GENETIC VARIABILITY AND DIVERGENCE IN GRAYLING, *THYMALLUS ARCTICUS*[1]

J. C. LYNCH AND E. R. VYSE

Department of Biology, Montana State University, Bozeman, Montana 59717

Manuscript received February 8, 1978
Revised copy received December 20, 1978

ABSTRACT

In North America there are two disjunct forms of grayling, Montana and arctic, which have been separated for approximately 75,000 to 100,000 years. Electrophoretic analysis of thirty-six protein loci in these forms has revealed: (1) levels of gene duplication comparable to other salmonids, (2) a level of heterozygosity similar to other salmonids, (3) a fast and a slow evolving set of proteins, and (4) no obvious relationship between genetic variability and enzyme function. The genetic divergence between these populations may warrant subspecific designations for these two forms.

NORTH American grayling were once common in the headwaters of the Missouri River, Montana, in northern Michigan and in the Arctic. These disjunct grayling populations were isolated by the last Wisconsin glaciation 75,000 to 100,000 years ago. Grayling were originally assigned to three species: *Thymallus signifer* found over large areas of arctic Canada and Alaska (RICHARDSON 1823), *T. tricolor* the Michigan form (COPE 1865) and *T. montanus*, the Montana form (MILNER 1873). That classification was principally based on geographical isolation and morphological characteristics such as size and shape of dorsal fin, maxillary length and color variation. Recently, WALTERS (1955) showed that the Canadian-Alaskan grayling were conspecific with two Asiatic forms (*T. arcticus pallasi* and *T. arcticus gruberi nation mertensi*) and designated this taxon as *T. arcticus signifer*. WALTERS (1955) further recognized the Montana-Michigan form as another subspecies *tricolor*. The validity of the subspecific distinctions in the North American forms has not been established (SCOTT and CROSSMAN 1973; NORDEN 1961), and at the present time no subspecies are recognized (MCPHAIL and LINDSEY 1970). MCCART and PEPPER (1971) found lateral line scale count differences between grayling populations on the northern and southern slopes of the Brooks Range in Alaska and suggested that subdivisions of *T. arcticus* should be investigated using additional criteria.

A genetic comparison of the three American forms of *T. arcticus* is impossible because the Michigan grayling has been extinct since 1936 (SCOTT and CROSSMAN 1973), and many populations of Montana grayling may have been altered

[1] This study represents part of a thesis submitted by the senior author to the Biology Department, Montana State University in partial fulfillment of the requirements for a M.S. degree.

genetically as a result of interbreeding with arctic grayling transplants. The Montana form as described by HENSHALL (1906) dwelt almost exclusively in rivers and streams; hence, it was an adfluvial form (stream dwelling, stream spawning) and only secondarily a lacustrine form (lake dwelling, stream spawning). Analysis of pure adfluvial populations of the Montana form is probably impossible because the Missouri River headwaters are virtually depleted of grayling excepting the Bighole River, which has been stocked with arctic grayling. On the other hand, the lacustrine form is widespread through alpine lakes in Montana and Wyoming. Many of these are pure populations of the Montana form and can be used to determine if specific or subspecific status should be assigned to the arctic and Montana forms.

We report the results of an electrophoretic survey of proteins from grayling populations in the Canadian Arctic and Yellowstone National Park.

MATERIALS AND METHODS

Sampling: Discrete populations of the Arctic form and the Montana form of *T. arcticus* were chosen for analysis. The populations representing the Arctic form were taken from the Donnelly River, N.W.T., and Fuse Lake, Montana. The Donnelly River population was sampled at Chick Lake (65° 52'N, 128°07'W) near the middle of the Mackenzie River drainage. Grayling are native to its water (MCPHAIL and LINDSEY 1970). Fuse Lake (Granite County, Montana), formerly devoid of fish, was stocked in 1952 with 25,000 grayling from the Fond du Lac River inlet of Black Lake, Saskatchewan (Saskatchewan Department of Fish and Game records).

The Fond du Lac River flows out of Black Lake into Lake Athabasca and is thus a tributary of the Mackenzie River. However, the source of Fuse Lake eggs is separated from the Donnelly River sampling site by at least 1200 miles of rivers and lakes in the Mackenzie River drainage system.

Montana grayling samples were obtained from Grebe and Wolf Lakes in Yellowstone National Park. Grebe Lake was stocked with 10^6 grayling (probably sac fry) in 1921. These were the progeny of Madison River fish grown in the state Anaconda hatchery, and/or Georgetown Lake. Grebe and Wolf Lakes are connected by 500 meters of the Gibbon River flowing from Grebe Lake to Wolf Lake. Wolf Lake presumably received grayling immigrants from Grebe Lake, but was stocked in the mid-1930's with fish from Grebe Lake (Yellowstone National Park records).

Yellowstone populations were sampled during spawning in late May and June. Grebe Lake was sampled at a small inlet stream (locally called Hatchery Creek) and Wolf Lake at the Gibbon River outlet. These sampling sites were selected with the expectation that behaviorally isolated and genetically distinct outlet and inlet spawning populations of grayling may exist. Distinct outlet and inlet spawning populations of *Salmo clarki* have been reported in Yellowstone Lake (RALEIGH and CHAPMAN 1971).

Fish were collected either by angling or with an electric backpack shocker. Length and weight measurements were taken immediately. If the sex of each fish could not be determined, they were classified as immature. Tissue samples of liver, muscle, heart and eye were taken immediately and placed on dry ice. Blood samples taken were stored on ice until the cells and serum could be separated by centrifugation. They were then stored with other tissues at —50°.

Tissue samples were ground, horizontal starch gel electrophoresis performed, and gels stained according to the methods published by SELANDER *et al.* (1971) and UTTER, HODGINS and ALLENDORF (1974). The starch used was Electrostarch Lot #*303* (11½%) from Electrostarch Co., Madison, Wisconsin.

Loci were designated 1 to 5 in the order of increasing anodal migration of the coded proteins. The alleles at a locus were numbered according to the relative mobility of their isozymes under

the electrophoretic conditions used. The most abundant allele was designed 1.00 and alleles corresponding to faster or slower migrating bands were assigned values relative to it. Enzymes with no detectable genetic variation were assumed to be coded by the minimum number of loci required to produce the number of isozymes found.

The buffer combinations used in the study are as follows: Buffer System A = Gel Buffer: pH 8.7, 0.076 M tris, 0.005 M citrate; Electrode buffer: pH 8.2, 0.30 M borate, sodium hydroxide. Buffer System B = Gel buffer pH 6.7, 0.008 M tris, 0.003 M citrate; Electrode buffer pH 6.3, 0.223 M tris, 0.086 M citrate. Buffer System C = Gel buffer pH 8.0, 0.0229 M tris, 0.0052 M citrate; Electrode buffer pH 8.0, 0.687 M tris, 0.157 M citrate. Buffer System D = Gel buffer pH 8.0, 0.3 M tris, 0.005 M citrate; Electrode buffer pH 8.3, 0.06 M lithium hydroxide, 0.3 M borate. Buffer System E = Stock solution A pH 8.1, 0.03 M lithium hydroxide, 0.19 M borate; stocks solution B pH 8.4, 0.05 M tris, 0.008 M citrate, Gel buffer 1:9 mixture stock solution A and B; Electrode buffer stock solution A. Buffer System F = Stock solution pH 8.6, 0.9 M tris, 0.5 M borate, 0.02 M EDTA; Gel buffer dilute stock solution 20:1; Electrode buffer dilute stock solution 4:1.

The buffer system used and the proteins surveyed electrophoretically are as follows: Buffer System A, Glucose-6-phosphate dehydrogenase = G6PD (1.1.1.49); Hexose-6-phosphate dehydrogenase = H6PD (1.1.1.47); Phosphoglucomutase = PGM (2.7.5.1); Xanthine dehydrogenase = XDH (1.1.1.25). Buffer System B, Isocitrate dehydrogenase = IDH (1.1.1.42); Malate dehydrogenase = MDH (1.1.1.37); Malic enzyme = ME (1.1.1.40). Buffer System C, Alphaglycerophosphate dehydrogenase = AGPD (1.2.1.12); Glutamate oxaloacetate transaminase = GOT (2.6.1.1). Buffer System D, Alcohol dehydrogenase ADH (1.1.1.1), Lactate dehydrogenase — LDH (1.1.1.27); Superoxide dismutase = SOD (1.15.1.1); Sorbitol dehydrogenase = SDH (1.1.1.14). Buffer System E, Esterase = Est (3.1.1.1), Transferrin = Tfn, Serum proteins = SP. Buffer System F, Hexokinase = Hk (2.7.1.1).

RESULTS

Proteins with monomorphic loci

Enzymes with only a single invariant band on electrophoretic gels included ADH, XDH, SDH, Hk and serum esterase. They were all assumed to be coded by a single locus (Table 1). This is a minimal estimate since an enzyme with only one invariant band might be the product of any number of loci with a common allele (ALLENDORF *et al.* 1977).

Enzymes with multiple isozymes and different tissue predominance, but no apparent genetic variability, included LDH, MDH, GOT, IDH_m, AGPD and ME.

LDH: These isozymes are presumably coded by five loci in salmonids (MARKERT, SHAKLEE and WHITT 1975; WRIGHT, HECKMAN and ATHERTON 1975). LDH isozyme patterns suggesting five loci are also found in *T. arcticus*. As in other salmonids, there appears to be differential tissue expression of these loci with different isozymes predominating in different tissues (Table 1).

MDH: The predominant muscle form of MDH is coded by two loci in salmonids (BAILEY *et al.* 1970). The predominant eye and liver form is also coded by two loci in brown trout (BAILEY *et al.* 1970; ALLENDORF *et al.* 1977), but this may not be true of all salmonids (SYLN'KO 1976; CLAYTON *et al.* 1975). The three-banded phenotype found in *T. arcticus* by MASSARO (1973) and confirmed by us in all tissues except liver suggests that there are at least two loci coding for cytoplasmic forms of MDH. Duplication of these loci would not be detected in the absence of genetic variation. A predominant band in liver tissue suggests

TABLE 1

Tissue distribution of loci, enzyme subunit structure and metabolic designation

Protein	Minimum No. loci	Tissue expression of loci* L.	M.	H.	E.	S.	No. of subunits†	Metabolic designation‡
AGPD	3	1,2,3	1,2,3				2	G,R
Hk	1	1	1,2,3				1	G,R
H6PD	1	1	1	1				G,NR
IDH_s	1	1					2	G,NR
IDH_m	2	1,2					2	G,R
G6PD	3	2,3	2,3	2,3	1,2,3		2	G,R
LDH	5	3,4	1,2,3,4	3,4	3,4,5		4	G,NR
MDH_s	2	1	1,2	1,2	1,2	1,2	2	G,NR
MDH_m	1	1	1	1	1		2	G,NR
ME_m	1	1					1	G,R
PGM	3	1,2,3	1,2				1	G,R
ADH	1	1	1,2				1	G,R
XDH	1	1	1				2	NG,R
SDH	1	1						NG,NR
GOT_s	2	1,2	1	1	2		2	NG,NR
GOT_m	2	1,2	1,2	1,2	1,2		2	NG,NR
Est	1					1	1	NG,VS
SOD	1	1	1				2	NG
Tfn	1					1	1	NG
SP	3					1,2,3		

* Number(s) of the locus/loci expressed in the tissue listed.
† Number of subunits after WARD (1977).
‡ Metabolic designation: G=glucose metabolizing, NG=nonglucose metabolizing (GILLESPIE and KOJIMA 1968); R=regulatory, NR=nonregulatory, VS=variable substrate (JOHNSON 1974).

differential expression of these MDH loci and supports the proposal of at least two loci (Table 1). A fourth weaker-staining MDH band was found close to the origin. This band, believed to be the mitochondrial form of MDH from correlation with zymograms from other salmonids (SYLN'KO 1976; ASPINWALL 1974; BAILEY *et al.* 1970), is probably coded by a separate locus.

GOT: This enzyme exists in mitochondrial and cytoplasmic forms, both of which are dimeric. In salmonids, cytoplasmic GOT is believed to be coded by two loci (SCHMIDTKE and ENGEL 1972; ALLENDORF, UTTER and MAY 1975; ALLENDORF and UTTER 1976). Asymmetrical three-banded phenotypes with opposite staining intensity were found in liver and eye samples of all grayling surveyed. This pattern suggests two loci coding for proteins of different electrophoretic mobility with differential activation in these tissues. The possibility of duplicated loci, one polymorphic and one monomorphic, is unlikely since all individuals display the same phenotype. In muscle tissue, only one GOT isozyme equivalent in electrophoretic mobility to GOT-1 in liver was found. The mitochondrial GOT is represented by two bands with no intermediate band, suggesting that there are two loci with no heterodimer formation occurring between their products. A similar pattern has been reported in brown trout (ALLENDORF *et al.* 1977).

IDH_m: Mitochondrial isocitrate dehydrogenase was represented by three bands in *T. arcticus*, presumably the result of duplicated loci coding for electrophoretically different subunits, as in other salmonids (ALLENDORF, UTTER and MAY 1975).

AGPDH: This enzyme has been reported to be coded by one, two or three loci in salmonids, but ALLENDORF, UTTER and MAY (1975) showed that the pattern found was buffer dependent. Using the buffer system of ALLENDORF, UTTER and MAY (1975), a six-banded pattern was found in *T. arcticus*, suggesting that there are three loci producing randomly associating subunits of a dimeric molecule.

ME: This enzyme has not been well characterized in fish, but two different bands were found in muscle and liver samples of rainbow trout (ALLENDORF, UTTER and MAY 1975). In *T. arcticus*, the supernatant form frequently appears as five bands of variable staining intensity, suggesting a tetrameric molecule coded by two loci. This enzyme was not included in the quantitative calculations because of inconsistent staining. The mitochondrial form appearing as a single diffuse band was assigned one locus.

Proteins with one or more polymorphic loci.

SOD: This is a dimeric enzyme, which appears to be coded by one locus with frequent polymorphism in salmonids (UTTER 1971; UTTER and HODGINS 1972; MAY, UTTER and ALLENDORF 1975). The SOD isozyme pattern in *T. arcticus* is similar to the pattern found in rainbow trout in which one band is produced by homozygotes and three bands by heterozygotes (UTTER 1971). SOD was polymorphic in three of the four populations studied (Table 2).

PGM: This enzyme has been postulated to be coded by three loci in rainbow trout (ROBERTS, WOHNUS and OHNO 1969). Polymorphisms of PGM have been found in this species (ROBERTS, WOHNUS and OHNO 1969; UTTER and HODGINS 1972) and in various species of salmon (MAY, UTTER and ALLENDORF 1975). Grayling liver tissue extracts produced three zones of activity indicative of three loci (PGM-1, PGM-2, PGM-3) coding for monomeric proteins. Only PGM-1 and PGM-2 were expressed in muscle tissue. PGM-1 and PGM-3 were monomorphic in all four populations, but PGM-2 was polymorphic in the Donnelly River population (Table 2). Individuals homozygous for either allele were represented by a single band, while heterozygous individuals exhibited two bands of activity.

IDH_s: The supernatant form of IDH behaves electrophoretically as a dimeric molecule (DARNALL and KLOTZ 1972). Allelic variation of IDH_s isozymes have been described in a variety of salmonid species (ALLENDORF, UTTER and MAY 1975; MAY, UTTER and ALLENDORF 1975; ROPERS, ENGEL and WOLF 1973; ENGEL, SCHMIDTKE and WOLF 1975), which confirms the dimeric structure in salmonid fish. Two disomic loci have been shown to code for IDH_s in rainbow trout (ALLENDORF and UTTER 1973; ENGEL, SCHMIDTKE and WOLF 1975; REINITZ 1977). In contrast, a single disomic locus encoding IDH_s has been reported for chum salmon (*Oncorhynchus keta*) (MAY, UTTER and ALLENDORF 1975). The liver IDH_s patterns of *T. arcticus* were polymorphic in three of the populations surveyed (Table 2). The expression of only three phenotypes in any

TABLE 2

Allele frequency and heterozygosity of polymorphic loci in Thymallus arcticus, *populations*

Locus	Alleles	Grebe	Wolf	Donnelly	Fuse
IDH_s-1	*1.00*	*0.980*	*0.983*	*0.970*	*1.000*
	1.10	*0.019*	*0.017*	*0.030*	—
	1.20	—	—	—	—
*(h)**		*0.039*	*0.033*	*0.058*	*0.000*
G6PD-3	*1.00*	*0.924*	*0.978*	*1.000*	*1.000*
	1.10	*0.076*	*0.022*	—	—
(h)		*0.140*	*0.043*	*0.000*	*0.000*
(P)†		*0.9*	*0.9*		
H6PD-1	*1.00*	*1.000*	*1.000*	—	—
	1.25	—	—	*1.000*	*1.000*
(h)		*0.000*	*0.000*	*0.000*	*0.000*
PGM-2	*1.00*	*1.000*	*1.000*	*0.887*	*1.000*
	1.10	—	—	*0.113*	—
(h)		*0.000*	*0.000*	*0.202*	*0.000*
(P)				*0.9*	
SOD-1	*1.00*	*0.647*	*0.562*	*0.646*	*1.000*
	0.50	*0.353*	*0.437*	*0.354*	—
(h)		*0.457*	*0.493*	*0.459*	*0.000*
(P)		*0.9*	*0.9*	*0.2*	
Tfn-1	*1.00*	*0.857*	*0.750*	*0.814*	*1.000*
	1.10	*0.197*	*0.166*	*0.186*	—
	1.20	*0.018*	*0.083*	—	—
(h)		*0.025*	*0.403*	*0.302*	*0.000*
(P)		*0.9*		*0.5*	
SP-1	*1.00*	*0.595*	*0.554*	*0.646*	—
	1.10	*0.405*	*0.445*	*0.354*	*1.000*
(h)		*0.482*	*0.505*	*0.457*	*0.000*
(P)		*0.6*	*0.9*	*0.7*	
SP-2	*1.00*	*1.000*	*1.000*	—	—
	1.10	—	—	*0.300*	*1.000*
	1.20	—	—	*0.700*	—
(h)		*0.000*	*0.000*	*0.420*	*0.000*
(P)				*0.6*	

* *h*=heterozygosity for each locus, calculated according to Nei (1975).

† *P*=probability values for comparison to expected Hardy-Weinberg equilibrium. *P* values for *IDH_s-1* and *Tfn* in Wolf Lake were not calculated because of the small class sizes of the rare alleles.

one population is indicative of a single disomic locus with a common and a variant allele. This postulate for *T. arcticus* is similar to that proposed for chum salmon, but is in contrast to the two disomic loci proposed for rainbow trout.

G6PD and H6PD: G6PD exists as two catalytically active forms, dimer and tetramer (Bonsignore *et al.* 1971), being mostly tetrameric in some organisms (Yamauchi and Goldberg 1973), but dimeric in others (Shaw and Koen 1968). In contrast, H6PD exhibits only a dimeric structure as evidenced by zymograms of allelic variants (Stegeman and Goldberg 1972).

STEGEMAN and GOLDBERG (1972) demonstrated that in Salvelinus, G6PD appears to be tetrameric (exhibiting five bands) with H6PD existing as a dimeric molecule. The existence of two co-dominant gene loci with different alleles has been postulated to code for G6PD in trout (OHNO *et al.* 1966; YAMAUCHI and GOLDBERG 1973; STEGEMAN and GOLDBERG 1972).

In liver tissue of *T. arcticus*, four bands of enzyme activity are found in the presence of glucose-6-phosphate, but only one of these bands stains in the presence of galactose-6-phosphate. Of the three G6PD bands, the middle one is most intense and appears to be a heterodimer derived from the subunits more anodal and cathodal to it. We assume that there are two loci with alleles that produce electrophoretically different subunits capable of combining randomly to produce an active G6PD dimer. This is supported by the discovery of a variant in the Yellowstone Park grayling populations in which presumed heterozygotes produced three bands in the region of the most anodal band and two bands in the middle band region. In eye tissue, an additional more cathodal G6PD band is produced that does not interact with the other G6PD isozymes (Tables 1 and 2).

The H6PD band, identified in liver samples by staining with the substrate galactose-6-phosphate, lies between the cathodal G6PD and the heterodimer isozymes. This enzyme clearly separates the Arctic and Montana forms of grayling, with the H6PD band in the Arctic populations slightly more anodal than the H6PD band in the Yellowstone Park populations.

Tfn: This monomeric B-globulin exhibits extensive polymorphism in salmonid species (HERSHBERGER 1970; UTTER and HODGINS 1972) reflecting co-dominant inheritance of alleles at a single locus. In grayling, the transferrin bands were identified as the protein bands having an electrophoretic mobility similar in position to those of rainbow trout (UTTER and HODGINS 1972; REICHENBACH-KLINKE 1973). Transferrin was polymorphic in the Donnelly River, Wolf Lake, and Grebe Lake populations with three different alleles being expressed (Table 2).

SP: Teleost species have been shown to possess intraspecies specificity of serum protein patterns (WOODS and ENGLE 1957; TSUYKI and ROBERTS 1966). Electropherograms of serum samples from *T. arcticus* revealed six zones of staining, numbered in order of decreasing anodal migration (Figure 1). Zone 2 was identified as transferrin, which has been dealt with previously. The only other zone adequately resolved was Zone 5. Individuals exhibited from three to five bands in this region. A total of 12 different electrophoretic phenotypes were observed (Figure 2). Further analysis revealed that the zone could be divided into two groups designated A (most anodal) and B (least anodal). Group A, in the Grebe and Wolf Lake populations, was represented by two intensely staining bands whose expression was independent of the B group. These bands were assumed to be the products of two loci, *SP-2* and *SP-3*. Group B proteins were represented by either one or two bands and were assumed to be coded by one locus (*SP-1*) with two alleles. The Yellowstone Park populations were polymorphic at the *SP-1* locus while the Donnelly River population was polymorphic at the *SP-1* and *SP-2* loci (Table 2). The presumptive *SP-2* locus identifies arctic

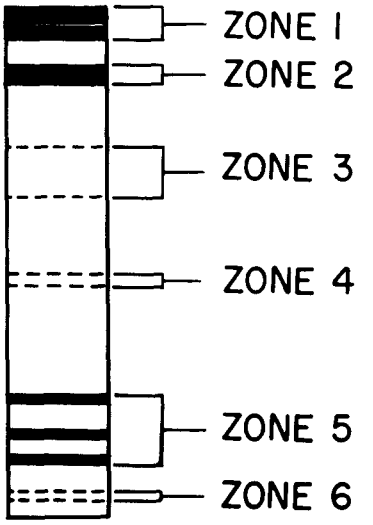

FIGURE 1.—Diagrammatic representation of electrophoretic pattern of serum proteins of *Thymallus arcticus*. Six Zones, numbered in order of decreasing anodal migration, are normally observed. Relative intensities are depicted in the diagram.

and Montana grayling. Arctic grayling have either or both *1.10* and *1.20* alleles, whereas the Yellowstone Park grayling are homozygous for the *1.00* allele.

Quantitative analysis of genetic variability

Allele frequencies and heterozygosity of the eight polymorphic loci for the four populations of *T. arcticus* surveyed are presented in Table 2. Allele frequencies were assigned directly from the gel phenotypes. The genetic basis of the observed polymorphisms were not verified through progeny studies, but the genetic interpretation in simple Mendelian terms is supported by the agreement between

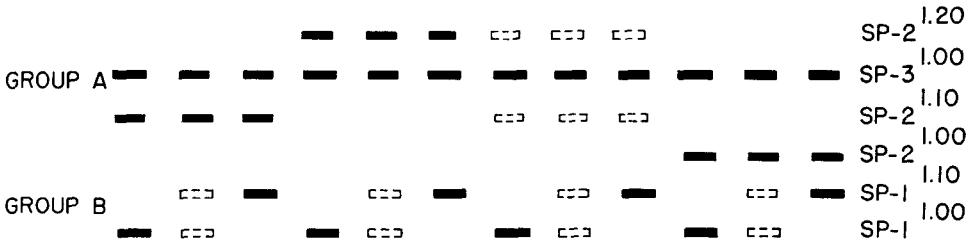

FIGURE 2.—The twelve observed phenotypic patterns of electropherograms of grayling serum proteins in zone 5. Columns 1–9, Donnelly River; Columns 10–12, Yellowstone Park Populations. The genotypes of the individuals are: 1—*SP-1(1.00/1.00)*, *SP-2(1.10/1.10)*; 2—*SP-1(1.00/1.10)*, *SP-2(1.10/1.10)*; 3—*SP-1(1.10/1.10)*, *SP-2(1.10/1.10)*; 4—*SP-1(1.00/1.00)*, *SP-2(1.20/1.20)*; 5—*SP-1(1.00/1.10)*, *SP-2(1.20/1.20)*; 6—*SP-1(1.10/1.10)*, *SP-2(1.20/1.20)*; 7—*SP-1(1.00/1.00)*, *SP-2(1.10/1.20)*; 8—*SP-1(1.00/1.10)*, *SP-2(1.10/1.20)*; 9—*SP-1(1.10/1.10)*, *SP-2(1.10/1.20)*; 10—*SP-1(1.00/1.00)*, *SP-2(1.00/1.00)*; 11—*SP-1(1.00/1.10)*, *SP-2(1.00/1.00)*; 12—*SP-1(1.10/1.10)*, *SP-2(1.00/1.00)*. All individuals are homozygous for the common allele (*1.00/1.00*) at the *SP-3* locus.

TABLE 3

Estimates of genetic variability in Thymallus arcticus

Population	Number of individuals	Number of loci	% Polymorphic loci (P)	Average heterozygosity ($\bar{H}$)
Grebe	102	33*	12.1	0.0268(±0.0161)
		36†	13.8	0.0382(±0.0197)
Wolf	58	33	12.1	0.0303(±0.0195)
		36	13.8	0.0418(±0.0223)
Donnelly	63	33	12.1	0.0318(±0.0177)
		36	16.6	0.0542(±0.0230)
Fuse	20	33	0.0	0.0000
		36	0.0	0.0000

* Excluding *SP-1*, *SP-2* and *SP-3*.
† Including *SP-1*, *SP-2* and *SP-3*.

the observed and expected proportions of genotypes, assuming Hardy-Weinberg equilibrium (Table 2). Percent polymorphic loci (P) and average heterozygosity ($\bar{H}$) (NEI and ROYCHOUDHURY 1974) were calculated separately for the 36 loci surveyed for each population, and for those same loci, excluding the three serum protein loci, which are not usually included in electrophoretic surveys (Table 3).

The $\bar{H}$ value for *T. arcticus* is compared to published data for other salmonids in Table 4. The average $\bar{H}$ for *T. arcticus* is in the range of other salmonids, but lower than the high averages of *Salmo gairdneri* and coastal *S. clarki*.

In Table 5, the unweighted averages of the mean population heterozygosities of loci coding for proteins classified according to enzyme function (GILLESPIE and KOJIMA 1968; JOHNSON 1974), subunit composition (WARD 1977) and evolutionary rate (SARICH 1977) are presented.

TABLE 4

Average heterozygosity of T. arcticus *and other Salmonids*

Species	No. of populations	Range of $\bar{H}$	Average $\bar{H}$ of populations
Oncorhyncus gorbuscha	6	0.034–0.047	0.039
O. keta	5	0.043–0.048	0.045
O. kisutchm	10	0.000–0.025	0.015
O. nerka	10	0.008–0.240	0.018
O. tshawytscha	10	0.024–0.052	0.035
Salmo clarki (Coastal)	6	0.022–0.077	0.063
S. clarki (Interior)	2	0.021–0.025	0.023
S. gairdneri	41	0.020–0.098	0.060
S. salar	2	0.020–0.028	0.024
Thymallus arcticus	4	0.000–0.054	0.034

Salmonid data are taken directly from ALLENDORF and UTTER (1978).

TABLE 5

Function and subunit composition of the protein products of the loci studied in T. arcticus

	G	NG		R	NR
No. of loci	23	9		17	15
Mean $\overline{H}$	0.00559	0.03915		0.00641	0.0021
S.E.	0.0029	0.0659		0.0036	0.0020
	Monomer	Dimer	Tetramer	Fast	Slow
No. of loci	6	20	8	6	30
Mean $\overline{H}$	0.00841	0.0420	0.0	0.166	0.00429
S.E.	0.0076	0.0266	0.0	0.050	0.00231

The genetic distance and genetic similarity (NEI 1975) among the four populations of *T. arcticus* is given in Table 6. These indices were calculated both with serum proteins included and excluded, since this separation allows for comparisons with other electrophoretic surveys in which serum proteins are generally excluded. The genetic distance/similarity indices indicate that the Montana and Arctic grayling interpopulational differences are greater than the differences between the two Montana or the two Arctic grayling populations studied.

DISCUSSION

Tetraploidy has been proposed for salmonids (OHNO, WOLF and ATKIN 1968) and gene duplication is extensive, although no loci have been shown to have tetrasomic segregation. ALLENDORF, UTTER and MAY (1975) and ALLENDORF *et al.* (1977) have reviewed the limitations of electrophoretic information to detect gene duplications and tetrasomic modes of inheritance. The criterion of a minimum of one locus per nonvariant band (ALLENDORF, UTTER and MAY (1975)

TABLE 6

Indices of similarity (below diagonal) and genetic distance (above diagonal) for four populations of Thymallus arcticus (NEI *1975*)

	Total (without serum)			
	Grebe	Wolf	Donnelly	Fuse
Grebe	0.0000	0.0007	0.0335	0.0368
Wolf	0.9993	0.0000	0.0337	0.0401
Donnelly	0.9670	0.9668	0.0000	0.0054
Fuse	0.9638	0.9606	0.9946	0.0000
	Total (with serum)			
	Grebe	Wolf	Donnelly	Fuse
Grebe	0.0000	0.0007	0.0359	0.0406
Wolf	0.9993	0.0000	0.0359	0.0405
Donnelly	0.9647	0.9647	0.0000	0.0183
Fuse	0.9602	0.9603	0.9818	0.0000

and ALLENDORF *et al.* (1977), in addition to the observed tissue predominance, allows the assignment of a minimum number of loci to each protein displaying more than one band on electrophoretic gels (Table 1). Variation in tissue distribution is presumably the result of regulation of duplicated loci as a product of evolution as described for LDH by MARKERT, SHAKLEE and WHITT (1975). The pattern of locus duplication in grayling is similar but not identical to that reported for rainbow and brown trout. These duplicated loci indicate that grayling should be included in those salmonids that have undergone tetraploidy during their evolutionary history. Duplicated loci that appear to be differentially regulated in *T. arcticus* include LDH, MDH, GOT, PGM and G6PD (Tables 1 and 2). Breeding data on the identified polymorphisms and more comparative electrophoretic studies would be helpful in identifying the evolutionary relationships of grayling to other salmonids.

The decline of grayling in Montana and Michigan has been attributed to their stringent habitat requirements and inability to adapt to disturbed environments (VINCENT 1962). VINCENT suggested that a lack of genetic diversity might be responsible for this ecologically narrow phenotype. When the $\overline{H}$ estimates for *T. arcticus* are compared with values for other salmonids (Table 4), *T. arcticus* is similar to many salmonid species, but is lower than the average $\overline{H}$ value of forty rainbow trout populations. Rainbow trout are considered to be ecologically plastic, occupying a wide range of habitats, while pacific salmon are considered to have a narrow habitat range (ALLENDORF and UTTER 1978). Thus, our results for *T. arcticus* are consistent with the observations of the above authors.

The lack of variability in the Fuse Lake population may be the result of many factors inclduing a low variability in the source population, a genetic "bottleneck" during egg collections from the source population, or a selection of genotypes after transplanting. The Grebe and Wolf Lake populations, although also established by stocking, have an $\overline{H}$ value that is not significantly different from that of the Donnelly River population in the main range of the species, suggesting that a reduction in genetic variability did not occur as a result of transplanting.

Extensive genetic variability has been found in invertebrates, small mammals, and many fish species, (POWELL 1975; SELANDER 1976), while recent studies in large mammals, with the exception of white-tailed deer, has found them to be relatively genetically depauperate (CAMERON and VYSE 1978). This genetic variability has been difficult to reconcile with evolutionary processes because of the genetic load required if the observed polymorphisms are maintained by natural selection. KIMURA (1968) and KING and JUKES (1969) have suggested that the majority of the observed variability is selectively neutral. In many species the variability was considered not to be distributed randomly amongst the loci surveyed utilizing electrophoretic techniques (POWELL 1975; SELANDER 1976).

Interlocus variability has been attributed to: (1) enzyme function in terms of substrate variability and function (GILLESPIE and KOJIMA 1968; GILLESPIE and LANGLEY 1974), (2) regulatory role (JOHNSON 1974), (3) primary structure

(ZOUROS 1975) and (4) quarternary structure (WARD 1977). When *T. arcticus* enzymes are classified in these categories (Table 5), no significant differences are found between glucose and nonglucose metabolizing enzymes. This result is similar to those reported by NAIR, BRNCIC and KOJIMA (1971) and FRYDENBERG and SIMONSEN (1973), but is in contrast to the result of other (KOJIMA, GILLESPIE and TOBARI 1970; AYALA and POWELL 1972; COHEN *et al.* 1973). The regulatory and nonregulatory classes of enzymes (JOHNSON 1974) also show no difference.

A comparison of heterozygosity levels of enzymes with presumed primary structure differences (ZOUROS 1975) or the variable substrate class of enzymes (JOHNSON 1974) is not included because only one esterase locus and no phosphatase or peptidase loci were studied.

Enzymes with monomeric subunit composition appear to be more variable than dimeric enzymes, but this difference is not significant ($P = 0.8$ to 0.9).

SARICH (1977) analyzed data from any organisms and found a slowly evolving set and a rapidly evolving set of proteins. The rapidly evolving set consists of secreted serum proteins and enzymes, while the slowly evolving set consists of intracellular proteins. In *T. arcticus*, there is a significant difference between the $\overline{H}$ values of secreted proteins and intracellular proteins. The differences in amino acid substitution may be attributed to the random walk of neutral alleles, as SARICH (1977) suggests. A more recent paper by FUERST, CHAKRABOTY and NEI (1977) has shown that all interlocus variability except some of the results in Drosophila can be accounted for on a theoretical basis by the stochastic mutation-drift hypothesis. Therefore, with the possible exception of some Drosophila loci, the interlocus variability observed may not have any biological significance.

The common usage of electrophoretic surveys for population analysis without progeny testing has resulted in the development of indices of genetic distance to aid in determining evolutionary relationships. These indices provide a standard index of genetic divergence between populations of the same genus, species or subspecies (NEI 1975), but by no means replaces the data obtained from intra- or interspecific crosses (LEWONTIN 1974).

The genetic distance measurements between the two Montana populations is probably not meaningful because of the common transplant origin and the possible migration between these two populations. There is no evidence for distinct inlet and outlet spawning populations from this analysis. The genetic distance estimates between the two arctic grayling populations may also have little meaning because the lack of variability in the Fuse Lake population could be the result of transplanting. The genetic distance estimate between the Donnelly River and the Yellowstone National Park populations probably reflects the actual genetic distance accrued since the isolation of Montana and arctic grayling. Even though the Yellowstone National Park populations are of transplant origin, the variability in those populations is not significantly reduced from that of the Donnelly River population. The genetic similarity estimate of 0.9647 is above the (0.77–0.99) range of subspecific distances measured in other taxons (AVISE 1976).

There are two protein loci (*SP-1* and *H6PD*) that have undergone complete allelic substitution and can be utilized to separate Montana and arctic grayling. Diagnostic alleles have been utilized as one criterion for the separation of subspecies in Drosophila (AYALA 1973; AYALA *et al.* 1974; AYALA and DOBZHANSKY 1974) and Dipodomys (PATTON, MACARTHUR and YANG 1976) suggesting that a subspecific designation of Montana and arctic grayling may be warranted. Since the taxon of arctic grayling at both the species and subspecific level is still in question, further analysis of genetic and meristic characters in other populations should help to clarify the taxonomic status.

We thank D. G. CAMERON for reviewing the manuscript and the National Park Research Center for financial support.

LITERATURE CITED

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

ALLENDORF, F. W. and F. M UTTER, 1973 Gene duplication within the family Salmonidae: Disomic inheritance of two loci reported to be tetrasomic in rainbow trout. Genetics **74:** 647–654. ——, 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. ——, 1978 Population Genetics of Fish. In: *Fish Physiology*. Edited by W. S. HOAR and D. J. RANDALL. Academic Press, New York. In press.

ALLENDORF, F. W., F. M. UTTER and B. P. MAY, 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. pp. 415–432. In: *Isozymes IV: Genetics and Evolution*. Edited by C. L. MARKERT. Academic Press, New York.

ASPINWALL, N., 1974 Genetic analysis of duplicate malate dehydrogenase loci in the pink salmon, *Oncorhynchus gorbuscha*. Genetics **76:** 65–72.

AVISE, J. C., 1976 Genetic Differentiation During Speciation. pp. 106–122. In: *Molecular Evolution*. Edited by F. J. AYALA. Sinauer Associates, Inc., Sunderland, Massachusetts.

AYALA, F. J., 1973 Two New Subspecies of the *Drosophila willistoni* Group. Pan-Pac. Entomol. **49:** 273–279.

AYALA, F. J. and T. DOBZANHSKY, 1974 A New Subspecies of *Drosophila pseudoobscura*. Pan-Pac. Entomol. **50:** 211–219.

AYALA, F. J. and J. R. POWELL, 1972 Enzyme variability in the *Drosophila willistoni* group. VI. Levels of polymorphism and the physiological function of enzymes. Biochem. Genet. **7:** 334–345.

AYALA, F. J., M. L. TRACEY, D. HEDGECOCK and R. C. RICHMOND, 1974 Genetic differentiation during the speciation process in *Drosophila*. Evolution **28:** 576–592.

BAILEY, G. S., A. C. WILSON, J. E. HALVER and C. L. JOHNSON, 1970 Multiple forms of supernatant malate dehydrogenase in salmonid fishes. J. Biol. Chem. **245:** 5927–5940.

BONSIGNORE, A., R. CANCEDDA, A. MICOLINI, G. DAMIANI and A. DEFLORA, 1971 Metabolism of human erythrocyte glucose-6-phosphate dehydrogenase. VI. Interconversion of multiple molecular forms. Arch. Biochem. and Biophys. **147:** 493–501.

CAMERON, D. G. and E. R. VYSE, 1978 Heterozygosity in Yellowstone Park Elk, *Cervus canadensis*. Biochem. Genet. **16:** 651–657.

CLAYTON, J. W., D. N. TRETIAK, B. N. BILLECK and P. IHSSEN, 1975 Genetics of multiple supernatant and mitochondrial malate dehydrogenase isozymes in rainbow trout (*Salmo gairdneri*). pp. 415–432. In: *Isozymes IV: Genetics and Evolution.* Edited by C. L. MARKERT. Academic Press, New York.

COHEN, P. T. W., G. S. OMENN, A. G. MOTULSKY, S. CHEN and E. R. GIBLETT, 1973 Restricted variation in glycolytic enzymes of human brain and erythrocytes. Nat. New. Biol. **241:** 228–233.

COPE, E. D., 1865 Partial catalogue of the cold blooded vertebrata of Michigan, Part II. Proc. Acad. Natur. Sci. Philadelphia. **17:** 78–88.

DARNALL, D. W. and I. M. KLOTZ, 1972 Protein subunits: A table (revised edition). Arch. Biochem. Biophys. **149:** 1–14.

ENGEL, W., J. SCHMIDTKE and U. WOLF, 1975 Diploid-tetraploid relationships in teleostean fishes. pp. 449–462. In: *Isozymes IV: Genetics and Evolution.* Edited by C. L. MARKERT. Academic Press, New York.

FUERST, P. A., R. CHAKRABOTY and M. NEI, 1977 Statistical studies on protein polymorphism in natural populations. I. Distribution of single locus heterozygosity. Genetics **86:** 455–483.

FRYDENBERG, O. and V. SIMONSEN, 1973 Genetics of *Zoarces* populations. V. Amount of protein polymorphism and degree of genic heterozygosity. Hereditas **75:** 221–232.

GILLESPIE, J. H. and K. KOJIMA, 1968 The degree of polymorphisms in enzymes involved in energy compared to that in non-specific enzymes in two *Drosophila ananassae* populations. Proc. Natl. Acad. Sci. U.S. **61:** 582–585.

GILLESPIE, J. H. and C. H. LANGLEY, 1974 A general model to account for enzyme variation in natural populations. Genetics **76:** 837–884.

HENSHALL, J. A., 1906 A list of the fishes of Montana. Bull. Univ. of Montana: No. **34(11):** 12 pp.

HERSHBERGER, W. K., 1970 Some physiochemical properties of transferrins in brook trout. Trans. Am. Fish. Soc. **99:** 207–218.

JOHNSON, G. B., 1974 Enzyme Polymorphism and Metabolism. Science **184:** 28–37.

KIMURA, M., 1968 Evolutionary rate at the molecular level. Nature **217:** 624–626.

KING, J. L. and T. H. JUKES, 1969 Non-Darwinian evolution: random fixation for selectively neutral alleles. Science **164:** 788–798.

KOJIMA, K., J. GILLISPIE and Y. N. TOBARI, 1970 A profile of *Drosophila* species' enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochem. Genet. **4:** 627–637.

LEWONTIN, R. C., 1974 *The Genetic Basis of Evolutionary Change.* Columbia University Press, New York.

MARKERT, C. L., J. B. SHAKLEE and G. S. WHITT, 1975 Evolution of a gene. Science **189:** 102–114.

MASSARO, E. J., 1973 Tissue distribution and properties of the lactate and malate dehydrogenase isozymes of the grayling *Thymallus arcticus* (Pallas). J. Exp. Zool. **186:** 151–158.

MAY, B. M., E. M. UTTER and F. W. ALLENDORF, 1975 Biochemical genetic variation in pink and chum salmon: Inheritance of intraspecies variation and apparent absence of interspecies introgression following massive hybridization of hatchery stocks. J. Heredity **66:** 227–232.

MCCART, P. and V. A. PEPPER, 1971 Geographic variation in the lateral line scale counts of the Arctic grayling, *Thymallus arcticus.* J. Fish. Res. Bd. Can. **28:** 749–754.

MCPHAIL, J. D. and C. C. LINDSEY, 1970 Freshwater fishes of northwest Canada and Alaska. Fish. Res. Bd. of Can. Bull No. 173.

MILNER, J. W., 1873 Notes on the graylings of North America. pp. 730–742. In: *Freshwater Fisheries of the United States.* U.S. Govt. Printing Office, Washington, D. C.

NAIR, P. S., D. BRNCIC and K. KOJIMA, 1911 II. Isozyme variations and evolutionary relationships in the mesophragmatica species group of *Drosophila.* pp. 17–28. In: *Studies in Genetics VI.* Univ. Texas Publ. No. 71030.

NEI, M., 1975 *Molecular Population Genetics and Evolution.* American Elsevier, New York.

NEI, M. and A. K. ROYCHOUDHURY, 1974 Sampling variances of heterozygosity and genetic distance. Genetics **76:** 379–390.

NORDEN, D. R., 1961 Comparative osteology of representative salmonid fishes with particular reference to the grayling (*Thymallus arcticus*) and its phylogeny. J. Fish. Res. Bd. Can. **18:** 679–791.

OHNO, S., H. W. PAYNE, M. MORRISON and E. BEUTLER, 1966 Hexose-6-phosphate dehydrogenase found in human liver. Science **153:** 1015.

OHNO, S., U. WOLF and W. B. ATKIN, 1968 Evolution from fish to mammals by gene duplication. Hereditas **59:** 169–187.

PATTON, J. L., H. MACARTHUR and SUH Y. YANG, 1976 Systematic relationship of the four-toed populations of *Dipodomys heermani.* J. Mammalogy **57:** 159–163.

POWELL, J., 1975 Protein variation in natural populations of animals. Evolutionary Biology **8:** 79–119.

RALEIGH, R. F. and D. W. CHAPMAN, 1971 Genetic control in lakeward migration of cutthroat trout fry. Trans. Amer. Fish. Soc. **100:** 33–40.

REINITZ, G. L., 1977 Inheritance of muscle and liver types of supernatant NADP—dependent isocitrate dehydrogenase in rainbow trout (*Salmo gairdneri*). Biochem. Genet. **15:** 445–454.

RICHARDSON, J. R., 1823 Notice of the fishes. pp. 705–728. In: *Narrative of a Journey to the Shores of the Polar Sea in the years 1819, 1820, 1821, 1822.* Edited by FRANKLIN, John Murray, London.

RIECHENBACH-KLINKE, H. H., 1973 Investigations on the serum polymorphism of trout and carp. pp. 315–318. In: *Genetics and Mutagenesis of Fish.* Edited by J. H. SCHROEDER. Springer-Verlag, Berlin.

ROBERTS, F. L., J. F. WOHNUS and S. OHNO, 1969 Phosphoglucomutase polymorphism in the rainbow trout, *Salmo gairdneri.* Experentia **25:** 1109–1110.

ROPERS, H. H., W. ENGEL and U. WOLF, 1973 Inheritance of the s-form of NADP-dependent isocitrate dehydrogenase polymorphism in rainbow trout. pp. 319–327. In: *Genetics and Mutagenesis of Fish.* Edited by J. H. SCHROEDER. Springer-Verlag, Berlin.

SARICH, V. M., 1977 Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature **265:** 24–28.

SCHMIDTKE, J. and W. ENGEL, 1972 Duplication of the gene loci coding for the supernatant aspartate aminotransferase by polyploidization in the fish family Cyprinidae. Experientia **28:** 976–978.

SCOTT, W. B. and E. J. CROSSMAN, 1973 Freshwater fishes of Canada. Fish. Res. Bd. Can. Bull. **184:** 300–305.

SELANDER, R. K., 1976 Genic variation in natural populations. pp. 21–45. In: *Molecular Evolution.* Edited by F. J. AYALA. Sinauer Assoc., Sunderland, Massachusetts.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON and J. B. GENTRY, 1971 Biochemical polymorphism and systematics in the genus *Peromyscus.* I. Variation in the old field mouse (*Peromyscus polionotus*). pp. 49–90. *Studies of Genetics VI.* Univ. Texas Publ. No. 7103.

SHAW, C. R. and A. L. KOEN, 1968 Glucose-6-phosphate dehydrogenase and hexose-6-phosphate dehydrogenase of mammalian tissues. Ann. New York Acad. Sci. **151**: 149–156.

STEGEMAN, J. J. and E. GOLDBERG, 1972 Inheritance of hexose-6-phosphate dehydrogenase polymorphism in brook trout, Biochem. Genet. **7**: 279–288.

SYLN'KO, V. I., 1976 Electrophoretic analysis of isoenzymes of malate dehydrogenase from fish of the Salmonidae family. Doklady Akad. Nauk. SSSR, **226**: 448–451.

TSUYUKI, H. and E. ROBERTS, 1966 Interspecies relationships within the genus *Oncorhynchus* based on biochemical systematics. J. Fish. Res. Bd. Can. **23**: 101–107.

UTTER, F. M., 1971 Tetrazloium oxidase phenotypes of rainbow trout (*Salmo gairdneri*) and Pacific salmon (*Oncorhynchus* spp.). Comp. Biochem. Physio. **39B**: 891–895.

UTTER, F. M. and H. O. HODGINS, 1972 Biochemical genetic variation at six loci in four stocks of rainbow trout. Tran. Amer. Fish. Soc. **101**: 494–502.

UTTER, F. M., H. O. HODGINS and F. W. ALLENDORF, 1974 Biochemical genetic studies of fishes: Potentialities and Limitations. pp. 213–238. In: *Biochemical and Biosphysical Perspectives in Marine Biology*. Edited by D. C. MALINS and J. R. SARGENT. Academic Press, New York.

VINCENT, R. E., 1962 Biogeographical and ecological factors contributing to the decline of arctic grayling, *Thymallus arcticus* Pallas, in Michigan and Montana. Ph.D. Thesis, Univ. of Michigan.

WALTERS, V., 1955 Fishes of western Arctic American and eastern Arctic Siberia: Taxonomy and zoogeography. Bull. Amer. Mus. Nat. Hist. **106**: 255–368.

WARD, R. D., 1977 Relationship between enzyme heterozygosity and quaternary structure. Biochem. Genet. **15**: 123–135.

WOODS, K. and R. ENGLE, 1957 Phylogenesis of plasma proteins and plasma cells. I. Starch gel zone electrophoresis of sera from marine invertebrates and fishes. Biol. Bull. **113**: 362–363.

WRIGHT, J. E., J. R. HECKMAN and L. M. ATHERTON, 1975 Genetic and developmental analysis of LDH isozymes in trout. pp. 375–401. In: *Isozymes III: Developmental Biology*. Edited by C. L. MARKERT. Academic Press, New York.

YAMAUCHI, T. and E. GOLDBERG, 1973 Glucose-6-phosphate dehydrogenase from brook, lake and splake trout: an isozymic and immunological study. Biochem. Genet. **10**: 121–134.

ZOUROS, E., 1975 Electrophoretic variation in allozymes related to function or structure? Nature **254**: 446–448.

Corresponding editor: M. NEI