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Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data

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Previously reported results on natural populations of brown trout (*Salmo trutta* L.) urged further studies using additional electrophoretically detectable loci. Samples of brain, eye, heart, kidney, liver, and muscle from approximately 50 specimens from each of two populations and a large number of muscle samples from several additional populations were examined. Electrophoretic and staining methods for the 37 enzymes studied are given in detail. Of a total of 69 loci detected, 54 loci were considered usable in population genetics screenings. The expression of the loci coding for these enzymes is described and interpreted. The results presented will serve as a basis for a more detailed examination of genetic variation in brown trout populations.

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An increasing interest has been focused on the study of fish populations during the last few years in the fields of evolution and population genetics. There are several reasons for this. In addition to the strong need for genetic data as an aid in the management of fish populations, fish are a uniquely useful tool in the study of basic population genetics (review by ALLENDORF and UTTER, 1977). The fact that fish are restricted to water often permits a very precise definition of the population studied. In many freshwater species, the presence (or absence) of passable streams connecting the inhabited lakes also present excellent examples of natural populations exhibiting the exact properties of theoretical "island" or "river bank" population genetics models. In many species, large numbers of mature eggs and sperm are easily removed and handled, permitting advanced and powerful breeding schemes or test crosses potentially producing large numbers of offspring. Furthermore, within many fish species the existence of a wide variety of life history patterns provides the evolutionary geneticist with an outstanding source of information (AVISE and SELANDER 1972; WILLIAMS et al. 1973; ASPINWALL 1974; AVISE and SMITH 1974).

In an earlier paper (ALLENDORF et al. 1976) we presented the first results of a genetic examination of population structure in Scandinavian brown trout (*Salmo trutta* L.). These results indicated a large amount of genetic differentiation within a small geographic area. There was also strong evidence of two genetically distinct populations of brown trout coexisting in a single very small lake. However, these results were based only on fish from four different lakes examined at ten electrophoretically detected loci. These preliminary results urged further studies, including an extended and detailed analysis of both additional loci and additional lakes. As the first step, we have concentrated on the search for additional loci.

For a detailed genetic analysis of population structure a large number of loci must be examined (LEWONTIN 1974; NEI and ROYCHOUDHURY 1972; NEI 1973). The extensive gene duplication found in salmonids, resulting in a large number of electrophoretically detectable loci, make the salmonids extremely well suited for population genetic studies.

On the other hand the existence of duplicated loci often confounds the genetic interpretation of electrophoretic data. We have at present no direct inheritance results aimed to identify the genetic control of

polymorphic loci in the brown trout. One of the authors (F. W. A.), however, has been involved in an ongoing series of experimental matings in other salmonids designed to clarify the inheritance of isozyme loci. We have used these studies as a basis for an extrapolation from those species to the brown trout (ALLENDORF and UTTER 1973, 1976; UTTER et al. 1973; ALLENDORF et al. 1975, 1976). In the present report, we present the results of an extensive screening for electrophoretically detectable loci in different tissues of brown trout.

Materials and methods

Tissue extracts were prepared and horizontal starch gel electrophoresis was accomplished following the methods of UTTER et al. (1974). Four different buffer systems were used:

A. Described by RIDGWAY et al. (1970). *Gel*: 0.03 M Tris—0.005 M citric acid, pH 8.5. *Electrode*: 0.06 M lithium hydroxide—0.3 M boric acid, pH 8.1. Gels were made using 99% gel buffer and 1% electrode buffer.
B. Described by CLAYTON and TRETIAK (1972). *Gel*: 0.002 M citric acid, pH 6.0. *Electrode*: 0.04 M citric acid, pH 6.1. Both buffers are pH adjusted with N-(3-Aminopropyl)-morpholine.
C. Described by SICILIANO and SHAW (1976). *Gel*: 0.009 M Tris—0.003 M citric acid, pH 7,0. *Electrode*: 0.13 M Tris—0.043 M citric acid, pH 7.0.
D. Described by SICILIANO and SHAW (1976). *Gel*: 0.05 M Tris—0.0016 M Versene—0.065 M boric acid, pH 8.0. *Electrode*: 0.5 M Tris—0.016 M Versene—0.65 M boric acid, pH 8.0.

Gels were made using 14 percent starch (Electrostarch Co., Madison, Wisconsin, U.S.A.) in the appropriate gel buffer. A potential of 200 volts was applied across the gel until a dye marker had migrated about 5 cm from the origin, i.e., about three hours. The gels were cooled either on the bottom with water cooled plates (4°C) or on the top with frozen ice packs.

The enzymes stained for, the abbreviations used, and the specific staining procedures are presented in Table 1. The staining techniques were modified from the following publications: AYALA et al. (1970), BREWER and SING (1970), HOPKINSON et al. (1970), JOHNSON et al. (1972), SELANDER et al. (1971), SHAW and PRASAD (1970), SICILIANO and SHAW (1976), and UTTER et al. (1973, 1974).

Seven different stain buffers were used, referred to by Roman numerals in Table 1.

I: 0.2 M Tris—HCl, pH 8.0
II: A solution of 1:4 of stain buffer I and water.
III: The A buffer system gel mixture, i.e., 99% A gel buffer and 1% A electrode buffer.
IV: The C buffer system tray buffer.
V: 0.2 M sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$), pH 5.0.
VI: 0.1 M phosphate ($NaH_2PO_4 \cdot H_2O$), pH 7.0.
VII: The A buffer system tray buffer.

As reported earlier (GOSSELIN-REY et al. 1968;

Table 1. Enzymes stained for, abbreviations, and stain recipies

The stain buffers I—VII are given in the text. If not stated otherwise, 50 ml of stain buffer was used. X in the NBT/PMS column indicates the presence of both NBT and PMS. 10 mg NBT, 5 mg PMS, and 5 mg of the cofactor was used for 50 ml of the stain buffer. 10 mg $MgCl_2$ is also included in all stains using NADP. ADP = adenosine diphosphate; ATP = adenosine triphosphate; EDTA = disodium ethylendiamine tetraacetate; NAD = β-nicotinamide adenine dinucleotide; NADH = β-diphosphopyridine nucleotide, reduced form; NADP = nicotinamide adenine dinucleotide phosphate; NBT = p-nitro blue tetrazolium; PMS = phenazine methosulfate

Enzyme	Abbreviation	NBT/PMS	Cofactor	Stain buffer	Other components
Aspartate aminotransferase	AAT			I	200 mg L-aspartic acid 100 mg α-ketoglutaric acid 1 mg Pyrodixal-5′-phosphate 300 mg Fast Blue BB salt
Adenosine deaminase	ADA	X		II (5 ml)	50 mg Arsenic acid 40 mg Adenosine 5 u Nucleoside phosphorylase 2 u Xanthine oxidase Dropwise on surface
Alcohol dehydrogenase	ADH	X	NAD	III	10 ml 95% ethanol

Table 1. Cont.

Enzyme	Abbreviation	NBT/PMS	Cofactor	Stain buffer	Other components
α-glycerophosphate dehydrogenase	AGP	X	NAD	III	1 g DL-α-glycerophosphate
Adenylate kinase	AK	X	NADP	II	100 mg Glucose 50 mg ADP 250 u HK 100 u G6PDH
Aldolase	ALD	X	NAD	II	250 mg Fructose-1, 6-diphosphate 75 mg Arsenic acid 40 u Glyceraldehyde-phosphate dehydrogenase
Creatine phosphokinase	CPK	X	NADP	III	300 mg Creatine phosphate 40 mg Glucose 30 mg ADP 60 u HK 10 u G6PDH
Diaphorase	DIA			III	1 mg 2,6-dichlorophenolindol-phenol 2.5 mg NADH 10 mg MTT tetrazolium
Esterase	EST			III	5 ml 1% α-naphtyl-acetate in 1:1 of acetone and water 150 mg Fast Blue BB salt
Fructose-1,6-diphosphatase	FDP		NADP	IV	125 mg $MgSO_4 \cdot 7\ H_2O$ 20 mg Fructose-1,6-diphosphate 1 mg PMS 10 mg MTT tetrazolium 20 u PGI 20 u G6PDH
Fumarase	FUM	X	NAD	II	400 mg Fumaric acid 300 u MDH
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	X	NAD	II	270 mg Fructose-1,6-diphosphate 75 mg Arsenic acid 100 u ALD
Glutamate dehydrogenase	GDH	X	NAD	III	2 g L-glutamic acid
Glycerol dehydrogenase	GLYDH		NAD	III	5 ml 0.1 M glycerol 10 mg MTT tetrazolium 1 mg PMS
Glutamate pyruvate transaminase	GPT			II (5 ml)	20 mg L-alanine 10 mg α-ketoglutaric acid 15 mg NADH 150 u LDH Dropwise on surface. Defluorescent
Glucose-6-phosphate dehydrogenase	G6PDH	X	NADP	II	200 mg Glucose-6-phosphate
β-glucoronidase	GUS			V	10 mg Naphtol-A-B-glucoronide 10 mg GBC Fast Garnet salt
Hexokinase	HK	X	NADP	III	50 mg Glucose 25 mg ATP 30 u G6PDH
Isocitrate dehydrogenase	IDH	X	NADP	II	200 mg DL-isocitric acid
Leucine aminopeptidase	LAP			IV	10 mg L-leucyl-β-naphtylamide 10 mg $MgCl_2$ 20 mg Black K salt
Lactate dehydrogenase	LDH	X	NAD	III	25 ml 0.5 M DL-Na-lactic acid

Table 1. Cont.

Enzyme	Abbreviation	NBT/PMS	Cofactor	Stain buffer	Other components
Malate dehydrogenase	MDH	X	NAD	III	25 ml 0.5 M DL-Na-malate pH 7.0
Malic enzyme	ME	X	NADP	III	25 ml 0.5 M DL-Na-malate pH 7.0
Nucleoside phosphorylase	NP	X		II	100 mg Inosine 100 mg Arsenic acid 2 u Xanthine oxidase
Peptidase	PEP			II	50 mg O-dianisidine 80 mg L-leucyl-L-alanine 10 mg Snake venom 50 mg $MgCl_2$ 500 u Peroxidase
6-phosphogluconate dehydrogenase	6PGDH	X	NADP	II	100 mg Ba-6-phosphogluconic acid
Phosphoglucose isomerase	PGI	X	NADP	III	25 mg Na-fructose-6-phosphate 10 u G6PDH
Phosphoglycerate kinase	PGK			II (5 ml)	10 mg Na-3-phosphoglyceric acid 15 mg NADH 10 mg ATP 5 mg $MgCl_2$ 1 mg EDTA 100 u Glyceraldehyde-phosphate dehydrogenase
Phosphoglucomutase	PGM	X	NADP	II	300 mg K-glucose-1-phosphate 50 u G6PDH 5 drops α-D-glucose-1,6 diphosphate
Pyruvate kinase	PK			II (5 ml)	25 mg Phospho(enol) pyruvate (Trisodium salt) 25 mg KCl 15 mg NADH 5 mg $MgCl_2$ 2.5 mg ADP 1 mg EDTA 1 mg Fructose-1,6 diphosphate 150 u LDH Dropwise on surface. Fluorescent
Phosphomannose isomerase	PMI	X	NADP	II	50 mg Ba-D-mannose-6-phosphate 100 u PGI 80 u G6PDH
Sorbitol dehydrogenase	SDH	X	NAD	I	100 mg D-sorbitol 2.5 mg MTT tetrazolium
Superoxide dismutase	SOD	X			
Succinate dehydrogenase	SUCDH	X	NAD	VI	150 mg Na-succinic acid 200 mg EDTA 25 mg ATP 35 mg MTT tetrazolium
Triose phosphate isomerase	TPI	X	NAD	II	2 g DL-α-glycerophosphate 1 g Na-pyruvic acid 250 mg Arsenic acid 500 u Glyceraldehyde-phosphate dehydrogenase 100 u Glycerophosphate dehydrogenase 150 u LDH
Xanthine dehydrogenase	XDH	X	NAD	VII	10 mg Hypoxanthine

UTTER et al. 1973; ALLENDORF et al. 1976), CPK is a major protein component of fish muscle extracts and can be routinely visualized using a general protein stain consisting of a 1% amidoblack solution in a 1:4:5 acetic acid-methanol-water mixture. This same mixture without amidoblack is then used for destaining the gels.

The results presented in the present paper are based on the following samples of brown trout.

(1) Samples of brain, eye, heart, kidney, liver and muscle from about 50 specimens from each of the two populations of Lake Bunnersjöarna, County of Jämtland, Sweden (cf. ALLENDORF et al. 1976). All combinations of buffer systems, staining procedures and tissues were tested on this material.

(2) Muscle samples from several lakes in the alpine areas of the Counties of Jämtland and Västerbotten in the central-northern parts of Sweden. This material represents about 3,000 individuals from some 30 natural lacustrine populations. Muscle samples from a few hatchery stocks from the Bonäshamn hatchery in the Jämtland county is also included in this material. Using the A buffer system, these samples were screened for AGP, CPK, LDH, MDH, and SOD.

The sample collection and storage techniques were those described by ALLENDORF et al. (1976). Notations about enzyme structure refer to DARNALL and KLOTZ (1972), if not stated otherwise.

We use the system of nomenclature proposed by ALLENDORF and UTTER (1977). An abbreviation is chosen to designate each protein, when in italics, these same abbreviations represent the loci coding for these proteins. In the case of multiple forms of the same enzyme, a hyphenated numeral is included; the form with the least anodal migration is designated one, the next two and so on. Allelic variants are designated according to the relative electrophoretic mobility. One allele (generally the most common one) is arbitrarily designated *100*. This unit distance represents the migration distance of the isozyme coded for by this allele. Other alleles are then assigned a numerical value representing their mobility relative to this unit distance. Thus, an allele of the least anodal LDH locus coding for an enzyme migrating one-half as far as the common allele product would be designated *LDH-1(50)*.

Results

Most of the results and interpretations are compiled in Table 2. Of the four buffer systems tested, the best resolution and enzyme activity was always obtained with the A or B buffer system. The majority of the enzymes were predominant in muscle, liver or eye extracts, as listed in Table 2. The tissues are presented in the order we consider them suitable for electrophoretic screening. Several of the enzyme forms are also found in extracts from brain, heart, or kidney, but for the sake of simplicity those tissues are only listed when a specific enzyme form is found or resolved in that tissue only.

Our estimates of the number of loci coding for different enzymes are conservative in that they reflect the minimum number of loci involved. For example, a single invariant band found in all individuals examined might represent the products of any number of loci with the same common allele coding for the protein considered. Therefore, the exact number of loci involved cannot be determined. In such situations we have chosen to treat the protein as if coded for by only one locus. Loci coding for enzymes judged to show activity and resolution good enough to make them usable in population screenings are also marked in Table 2.

ADH, DIA, GDH, XDH. — These enzymes, predominantly or only expressed in liver, were all represented by a single invariant band. As mentioned above, nothing can be concluded about the number of loci; each of these enzymes will be designated as if coded for by one locus. There is evidence that ADH is coded for by a single disomic locus in the rainbow trout (*Salmo gairdneri*) (ALLENDORF et al. 1975).

FUM, GAPDH, G6PDH, GUS, HK, IDH, LAP, PEP, 6PGDH, PGM, PMI. — Each of these enzymes was represented by two recognizable forms with different electrophoretic mobility and tissue predominance. So far, no variation has been observed in the brown trout. Thus, the number of loci cannot be determined. In the designation of each of these enzymes we have assumed that each is coded for by two loci. PGM has been shown to be coded for by a single locus in other salmonid species (UTTER et al. 1973; ALLENDORF et al. 1975). In the brown trout there is no doubt that this monomeric enzyme is represented by two distinct zones indicating the existence of a second locus (*PGM-2*). However, the products of this second locus resolve poorly and cannot be reliably screened for in population surveys. There is evidence that 6PGDH is coded for by a single locus in other closely related salmonids (UTTER, pers. comm.).

Each of the muscle and liver forms of IDH have been shown to be coded for by two loci in the rainbow trout (UTTER et al. 1974; ALLENDORF et al. 1975). Thus, in that species four loci are involved in the

Table 2. Designation of loci coding for different enzymes. Tissues and buffer systems for best resolution are also shown

X incidates that the locus is considered usable for population surveys. B = brain, E = eye, H = heart, K = kidney, L = liver, and M = muscle. See text for further explanation

Enzyme	Locus designation (if multiple)	Usable in population genetic screenings	Buffer system	Best activity and resolution
AAT	1		A,B	LM
	2		A,B	LM
	3		A,B	ML
	4		A,B	ML
	5		A,B	E
	6		A,B	L
ADH		X	A	L
AGP	1		A	M
	2	X	A	M
	3		A	L
AK	1		B	L
	2	X	B	L
	3	X	B	LME
ALD	1	X	B	M
	2		B	EH
	3		B	EB
CPK	1	X	A	M
	2	X	A	M
	3		A	E
DIA		X	A	L
EST	1	X	A	L
	2	X	A	LME
	3	X	A	L
FDP	1	X	A	L
	2	X	A	L
FUM	1	X	A	M
	2	X	A	E
GAPDH	1	X	A,B	M
	2	X	A,B	E
GDH		X	B	L
G6PDH	1	X	B	LE
	2	X	B	K
GUS	1	X	A	ML
	2	X	A,B	L
HK	1	X	B	L
	2	X	B	LME
IDH	1	X	B	ME
	2	X	B	LE
LAP	1	X	A	M
	2	X	A	L
LDH	1	X	A	MK
	2	X	A	MK
	3	X	A	HMK
	4	X	A	LMK
	5	X	A	E

Table 2. Cont.

Enzyme	Locus designation (if multiple)	Usable in population genetic screenings	Buffer system	Best activity and resolution
MDH	1	X	B	LE
	2	X	B	LE
	3	X	A	EM
	4	X	A	EM
ME	1	X	B	EM
	2	X	B	EM
	3	X	B	LEM
PEP	1	X	A	MLE
	2	X	A	MLE
6PGDH	1	X	B	LM
	2	X	B	L
PGI	1	X	A	M
	2	X	A	M
	3	X	A	MLE
PGM	1	X	A	ML
	2		A	EL
PMI	1	X	B	L
	2	X	B	LME
SDH	1	X	A	L
	2	X	A	L
SOD		X	A	L
TPI	1		B	M
	2		B	E
XDH		X	A	L

control of IDH. The general degree of genetic similarity between different *Salmo* species suggests this to be true for the brown trout also. However, in the absence of further evidence IDH is designated as coded for by two loci in the brown trout.

G6PDH has previously been reported to be coded for by two loci in brook and lake trout (*Salvelinus fontinalis* and *S. namaycush*) (YAMAUCHI and GOLDBERG 1975). However, the heterotetrameric isozymes reported in those species were not observed in the brown trout.

AK, ALD, FDP. — These enzymes were all represented by three distinct invariant bands. The monomeric structure of AK suggests that this enzyme is coded for by three loci. The poor resolution of the least anodal locus only permits screening for two loci. Using the B buffer system ALD was represented by a single cathodal band in muscle extracts, a slow anodal band in heart, and a fast anodal band in brain. Both the anodal bands were present in eye extracts. On this basis, ALD is assumed to be coded for by three loci, but both the anodal loci are too weakly expressed to be used in population surveys. FDP was represented by three bands in liver extracts. The dimeric structure of this enzyme suggests that it is coded for by two loci fixed for different alleles and thus displaying a fixed heterozygote effect.

ME. — Three loci are assumed to code for this tetrameric enzyme. The most anodal locus (*ME-3*) was represented by a single band in all the tissues examined. *ME-1* and *ME-2* are predominantly expressed in eye, heart, and muscle and are represented by five distinct bands indicating that they are fixed for different alleles. No heterotetramers involving the gene products of the *ME-3* locus were observed.

PGI. — ENGEL et al. (1975) have reported this dimeric enzyme to be coded for by two loci in the brown trout. Our data, however, clearly indicates the presence of three loci. The most anodal locus (*PGI-3*) is expressed in liver, muscle, and eye while *PGI-1* and *PGI-2* are primarily expressed in muscle. Heterodimers involving the gene products from all three loci are formed giving a six-banded fixed heterozygote effect in muscle extract.

AGP, CPK, LDH, MDH, SOD. — A previous publication from our group (ALLENDORF et al. 1976) has reported the brown trout muscle expression of these enzymes. The results presented in that paper are extended in the present report.

ENGEL et al. (1971) showed the dimeric enzyme AGP to be coded for by three loci (A, B, and C) in brown trout. The A, B, and C loci correspond to the *AGP-1*, *AGP-2*, and *AGP-3* loci, respectively, in our nomenclature. The *AGP-1* and *AGP-3* gene products had very poor activity in our material and were only occasionally detected. The previously reported alleles *AGP(100)* and *AGP(50)* are now designated *AGP-2(100)* and *AGP-2(50)* in the extended nomenclature. These alleles seem to be identical to the B and B′ alleles reported by ENGEL et al. A third allele with the same mobility as *AGP-2(50)* but with highly reduced activity has also been observed. The homodimeric isozymes originating from that allele are not visualized on the gels; muscle extracts from fish heterozygous for the low-active and the common *AGP-2(100)* allele express a two-banded electrophoretic pattern.

The muscle forms of CPK have previously been reported to be coded for by two loci. Two alleles, *CPK-1(100)* and *CPK-1(115)*, have been observed in the least anodal locus (ALLENDORF et al. 1976). The existence of a third locus is indicated by an additional band in eye extracts.

LDH has been shown to be coded for by five loci in many salmonids (WRIGHT et al. 1975). In the brown trout *LDH-1* and *2* predominate in muscle, *LDH-3* in heart, *LDH-4* in liver, and *LDH-5* in eye. As reported previously the muscle LDH pattern is polymorphic; the polymorphism is assumed to be caused by the segregation of a fast or null allele (*LDH-1(240)*) at the least anodal muscle locus (ALLENDORF et al. 1976). In the course of the present study *LDH-5* was also found to be polymorphic. So far only two phenotypes have been observed and we are not certain about the genetic interpretation of this variation. The deviating phenotype exhibits a faster band than the common phenotype. Though the muscle, liver, and heart LDH forms resolve excellently the eye form does not; it has so far been impossible to identify the total subunit composition of the variant phenotype. The assumed variant allele has been designated *LDH-5(105)*. We will estimate the allele frequencies at this locus from the square root of the frequency of the phenotype which has so far been most commonly observed until more definite results are collected.

BAILEY et al. (1970) showed that the predominant muscle MDH, the B form, is coded for by two loci in salmonids. In the brown trout these loci, *MDH-3* and *4* share alleles with identical electrophoretic mobility most commonly represented by a single band on the gel. We have reported the existence of a slow allele at one of these loci. Although it is impossible to assign that allele to a specific locus (i.e. *MDH-3* or *MDH-4*) it was designated *MDH-3(80)* for the sake of simplicity (ALLENDORF et al. 1976). In the present study a fast allele was also observed at one (or both) of these loci. At present we cannot determine whether these two variant alleles belong to the same locus (loci) or not; arbitrarily we have chosen to designate the fast allele *MDH-4(125)*. The *MDH-3* and *4* loci are both expressed in the eye; we prefer the resolution obtained using that tissue.

BAILEY et al. (1970) also presented evidence that the A form of MDH, predominant in eye and liver, is coded for by two loci in the brown trout. The present study support that conclusion, MDH-A was represented by three invariant bands indicating the existence of two loci fixed for different alleles. These loci are designated *MDH-1* and *2*.

SOD has been reported to be coded for by a single disomic locus in other salmonids (UTTER et al. 1973). A slow allele, *SOD(50)*, was observed in the present study. Although SOD activity is present in several tissues, we recommend this enzyme to be routinely screened for using liver extracts.

EST. — The genetic interpretation of esterase zymogram patterns has been reported to be confused by environmental or ontogenetic artifacts in other salmonids (UTTER et al. 1974; ALLENDORF et al. 1975). In the present study three zones of EST activity were observed; they are assumed to represent the expression of three loci, *EST-1*, *2*, and *3*. Two alleles were observed at the *EST-2* locus, *EST-2(100)* and *EST-2(92)*. However, in view of the lack of breeding data and the warning put forward by UTTER et al. (1974) the genetic basis of this phenotypic variation must be accepted with apprehension.

SDH. — This tetrameric enzyme has been shown to be coded for by two loci in the brown trout (ENGEL et al. 1970). These loci have different electrophoretic mobility and the most common phenotype is five banded. Using the A buffer system one locus migrates cathodally (*SDH-1*) and the other one anodally (*SDH-2*). In addition to the common cathodal allele at *SDH-1*, a variant allele (*SDH-1(0)*) was observed in the present study. The mobilities of both loci are very susceptible to even minor changes of pH.

AAT. — We have obtained very poor resolution for this dimeric enzyme in brown trout, using techniques which work well with other salmonids

(ALLENDORF and UTTER 1976). The enzyme activity is good in all tissues but the resolution is very poor. Five different zones of activity were observed. Further, the previously reported polymorphism (ALLENDORF et al. 1976) suggests that one of these zones represents a duplicated locus. Altogether the observations indicate the existence of as many as six loci. At present, however, none of the loci coding for AAT in the brown trout can be used for population genetic studies.

TPI. — Two zones of activity which were visualized in extracts from different tissues suggested the existence of two loci. Because of the unreliable resolution, however, they are not recommended for population surveys.

ADA, GPT, NP, PK, PGK. — These enzymes were not resolved clearly enough to permit any conclusions about their genetic control.

GLYDH, SUCDH. — Staining for these enzymes revealed little or no activity. However, LDH activity was detectable with both of these staining solutions.

Discussion

As previously mentioned, the present investigation was instigated by the findings of a previous paper (ALLENDORF et al. 1976). It was concluded that both from an evolutionary and a fishery management perspective there is a strong need for a detailed examination of the patterns of genetic diversity in the brown trout.

As discussed by LEWONTIN (1974), NEI (1973), and NEI and ROYCHOUDHURY (1972, 1974) reliable estimates of genetic variation in natural populations require examination of a large number of loci. Studies of genetic variability between and within populations should be based on a random sample of the genome. This requires a sample which is both diverse with regard to biological function of loci and unbiased with regard to monomorphism versus polymorphism. Further, because of the considerable variation in heterozygosity found between and within loci, a large sample of loci is often much more important than a large sample of individuals.

In the present paper we present the genetic interpretation of electrophoretic data in the brown trout. If possible, these interpretations should, of course, be based on inheritance experiments. In many wildlife species, however, this is very difficult or even impossible. This must not make us refrain from genetic analyses of such species. On the contrary there is a strong need for genetic studies of population structure in many species which are not easily stocked. In the management of both endangered species and fish and wildlife populations which are commercially harvested, a clear picture of the genetic structure of the population is a basic prerequisite for the construction of proper management programs (SMITH et al. 1976; ALLENDORF and UTTER 1977; RYMAN et al. 1977). Further, for a correct understanding of the genetic basis of evolutionary changes, the patterns of genetic diversity present in many species must be examined: a general picture of genetic variation in natural populations cannot be obtained from studies which are restricted to organisms easily bred in the laboratory.

With regard to the brown trout, there are no breeding data available. The interpretations presented in this paper are based on comparisons and extrapolations of results obtained in other salmonids, population data, tissue distributions, and known enzyme structures. It should also be pointed out that for the majority of loci only two populations have been examined (i.e. the two populations of lake Bunnersjöarna). Therefore, the present material is not sufficient for an estimate of the fraction of polymorphic loci in brown trout. Further studies of additional populations will probably reveal polymorphisms at loci which were monomorphic in the present study.

We have concluded that 54 of the 69 loci detected are suitable for population screenings; a few remarks should be made, however. The first one concerns the estimation of allele frequencies. At a duplicated locus coding for a dimeric enzyme and segregating for two alleles there is more than one three-banded genotype. There are only quantitative differences between these banding types which for some loci might complicate the identification of genotypes. Of course, similar complications also occur at all duplicated loci. Nevertheless, unbiased allele frequency estimates can be obtained from the frequency of individuals exhibiting a genetically defined homozygous phenotype (see ALLENDORF et al. 1975). The statistical precision of such estimates is, of course, weaker than if estimates are obtained by direct "allele counting". However, being aware of this problem allows the adjustment of sample sizes to give the precision desired.

Tests of linkage disequilibrium are also less powerful in situations where it is impossible to identify all the genotypes. They are, however, still possible to perform and to some extent the loss of power can be compensated for by an increased sample size (HILL 1974; BROWN 1975).

With regard to the loci represented by a mono-

morphic banding pattern, the estimate of the number of loci is probably low. In turn, this underestimate will to some extent bias estimates of average heterozygosity and genetic distance. The magnitude of that bias should be small, however. First, it does not at all affect comparisons between different populations within the species. Second, adding of a few monomorphic loci will only result in a minor change of the absolute number of loci examined. Therefore, comparisons between the brown trout and other species should still be reliable.

In conclusion, we think that the loci identified in the present study provide a basis for a detailed analysis of genetic variation in brown trout. We intend to refer to the nomenclature used in the present paper in future reports. These studies are currently in progress.

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Literature cited

ALLENDORF, F. W. and UTTER, F. M. 1973. Gene duplication within the family Salmonidae: Disomic inheritance of two loci reported to be tetrasomic in rainbow trout. — *Genetics 74*: 647—654

ALLENDORF, F. W. and UTTER, F. M. 1976. Gene duplication in the family Salmonidae. III. Evidence of linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout. — *Hereditas 82*: 19—24

ALLENDORF, F. W. and UTTER, F. M. 1977. Population genetics of fish. — *Fish Physiol. 8 (in press)*

ALLENDORF, F. W., UTTER, F. M. and 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 IV: Genetics and Evolution* (*Ed.* C. L. MARKERT), *Acad. Press, New York*, p. 415—432

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

ASPINWALL, N. 1974. Genetic analysis of North American populations of the pink salmon, *Oncorhynchus gorbuscha*, possible evidence for the neutral mutation-random drift hypothesis. — *Evolution 28*: 295—305

AVISE, J. C. and SELANDER, R. K. 1972. Evolutionary genetics of cave-dwelling fishes of the genus *Astyanax*. — *Evolution 26*: 1—19

AVISE, J. C. and SMITH, M. H. 1974. Biochemical genetics of sunfish. II. Genetic similarity between hybridizing species. — *Am. Natur. 108*: 458—472

AYALA, F. J., POWELL, J. R., TRACEY, M. L., MOURÃO, C. A. and PÉREZ-SALAS, S. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. — *Genetics 70*: 113—139

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

BREWER, G. J. and SING, C. F. 1970. An Introduction to Isozyme Techniques. — *Acad. Press, New York*

BROWN, A. H. D. 1975. Sample sizes required to detect linkage disequilibrium between two or three loci. — *Theoret. Pop. Biol. 8*: 184—201

CLAYTON, J. W. and TRETIAK, D. N. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. — *J. Fisheries Res. Board Can. 29*: 1169—1172

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

ENGEL, W., OP'T HOF, J. and 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. and WOLF, U. 1971. Genetic variation of α-glycerophosphate-dehydrogenase isoenzymes in clupeoid and salmonoid fish. — *Experientia 27*: 1489—1491

ENGEL, W., SCHMIDTKE, J. and WOLF, U. 1975. Diploid-tetraploid relationships in teleostean fishes. — In *Isozymes IV: Genetics and Evolution* (*Ed.* C. L. MARKERT), *Acad. Press. New York*, p. 449—462

GOSSELIN-REY, C., HAMOIR, G., and SCOPES, R. K. 1968. Localization of creatine kinase in the starch-gel and moving-boundary electrophoretic patterns of fish muscle. — *J. Fisheries Res. Board Can. 15*: 2711—2714

HILL, W. G. 1974. Estimation of linkage disequilibrium in randomly mating populations. — *Heredity 33*: 229—239

HOPKINSON, D. A., CORNEY, G., COOK, P. J. L., ROBSON, E. B. and HARRIS, H. 1970. Genetically determined electrophoretic variants of human red cell NADH diaphorase. — *Ann. Hum. Genet. 34*: 1—10

JOHNSON, A. G., UTTER, F. M. and NIGGOL, K. 1972. Electrophoretic variants of aspartate aminotransferase and adductor muscle proteins in the native oyster (*Ostrea lurida*). — *Anim. Blood. Groups Biochem. Genet. 3*: 109—113

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

NEI, M. 1973. Analysis of gene diversity in subdivided populations. — *Proc. Nat. Acad. Sci. 70*: 3321—3323

NEI, M. and ROYCHOUDHURY, A. K. 1972. Gene differences between Caucasian, Negro, and Japanese populations. — *Science 177*: 434—436

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

RIDGWAY, G. J., SHERBURNE, S. W. and LEWIS, R. D. 1970. Polymorphisms in the esterases of Atlantic herring. — *Trans. Am. Fisheries Soc. 99*: 147—151

RYMAN, N., BECKMAN, G., BRUUN-PETERSEN, G. and REUTERWALL, C. 1977. Variability of red cell enzymes and genetic implications of management policies in Scandinavian moose (*Alces alces*). — *Hereditas 85*: 157—162

SELANDER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E.

and GENTRY, J. B. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). — *Studies in Genetics. VI. Univ. Texas Publ. 7103*, p. 49—90

SHAW, C. R. and PRASAD, R. 1970. Starch gel electrophoresis of enzymes. — A compilation of recipes. — *Biochem. Genet. 4*: 297—320

SICILIANO, M. J. and SHAW, C. R. 1976. Separation and visualization of enzymes on gels. — In *Chromatographic and Electrophoretic Techniques, Vol. 2, Zone Electrophoresis (Ed.* I. SMITH), *Heinemann, London*, p. 185—209

SMITH, M. H., HILLESTAD, H. O., MANLOVE, M. N. and MARCHINTON, R. L. 1976. Use of population genetics data for the management of fish and wildlife populations. — *Trans. 41 North Am. Wildlife Natural Resources Conf.*, p. 119—133

UTTER, F. M., ALLENDORF, F. W. and HODGINS, H. O. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. — *Syst. Zool. 22*: 257—270

UTTER, F. M., HODGINS, H. O. and ALLENDORF, F. W. 1974. Biochemical genetic studies of fishes: Potentialities and limitations. — In *Biochemical and Biophysical Perspectives in Marine Biology (Eds.* D. C. MALINS and J. R. SARGENT), *Acad. Press, New York*, p. 213—238

WILLIAMS, G. C., KOEHN, R. K. and MITTON, J. B. 1973. Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. — *Evolution 27*: 192—204

WRIGHT, J. E., HECKMAN, J. R. and ATHERTON, L. M. 1975. Genetic and developmental analyses of LDH isozymes in trout. — In *Isozymes III: Developmental Biology (Ed.* C. L. MARKERT), *Acad. Press, New York*, p. 375—401

YAMAUCHI, T. and GOLDBERG, E. 1975. Glucose-6-phosphate dehydrogenase in brook, lake, and splake trout: an isozymic and developmental study. — In *Isozymes III: Developmental Biology (Ed.* C. L. MARKERT), *Acad. Press, New York*, p. 403—415