptidase: Two invariant zones appeared in all the fish tested and were assumed to represent the products of two loci.

Acid phosphatase: A single slow migrating invariant enzyme was observed using methylumbelliferyl phosphate as substrate.

Adenosine deaminase: Three loci have been provisionally identified as coding for this enzyme in the salmon. Both muscle and liver tissue showed the same invariant fast moving isozyme, the product of *Ada-3*. Liver tissue expressed in addition the *Ada-2* locus, which gave a well stained band preceded by a weaker satellite zone. A single heterozygote with two major bands was observed and designated $Ada\text{-}2^{100}/Ada\text{-}2^{90}$. The individual remained single banded for the ADA-3

isozyme. It should be noted that only 18 parr were scored for *Ada-2*; thus the frequency of variants at this locus could not be accurately estimated. A slow moving invariant zone, expressed in muscle tissue, has been identified tentatively as the product of a third locus, *Ada-1*, but since it appeared to stain weakly when xanthine oxidase was omitted from the staining mixture, it may not in fact be an ADA isozyme.

Glyoxylase I (preferred name *Lactoyl-glutathione lyase*): A single invariant zone was observed in muscle electropherograms of all but one of the specimens tested. The three bands of the heterozygote, designated Glo^{115}/Glo^{100}, were consistent with the reported dimeric nature of this enzyme (Kömpf *et al.* 1975), and activities in the approximate ratio of 1:2:1 indicated that a single locus was responsible for the observed activity.

Phosphomannose isomerase: A fast moving single banded invariant enzyme was observed.

Phosphoglucose isomerase: Electrophoresis of salmon tissues gives the typical Teleost fish pattern of two loci whose products hybridize to give three bands (Avise & Kitto, 1973). The fast system predominates in liver, the slow in muscle, but both are invariant. There is no evidence in *S. salar* for the third PGI locus described in *S. trutta* (Allendorf *et al.* 1977). Khanna *et al.* (1975*a*) described a third PGI locus in *S. salar* but appear to have misidentified the secondary isozymes which are present anodal to PGI-1 in liver zymograms, as the products of a separate locus.

Muscle proteins: Five well-separated invariant bands were observed. The two least anodal of these zones were homologous with the two CK muscle isozymes. The three most anodal zones were interpreted as the products of three monomorphic loci, though in the absence of variants this interpretation cannot be confirmed.

Transferrins: Data for the polymorphic *Tf* locus has been published for the Blackwater population by Child *et al.* (1976). Allele frequencies are given in Table 3.

4. DISCUSSION

The fish families Salmonidae, Cyprinidae and Catostomidae have been the subject of extensive investigations in recent years. Measures of DNA content and numbers of chromosomes suggested that members of these families had arisen from tetraploid ancestors. (Ohno, 1970; Uyeno & Smith, 1972), and surveys based upon the electrophoretic separation of proteins have supported such theories. Despite loss of function at many of the original loci, approximately 50% duplicate gene expression has been retained in representatives of the Salmonidae and Catostomidae (Allendorf, 1978; Ferris & Whitt, 1977).

The enzymes which have retained duplicate gene expression in *Salmo salar* include supernatant and mitochondrial AAT, supernatant MDH, mitochondrial ME, α-GPDH, SDH, the products of two of the three original LDH loci and the two liver IDH loci, the major muscle CK and, probably, ADA and mitochondrial

MDH. Proteins which have lost duplicate expression are GLO and TF. We are not able to ascertain which of the following invariant enzymes have retained or lost duplicate expression: PGM, muscle and liver PGI, 6-PGDH, muscle and eye G3PDH, supernatant and mitochondrial SOD, supernatant ME, muscle IDH, ADH, ODH, EST-D, other esterases, ACP, PMI, and two APs. Ferris & Whitt (1977) estimate that in the 50 million year history of the catostomids, only 5% of loci will have retained duplicate non-divergent gene expression, an estimate supported by their own electrophoretically derived data. The proportion of loci that have retained duplicate non-divergent expression in salmonids may be higher. In the Atlantic salmon *at least* two pairs of loci share electrophoretically identical common alleles, Mdh_s-*3* and Mdh_s-*4*, and *Sdh-1* and *Sdh-2*. Allendorf & Utter (1976) have pointed out that divergence of duplicated loci cannot begin until disomic inheritance has been established; they further suggest that the absence of detectable divergence at a number of salmonid loci known to be present in a duplicate state indicates that disomic inheritance at these loci has probably been established only comparatively recently. If the assumption is made that the third list of enzymes given above consists largely of enzymes that have lost duplicate expression, then these lists agree well with similar lists given for the rainbow trout, *S. gairdneri* (Allendorf *et al.* 1975; Allendorf, 1978). An exception is ME, considered to have lost duplicate expression in *S. gairdneri* but which, in mitochondrial form at least, is encoded by duplicate loci in *S. salar* (Cross *et al.* 1979), *Salvelinus fontinalis* (Stoneking, May & Wright 1979), and, probably, *S. trutta* (Allendorf *et al.* 1977).

Ferris & Whitt (1978) used the degree of loss of duplicate gene expression in different species to study the systematics of the catostomid fishes. The closest congener to *S. salar* is *S. trutta*. The former is a western European/eastern North American form, whereas the latter species is endemic to Europe. The more distantly related rainbow trout, *S. gairdneri*, and species of the genus *Oncorhynchus* are endemic to western North America. The presence of duplicate mitochondrial ME loci in *S. salar* and *S. trutta* but, probably, a single locus in *S. gairdneri* is consistent with the likely evolutionary history of these fishes. Divergence between *S. salar* and *S. trutta* is evidenced by the apparent presence of two invariant loci for PGI in the former, whereas this enzyme is encoded by three loci in the latter species (Allendorf *et al.* 1977). It may be that three loci do code for this enzyme in *S. salar*, but that two of these loci have not diverged sufficiently to give electrophoretically distinguishable products. More information is required before such an approach will be of use in studying the systematics of salmonids.

On the basis of electrophoretic mobility of different proteins examined, we have estimated the mean heterozygosity per locus in the River Funshion population to be $0{\cdot}033 \pm 0{\cdot}015$, and the proportions of polymorphic loci as 0·136 and 0·068 using the 0·99 and 0·95 criteria of polymorphism respectively (Table 5). However, the number of loci coding for monomorphic non-divergent proteins may have been underestimated, leading us to slightly overestimate the true levels of variation. The same error occurs in all such estimates in salmonids, and thus we

can directly compare our estimates with those of other workers on this group. In the review of Allendorf & Utter (1979), average heterozygosities of 0·039, 0·045, 0·015, 0·018, 0·035, 0·000, 0·043, 0·060 and 0·024 were given for *O. gorbuscha*, *O. keta*, *O. kisutch*, *O. nerka*, *O. tshawytscha*, *S. apache*, *S. clarki*, *Sl. gairdneri*, and *S. salar* respectively. (No further details have been published by Allendorf and Utter on genetic variation in *S. salar*, but the two estimates of heterozygosity agree fairly closely with one another.) Allendorf *et al.* (1977) describe variation at only nine out of 58 loci in a Scandinavian sample of *S. trutta*, but do not provide an heterozygosity estimate. Judging from the description of numbers of variant

Table 5. *Levels of protein variation in Atlantic salmon parr from the River Blackwater population*

		Proportion of loci polymorphic		
	N	Criterion *A*	Criterion *B*	Mean heterozygosity ± S.E.
Enzymes	55	0·127	0·073	0·035 ± 0·016
Other proteins	4	0·125	0·000	0·009 ± 0·009
All proteins	59	0·136	0·068	0·033 ± 0·015

Criterion A: frequency of most common allele ⩽ 0·99. Criterion B: frequency of most common allele ⩽ 0·95.

enzyme phenotypes (Allendorf *et al.* 1977) the mean heterozygosity will clearly be low. Thus the mean heterozygosity per locus over all salmonid species examined is about 0·03, a value low in comparison with most other fish species. Powell (1975) and Selander (1976) give average heterozygosities of approximately 0·06 and 0·08 respectively for a large range of fish species. The low heterozygosity of *Salmo salar* may be associated with the relatively small population sizes typical of the species (E. Twomey – personal communication – estimates the number of pairs of adult salmon breeding in the River Funshion to be 100) and with the reproductive isolation of the populations of different rivers (Cross, Healy & O'Rourke, 1978). This reproductive isolation is maintained by accurate homing of salmon to their natal rivers (Went, 1964). Allendorf & Utter (1979) note that low heterozygosity in *Oncorhynchus* species cannot be attributed to small extant populations. It does seem possible, however, that these species and also *S. salar* may have experienced population bottlenecks during geological history when their habitats were subjected to glaciation. The low variability in the salmonids contrasts with the high variability observed in, for example, members of the flatfish family Pleuronectidae, which, in general, have large population sizes and are reproductively panmictic over wide geographic areas. Here mean heterozygosities for six Atlantic species are 0·114, 0·113, 0·086, 0·091, 0·069 and 0·065 (Galleguillos & Ward, unpublished; for earlier data on three of these species see Ward & Beardmore, 1977; Ward & Galleguillos, 1978): the overall mean for the six species is 0·090.

Our results for Blackwater River Atlantic salmon can be compared with those

of Khanna *et al.* (1975*a*, *b*), who assayed three Swedish stocks of Atlantic salmon for a number of proteins. Differences are evident between the results of the two surveys. The enzymes mitochondrial ME-2, ADA-2 and GLO, variable in our material, were not screened in the Scandinavian study. Supernatant AAT-2 was not adequately enough resolved to be typed in the investigation of Khanna *et al.*, nor was SDH, though in the latter case indications of variations were noted. However, Khanna *et al.* found, in contrast to our results, the eye-specific *Ldh* locus and six *Est* loci to be variable, but *Mdhs-3* and *-4* and the liver specific *Idh-3* to be monomorphic. Wilkins (1971) reported absolute differences between liver esterase phenotypes in *S. salar* from different areas, a finding consistent with the results of Khanna *et al.*, yet we, using the same buffer as Khanna *et al.*, found all four liver esterases to be monomorphic both in the present study and in a sample of salmon livers from West Greenland (Cross, unpublished results). Clearly this question requires resolving, but it seems appropriate to mention the warning of Utter, Hodgins & Allendorf (1974) who stated that great care should be taken with interpretation of aspecific esterase patterns since they are particularly subject to variation due to physiological, ontogenetic or denaturing effects. Nonetheless, there would appear to be genetic differences between Scandinavian and Irish salmon which would make detailed comparative studies interesting. Such differences might mean that moving of salmon from one area to the other, for example for use in restocking rivers, could be unproductive, if the degree of genetic divergence is indicative of adaptational differences between populations from Scandinavia and Ireland.

It is interesting to note that the majority of polymorphic enzymes in *S. salar* are either tetramers or dimers (Table 1). This contrasts with the general trend observed in vertebrates where the highest heterozygosity is observed in enzymes of the lowest sub-unit number (Ward, 1977; Ward & Galleguillos, 1978).

Investigation of the sub-cellular localization of different forms of enzymes as undertaken here for MDH, ME, AAT and SOD seems particularly useful in the case of the salmonid fishes where the large array of isozymes for many enzymes makes interpretation difficult. Designation of enzymes by sub-cellular location also makes inter-specific comparison easier. Of the gene markers detected in the present study, Me_m-*2*, Aat_s-*2*, *Sdh-1* and *2* and *Idh-3* should prove most useful in population studies since at these loci the frequency of the common allele is less than 0·9, while Mdh_s-*3* and/or *4*, *Ada-2*, *Glo* and additionally *Tf* (Child *et al.* 1976) may be of more limited use because of the high frequency of the common allele. The close similarity of *Idh-3* frequencies between the 1977 sample of parr and a sample of adults taken at the mouth of the River Blackwater in 1975 (Cross & Payne, 1977) suggests that, at least for the *Idh* locus, allele frequencies are relatively constant over generations (Table 4). Furthermore, the lack of deviation of observed numbers from the expectations of the Hardy-Weinberg equilibrium in the case of all the polymorphic systems described is consistent with the salmon of the River Funshion behaving as a single inter-breeding population. These gene markers, particularly when considered together, should prove useful in population and racial

studies, and in the study of applied problems such as stock discrimination, genetic comparison of grilse and large salmon, investigation of the degree of inbreeding associated with hatchery propagation and in selection of the most suitable strains for cage rearing in the sea.

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REFERENCES

ALLENDORF, F. W. (1978). Protein polymorphism and the rate of loss of duplicate gene expression. *Nature* **272**, 76–78.

ALLENDORF, F. W. & UTTER, F. M. (1976). Gene duplication in the family Salmonidae. III. Linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout (*Salmo clarki*). *Hereditas* **82**, 19–24.

ALLENDORF, F. W. & UTTER, F. M. (1979). Population genetics. In *Fish physiology*, vol. VIII (ed. D. J. Randall, J. S. Hoar and R. Brett), pp. 407–454. New York: Academic Press.

ALLENDORF, F. W., UTTER, F. M. & MAY, B. P. (1975). Gene duplication within the family Salmonidae. II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. In *Isozymes*, vol. IV *Genetics and Evolution* (ed. C. L. Markert), pp. 415–432. New York: Academic Press.

ALLENDORF, F. W., RYMAN, N., STENNEK, A. & STAHL, G. (1976). Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations. *Hereditas* **83**, 73–82.

ALLENDORF, F. W., MITCHELL, N., RYMAN, N. & STAHL, G. (1977). Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. *Hereditas* **86**, 179–190.

AVISE, J. C. & KITTO, G. B. (1973). Phosphoglucose isomerase gene duplication in the bony fishes: an evolutionary history. *Biochemical Genetics* **8**, 113–132.

BAILEY, G. S., TSUYUKI, H. & WILSON, A. C. (1976). The number of genes for lactate dehydrogenase in salmonid fishes. *Journal of the Fisheries Research Board of Canada* **33**, 760–767.

BAILEY, G. S., WILSON, A. C., HALVER, J. E. & JOHNSON, C. L. (1970). Multiple forms of supernatant malate dehydrogenase in salmonid fishes. *Journal of Biological Chemistry* **245**, 5927–5940.

CHILD, A. R., BURNELL, A. M. & WILKINS, N. P. (1976). The existence of two races of Atlantic salmon (*Salmo salar* L.) in the British Isles. *Journal of Fish Biology* **8**, 35–43.

CLAYTON, J. W., FRANZIN, W. G. & TRETIAK, D. N. (1973). Genetics of glycerol-3-phosphate dehydrogenase isozymes in white muscle of lake whitefish (*Coregonus clupeaformis*). *Journal of the Fisheries Research Board of Canada* **30**, 187–193.

CLAYTON, J. W., TRETIAK, D. N., BILLECK, B. N. & IHSSEN, P. (1975). Genetics of multiple supernatant and mitochondrial malate dehydrogenase isozymes in rainbow trout (*Salmo gairdneri*). In *Isozymes*, vol. IV. *Genetics and Evolution* (ed. C. L. Markert), pp. 433–448. New York: Academic Press.

CROSS, T. F. & PAYNE, R. H. (1977). NADP-isocitrate dehydrogenase polymorphism in the Atlantic salmon, *Salmo salar*. *Journal of Fish Biology* **11**, 493–496.

CROSS, T. F., HEALY, J. A. & O' ROURKE, F. J. (1978). Population discrimination in Atlantic salmon from Irish rivers using biochemical genetic methods. *Mimeograph of ICES committee meeting* 1978/M:2.

CROSS, T. F., WARD, R. D. & ABREU-GROBIOS, A. (1979). Duplicate loci and allelic variation for mitochondrial malic enzyme in the Atlantic salmon, *Salmo salar* L. *Comparative Biochemistry and Physiology* **62B**, 403–406.

ENGEL, W., OP'T HOF, J. & WOLF, U. (1970). Genduplikation durch polyploide Evolution: die Isoenzyme der Sorbitdehydrogenase bei herings- und lachsartigen Fischen (Isospondyli). *Humangenetik* **9**, 157–163.

Engel, W., Schmidtke, J. & Wolf, U. (1971). Genetic variation of α-glycerophosphate dehydrogenase isoenzymes in Clupeid and Salmonid fish. *Experientia* **27**, 1489–1491.

Ferris, S. D. & Whitt, G. S. (1977). Loss of duplicate gene expression after polyploidisation. *Nature* **265**, 258–260.

Ferris, S. D. & Whitt, G. S. (1978). Phylogeny of tetraploid catostomid fishes based on the loss of duplicate gene expression. *Systematic Zoology* **27**, 189–206.

Hansen, P. M. (1965). Report on recaptures in Greenland waters of Salmon tagged in rivers in America and Europe. *International Commission for Northwest Atlantic Fisheries. Redbook* Part 3, 194.

Harris, H. & Hopkinson, D. A. (1976). *Handbook of Enzyme Electrophoresis in Human Genetics*. Amsterdam, New York and Oxford: North Holland.

Hopkinson, D. A., Edwards, Y. H. & Harris, H. (1976). The distribution of subunit numbers and subunit sizes of enzymes: a study of the products of 100 human gene loci. *Annals of Human Genetics* **39**, 383–411.

Khanna, N. D., Juneja, R. K., Larsson, B. & Gahne, B. (1975*a*). Electrophoretic studies on proteins and enzymes in the Atlantic salmon, *Salmo salar* L. *Swedish Journal of Agricultural Research* **5**, 185–192.

Khanna, N. D., Juneja, R. K., Larsson, B. & Ghane, B. (1975*b*). Electrophoretic studies on esterases in the Atlantic salmon, *Salmo salar* L. *Swedish Journal of Agricultural Research* **5**, 193–197.

Kömpf, J., Bissbort, S., Gussmann, S. & Ritter, H. (1975). Polymorphism of red cell glyoxalase I (EC 4.4.1.5). A new genetic marker in man. *Humangenetik* **27**, 141–143.

May, B., Wright, J. E. & Stoneking, M. (1979). Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvelinus* and a review of the literature on other species. *Journal of the Fisheries Research Board of Canada* **36**, 1114–1128.

Møller, D. (1970). Transferrin polymorphism in Atlantic salmon (*Salmo salar*). *Journal of the Fisheries Research Board of Canada* **27**, 1617–1625.

Ohno, S. (1970). *Evolution by Gene Duplication* New York: Springer Verlag.

Payne, R. H. (1974). Transferrin variation in North American populations of the Atlantic salmon, *Salmo salar*. *Journal of the Fisheries Research Board of Canada* **31**, 1037–1041.

Payne, R. H. & Cross, T. F. (1977). Liver aspartate aminotransferase polymorphism: a new tool for estimating proportions of European and North American salmon at West Greenland. *Mimeograph of ICES committee meeting*, 1977/M:10.

Payne, R. H., Child, A. R. & Forrest, A. (1971). Geographic variation in the Atlantic salmon. *Nature* **231**, 250–252.

Powell, J. R. (1975). Protein variation in natural populations of animals. *Evolutionary Biology* **8**, 79–119.

Selander, R. K. (1976). Genic variation in natural populations. In *Molecular Evolution* (ed. F. J. Ayala), pp. 21–45. Sunderland, Mass.: Sinauer Associates.

Shaw, C. R. & Prasad, R. (1970). Starch gel electrophoresis of enzymes – a compilation of recipes. *Biochemical Genetics* **4**, 297–320.

Stoneking, M., May, B. & Wright, J. E. (1979). Genetic variation, inheritance and quaternary structure of malic enzyme in brook trout, (*Salvelinus fontinalis*). *Biochemical Genetics* **17**, 599–619.

Utter, F. M. & Hodgins, H. O. (1972). Biochemical genetic variation at six loci in four stocks of rainbow trout. *Transactions of the American Fisheries Society* **101**, 494–502.

Utter, F. M., Hodgins, H. O. & Allendorf, F. W. (1974). Biochemical genetic studies of fishes: Potentialities and limitations. In *Biochemical and Biophysical Perspectives in Marine Biology* (ed. D. C. Mallins and J. R. Sargent), pp. 213–238. New York: Academic Press.

Utter, F. M., Allendorf, F. W. & May, B. (1979). Genetic basis of creatine kinase isozymes in skeletal muscle of salmonid fishes. *Biochemical Genetics* **17**, 1079–1091.

Uyeno, T. & Smith, G. R. (1972). Tetraploid origin of the karyotype of catostomid fishes. *Science* **175**, 644–646.

Ward, R. D. (1977). Relationship between enzyme heterozygosity and quaternary structure. *Biochemical Genetics* **15**, 123–135.

Ward, R. D. & Beardmore, J. A. (1977). Protein variation in the plaice, *Pleuronectes platessa* L. *Genetical Research* **30**, 45–62.

WARD, R. D. & GALLEGUILLOS, R. A. (1978). Protein variation in the plaice, dab and flounder, and their genetic relationships. In *Marine Organisms: Genetics, Ecology and Evolution* (ed. B. Battaglia and J. A. Beardmore), pp. 71–93. New York and London: Plenum.

WENT, A. E. J. (1964). A review of salmon investigations in Ireland up to 1963. *Scientific Proceedings of the Royal Dublin Society, Series A*, I. No. 15.

WILKINS, N. P. (1971). Biochemical and serological studies on Atlantic salmon *Salmo salar* L. *Rapports et Procès-verbaux, International Council for the Exploration of the Sea* **161**, 91–95.

WILKINS, N. P. (1972*a*). Biochemical genetics of the Atlantic salmon *Salmo salar L.* I. A review of recent studies. *Journal of Fish Biology* **4**, 487–504.

WILKINS, N. P. (1972*b*). Biochemical genetics of the Atlantic salmon *Salmo salar* L. II. The significance of recent studies and their application in population identification. *Journal of Fish Biology* **4**, 505–517.

WRIGHT, J. E., HECKMAN, J. R. & ATHERTON, L. M. (1975). Genetic and developmental analyses of LDH isozymes in trout. In *Isozymes*, vol. III: *Developmental Biology* (ed. C. L. Markert), pp. 375–401. New York: Academic Press.