1976

Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations

Fred W. Allendorf
University of Montana - Missoula, Fred.Allendorf@umontana.edu

Nils Ryman
Institute of Genetics, University of Stockholm

A. Stennek

G. Stahl

Hereditas 83: 73—82 (1976)

Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations

F. ALLENDORF,[1] N. RYMAN, A. STENNEK AND G. STÅHL

Institute of Genetics, University of Stockholm, Sweden
Department of Genetics, University of Århus, Denmark

ALLENDORF, F., RYMAN, N., STENNEK, A. and STÅHL, G. 1976. Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations. — *Hereditas 83:* 73—82. Lund, Sweden. ISSN 0018-0661. Received March 11, 1976

Large differences in certain morphological traits between populations of brown trout (*Salmo trutta* L.) in the lakes of northern Sweden led us to initiate an electrophoretic examination in an effort to understand the genetic structure of these populations. A total of 549 brown trout from four lakes were examined at 10 electrophoretically detected loci. Genetic variation was found at only three loci, coding for lactate dehydrogenase (LDH), alpha-glycerophosphate dehydrogenase (AGP), and creatine phosphokinase (CPK). A total absence of heterozygotes at the *LDH-1* locus in Lake Bunnersjöarna, coupled with significant correlations between *LDH-1* type and both *CPK-1* allele frequencies and body size, is interpreted as evidence of two genetically isolated populations of brown trout in this lake. This finding, in addition to large allele frequency differences between brown trout from different lakes, is evidence of a large amount of genetic differentiation in these populations within a small geographical area. The implications of these results are discussed from both an evolutionary and a fishery management perspective. Further studies are planned to determine if these two sympatric populations are representatives of two widespread sibling species or are a locally evolved phenomenon. We also intend to detail the biochemical genetic, behavioral, and ecological differences between the two distinct populations in Lake Bunnersjöarna.

Nils Ryman, Institute of Genetics, University of Stockholm, Box 6801, S-11386 Stockholm, Sweden

The pattern of genetic diversity present in fish species of the family Salmonidae presents the evolutionary biologist with a special problem. The repeated advance and retreat of glaciation in the northern hemisphere has created a situation in which the isolation of populations of fish for periods of approximately 1000 years is followed by the secondary contact of these populations during reinvasion of the previously glaciated waters (BEHNKE 1972). Another complicating factor in salmonid systematics is the tendency for salmonids to evolve genetically discrete, ecologically specialized populations, which are differentiated on the basis of such life-history characters as time and place of spawning (reviewed by BEHNKE 1972). The strong homing behavior of most salmonids is an important factor in the maintenance of this genetic diversity. The overall result of these factors is the present existence of reproductively isolated sympatric populations within a number of salmonid species (BEHNKE 1972; NYMAN 1972; SVÄRDSON 1970).

The pattern of genetic diversity present in these populations makes it virtually impossible to classify them by means of the binomial system of nomenclature, reflecting the genetic diversity and evolutionary relationships of the populations. As pointed out by BEHNKE (1972), the current taxonomic designation of a species may include "a multitude of populations with actual or potential reproductive isolation and diverse life histories". BEHNKE has also emphasized the fact that such a system of classification has the danger of promoting the typological species concept among fishery biologists so that the actual range of genetic diversity present within a species is neither comprehended nor preserved.

The temporally fluctuating isolation caused by glaciation may bring about the recontact of formerly isolated populations before efficient reproductive isolating mechanisms have evolved. Such zones of secondary contact between allopatrically evolved populations are of major interest to the evolutionary

[1] Present address: Department of Zoology, University of Montana, Missoula, Montana 59801, U.S.A.

geneticist. The present techniques of electrophoresis allow the detailed examination of the interaction of such populations, including an estimation of both the initial genetic divergence present and the patterns of introgression of alleles between the former isolates.

The existence of genetically distinct sympatric populations also presents an important problem to those responsible for the management of fish populations. Many unique and potentially valuable gene pools have been lost by improper management. BEHNKE has estimated that 99% of the original populations of cutthroat trout (*Salmo clarki*) in the interior of the U.S.A. have been lost in the last 100 years (BEHNKE 1968). Electrophoresis provides an effective tool for delineating the genetic diversity present within a species. Such an understanding is necessary if fish management programs are going to take advantage of potentially useful ecological adaptions in future fish management and breeding programs.

Many of the distinct life history patterns differing between populations of fish are potentially very important from the perspective of fish management (e.g. age at maturity, maximum life span, habitat and food preference, and growth rate). Within many species there are considerable phenotypic differences between different natural populations. Usually the most striking variation is in body size but differences in other characters such as body shape, color and spawning age are also common. In Scandinavia, the variation in body size is most accentuated in species of brown trout (*Salmo trutta* L.), char (*Salvelinus* spp.), perch (*Perca fluviatilis* L.) and whitefish (*Coregonus* spp.), which often occur in dwarfed and low yielding populations of poor economic value.

Very little is known about the causes of these differences in size distribution in wild populations of fish. The effect of the environment has been studied in several transplantation experiments where fish have been removed from "good" environments to "bad" ones and vice versa (ALM 1946, 1949, 1959). In all of these transplantation experiments environmental effects were observed. The results do not explain, however, why closely located and environmentally very similar lakes often exhibit populations of the same species with quite different size distributions. Furthermore, temporal changes in size distribution are sometimes observed within populations of such species as the brown trout in very remote and virgin lakes in the Norwegian and Swedish alpine areas.

In char and whitefish the differences in population structure have been explained by the species being subdivided into several sibling species, which in some lakes occur sympatricly (NYMAN 1972; SVÄRDSON 1970). However, as shown by LINDSTRÖM (1973), there is no systematic difference between the size distributions of the different whitefish sibling species. Therefore the existence of sibling species can only partly explain the population differences within a species complex.

The genetic components of growth rate and body size have been examined in several domesticated fish species such as the carp (CHERFAS 1972; KIRPICHNIKOV 1968; MOAV et al. 1975; NENASHEV 1966; WOLFARTH et al. 1975), guppy (RYMAN 1972b, 1973), rainbow trout (DONALDSON and OLSON 1955) and Atlantic salmon (RYMAN 1972a). Very little, however, is known about the genetic basis of the morphological differences between wild populations of fish.

The aim of the present investigation is to estimate, from electrophoretic data, the degree of genetic variation between populations of brown trout showing considerable morphological differences between different populations. The determination of the genetic diversity will help in the understanding of the existing phenotypic differences. As discussed previously, this diversity is an important consideration from both an evolutionary and a fishery management perspective.

The brown trout was chosen for several reasons. This species is represented by three ecological forms — anadromous, lacustrine, and fluviatile (BEHNKE 1972). Our initial interest is concentrated on the populations of the lacustrine brown trout in the mountain lakes of Sweden. This group of populations exhibits considerable phenotypic differences between populations in regard to body size as well as other morphological characters. In addition, they have a wide geographical distribution, occurring in virgin lakes of the alpine areas as well as in the lakes of the coastal region.

Materials

Sample collection

Several lakes of the alpine areas of Jämtland county in the middle of Sweden were sampled. The lakes described in the present report are Lake Bunnersjöarna, Lake Lågsjön, Lake Trollsvattnet, and Lake Övre Hemsjön. The geographic locations of the lakes are shown on the map in Fig. 1, which also shows the main drainage systems. All the lakes are very isolated, with the only access being a walk of at least one day from the nearest road. They are quite pristine, and as

far as known, no planting of fish has been carried out in any of them. As indicated by the Swedish name (plural) Lake Bunnersjöarna is in reality two different lakes; these will be referred to as the northern and the southern lakes of Lake Bunnersjöarna. They are connected through a 50 m long non-flowing channel with a depth of about 1 m. Both lakes have an area of approximately one half square kilometer. The southern lake is very shallow (0.5 m) with a deeper hole only in the middle of it; the northern lake is deeper (2 m) and is largely without shallow banks.

The majority of the fish were caught with gill nets of varying mesh sizes, except for Lake Trollsvattnet where all sampling was performed by angling. A section of skeletal muscle was cut off behind the head of every specimen and brought to the laboratory for freeqing (−60° C). Sexing, otolith preparation, and length and weight measurements were performed in the field. The sampling was carried out during the summer 1975, and the electrophoretic analysis during the autumn the same year. Sampling at each lake took several days; during that time, the samples were kept in snow, if available, or buried in the ground. In this way samples from the fish first caught sometimes were kept up to six days before they reached the freezer. However, there were no detectable differences with regard to enzyme activity between samples stored for different periods in the field.

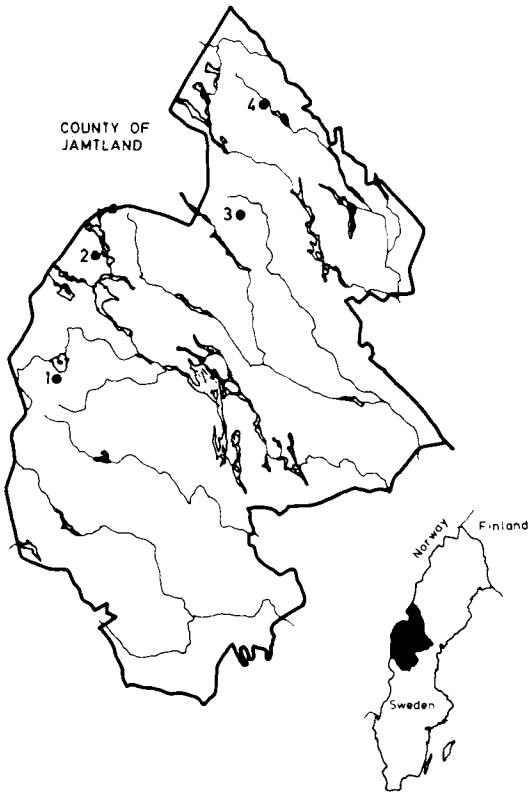

Fig. 1. Map showing the main drainage systems of the County of Jämtland and the locations of the lakes investigated. The lakes are too small to be seen in this scale and they are therefore indicated by black dots.
1. Lake Bunnersjöarna
2. Lake Lågsjön
3. Lake Trollsvattnet
4. Lake Övre Hemsjön
The location of the County of Jämtland is indicated on the lower right map of Sweden.

Electrophoresis

Muscle extracts were prepared and horizontal starch gel electrophoresis was accomplished following the methods of UTTER et al. (1974). Gels were made using 14% starch in a buffer consisting of 99% tris (0.03 M) and citric acid (0.005 M) and 1% of the bridge buffer. The bridge buffer consisted of lithium hydroxide (0.06 M) and boric acid (0.03 M) (RIDGWAY et al. 1970). A potential of 200 volts was applied across the gel until the borate boundary was 6 cm from the origin, i.e. about three hours. The gels were cooled on the bottom with cooling plates in which water at 5° C circulated.

The staining solutions for the enzymes alpha-glycerophosphate dehydrogenase (AGP), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) consisted of 100 ml of the gel buffer described above, 15 mg NAD, 15 mg p-nitroblue tetrazolium (NBT), 10 mg phenazine methosulfate (PMS), and the appropriate substrate. The substrates used for these enzyme staining solutions were 50 mg DL-alpha-glycerophosphate, 20 ml 0.5 M DL-sodium lactate, and 20 ml 0.5 M DL-sodium malate respectively. Superoxide dismutase (SOD) activity was best visible as a heavy white negative staining band on gels stained for MDH. Aspartate aminotransferase (AAT) activity was stained for using the method of JOHNSON et al. (1972). Specific creatine phosphokinase (CPK) activity was stained for with a solution consisting of 100 ml of the gel buffer previously described, 15 mg NADP, 15 mg NBT, 10 mg PMS, 500 mg creatine phosphate, 30 mg adenosine diphosphate, 20 mg glucose, 100 units of hexokinase, and 20 units of glucose-6-phosphate dehydrogenase. As reported previously (UTTER et al. 1973; GOSSELIN-REY et al. 1968), CPK is a major protein component of fish muscle extracts and can be scored for on a starch gel using a general protein stain. In the course

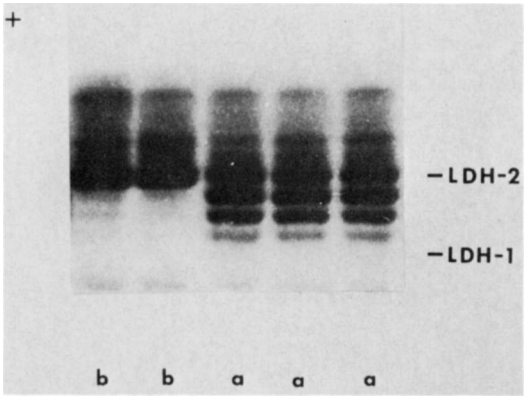

Fig. 2. Two different LDH phenotypes seen in Lake Bunnersjöarna; a: *LDH-1(100/100)*; b: *LDH-1(240/240)*. The mobility of the homotetrameric isozymes of the *100* alleles of the two muscle LDH loci are indicated.

of this study CPK was routinely visualized on the gels 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 the admidoblack was used for destaining the gels.

Results

Description of genetic systems

The extensive gene duplication found in salmonids often confounds the genetic interpretation of observed electrophoretic variation. Because of the critical importance of understanding this genetic basis one of the authors (FWA) has been involved in a series of inheritance studies directed towards the identification of the genetic control of polymorphic loci in salmonids (ALLENDORF and UTTER 1973, 1976; UTTER et al. 1973; ALLENDORF et al. 1975). Although these inheritance experiments have not involved the brown trout there is sufficient homology of the biochemical genetic systems involved to allow direct extrapolation of the results with other *Salmo* species to the brown trout.

A brief examination of the literature will reveal a wide range of systems of nomenclature used to describe electrophoretically detected genetic variation. In the present paper, we have adopted a system as proposed by UTTER and ALLENDORF (1976) for the description of such genetic systems in populations of fish.

This proposed uniform system of nomenclature is similar to that used by RICHMOND (1972). 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 their 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 most cathodal LDH locus coding for an enzyme migrating one-half as far as the common allele would be designated *LDH-1(50)*.

The dimeric enzyme AGP was found to be coded for by a single polymorphic locus in the brown trout. During this work the buffer system described in this paper was used. The two alleles found at this locus were *AGP(100)* and *AGP(50)*. The enzyme SOD has also been shown to be coded for by a single locus in other salmonid species (UTTER et al. 1973). However, no variants were observed for SOD in the course of the present study.

The muscle form of MDH, designated MDH-B (BAILEY et al. 1970), has been shown to be coded for by two disomic loci having common alleles of identical electrophoretic mobility in the rainbow trout (ALLENDORF et al. 1974). No variants were found in the populations reported in this paper. However, a variant allele, *MDH-3(80)*, was seen in a preliminary examination of a brown trout population not included in this report.

The supernatant form of AAT has been shown to be coded for by two disomic loci in salmonids (SCHMIDTKE and ENGEL 1972; ALLENDORF and UTTER 1976). This enzyme has been found to be very labile under storage conditions (MAY et al. 1975). Reliable phenotypes could not be obtained during the major part of the electrophoretic examination reported in this paper. However, a preliminary survey revealed a polymorphism for this enzyme with the variant allele being *AAT-1(119)*.

Previous reports have shown LDH to be coded for by five loci in salmonids (WRIGHT et al. 1975). All of these loci except for the locus expressed only in the eye (*LDH-5*) were examined in the present study. The only phenotypic variation observed was for the *LDH-1* locus as shown in Fig. 2. The variant allele at *LDH-1* causing this phenotypic variation could either be a null allele (i.e. an allele not producing an active enzyme subunit) or an allele with

mobility identical to that of the common allele of *LDH-2*. For the sake of simplicity, we have designated this allele under the assumption that it has a mobility identical to that of the common *LDH-2* allele.

The *LDH-1* and 2 loci together produce the predominant LDH found in the muscle of salmonids and for this reason are commonly referred to as the muscle LDH loci (WRIGHT et al. 1975). The variant single banded muscle LDH phenotype was first found in Lake Bunnersjöarna where it and the five banded phenotype were at approximately equal frequencies. No intermediate types were observed that could be interpreted as heterozygotes (i.e. *LDH-1(100/240)*.

It has been reported that the *LDH-1* subunits are not as strongly expressed as those of *LDH-2* (UTTER et al. 1973); our results confirm this observation in that the *LDH-2* homotetrameric isozyme was always much darker staining than that of the *LDH-1* homotetramer (see Fig. 2). The phenotype expected of a *LDH-1(100/240)* heterozygote would be multiple banded but would have a dramatic shift in the pattern towards the *LDH-2* homotetramer. Such a phenotype was not seen; the multiple-banded muscle LDH types in Lake Bunnersjöarna were identical to those in lakes in which the single-banded muscle LDH type was not found. If the fish in Lake Bunnersjöarna were a single panmictic population so that this polymorphism was in Hardy-Weinberg proportions, these heterozygotes would be expected at a frequency of approximately 40%.

On this basis, it was concluded that the fish sampled from Lake Bunnersjöarna represented two separate populations with very little (if any) hybridization between the two groups. Subsequent differences between these two groups in allele frequencies at another locus and in body size support this concept.

The dimeric enzyme CPK in salmonids has been found to be coded for by two gene loci in both *Salmo* and *Onchorhynchus* species (ALLENDORF, UTTER and MAY, in prep.). These authors have presented a model based on inheritance studies and previous biochemical reports (EPPENBERGER et al. 1971) of two loci, each of which produces an enzyme subunit which does not form heterodimers with the products of the other locus. However, the active enzyme produced by each locus is altered in vivo so that each allele is represented by two electrophoretically distinct bands.

The common three-banded CPK type seen in brown trout (Fig. 3) is identical to that seen in rainbow trout. This type is assumed to be the result of two loci with the middle band representing two isozymes with identical mobility, in that the fast isozyme

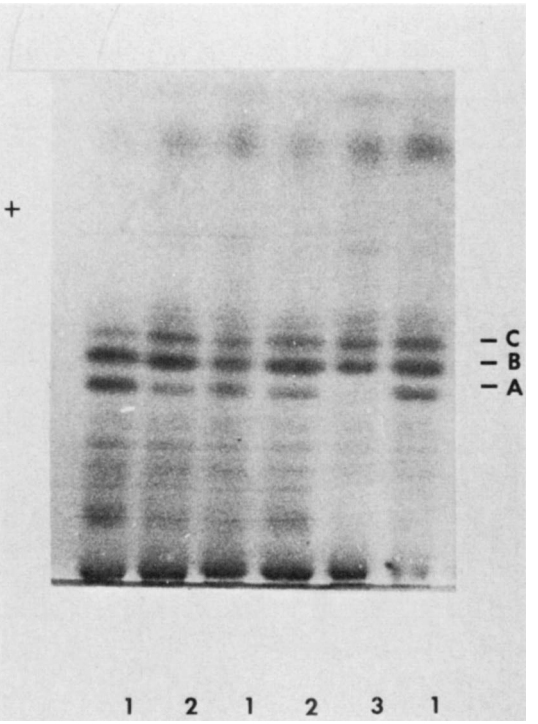

Fig. 3. The three different CPK phenotypes observed; 1: *CPK-1(100/100)*; 2: *CPK-1(100/115)*; 3: *CPK-1(115/115)*. The bands designated A, B, and C are those isozymes which have specific CPK activity.

produced by *CPK-1* has the same mobility as the slow *CPK-2* isozyme. The two-banded types seen in Fig. 3 are interpreted as homozygotes for a *CPK-1* allele which is either a null allele or has the same mobility as the common *CPK-2* allele. The heterozygote for this locus, *CPK-1(100/115)*, should also have three bands but should have a shift in relative activity so that the slowest band is much reduced in activity compared to the *CPK-1(100/100)* homozygote. Although these types could be identified as shown in Fig. 3, they could not be reliably routinely scored on the basis of this slight quantitative difference. Therefore, the frequency of the variant allele was estimated by assuming Hardy-Weinberg proportions and letting the frequency of *CPK-1(115)* equal the square root of the frequency of the *CPK-1(115/115)* genotype in the sample.

Because of the nature of the additional gene duplication found in salmonids it is not surprising to find the situations as described here for both LDH and CPK. A significant portion of the loci so far examined in salmonids show no evidence of duplica-

Table 1. Comparisons of the frequencies of *CPK-1(115/115)* homozygotes, mean lenghts (cm) and mean weights (g) among the two *LDH-1* genotypes found in the two lakes comprising Lake Bunnersjöarna

The standard errors of the means are given within brackets. The χ^2-values connected with the lengths and weights refer to nonparametric comparisons of the corresponding distributions with regard to the quartiles in the pooled material. The asterisks indicate the level of significance.

	Lake	*LDH-1(100/100)*	*LDH-1(240/240)*	
Frequency of *CPK-1(115/115)*	Northern	0.21	0.57	$\chi^2_1 = 6.84^{**}$
	Southern	0.18	0.46	$\chi^2_1 = 6.02^{*}$
	Total	0.19	0.49	$\chi^2_1 = 13.52^{***}$
Mean length	Northern	35.0 (±1.0)	32.3 (±1.6)	$\chi^2_3 = 7.01$
	Southern	32.6 (±0.7)	29.9 (±0.7)	$\chi^2_3 = 8.73^{*}$
	Total	33.8 (±0.7)	30.6 (±0.7)	$\chi^2_3 = 14.50^{**}$
Mean weight	Northern	417 (±31)	358 (±46)	$\chi^2_3 = 9.43^{*}$
	Southern	349 (±21)	275 (±18)	$\chi^2_3 = 8.33^{*}$
	Total	386 (±20)	299 (±19)	$\chi^2_3 = 16.45^{***}$
Number of fish	Northern	39	23	
	Southern	33	56	
	Total	72	79	

tion (ALLENDORF et al. 1975). Under the assumption that all salmonid loci were duplicated by a tetraploid event (OHNO 1974), the simplest explanation of this loss of a locus is the fixation of a null allele at one of the duplicated loci. Therefore, the finding of possible null alleles at the frequencies found for *LDH-1* and *CPK-1* is not unexpected. On the other hand, there are many examples of distinct duplicate loci in salmonids sharing electrophoretically identical alleles e.g. MDH in rainbow trout (ALLENDORF et al. 1974) and AAT in cutthroat trout (ALLENDORF and UTTER 1976). It is therefore also not unexpected to find possible shared alleles such as those postulated at the duplicated LDH and CPK loci. It is not possible (at this time) to distinguish between the two possible models (i.e. a null allele or shared alleles) which can explain the phenotypic variation for LDH and CPK.

Population differences

No genetic variants were seen in the populations reported in this paper for the following loci — *CPK-2*, *LDH-2*, *LDH-3*, *LDH-4*, *MDH-3*, *MDH-4*, and *SOD*.

As mentioned previously, only two *LDH-1* phenotypes were found, interpreted as the homozygotes of the *100* and *240* alleles, respectively. The intermediate frequencies of these two *LDH* types in Lake Bunnersjöarna permits a comparison of other characters between these two types. A comparison of the length and weight distributions and the frequency of the *CPK-1 (115/115)* types in these two groups is presented in Table 1. Several important points can be concluded from these data. There is a non-random distribution of the two groups in the two connected lakes. This difference appears to be caused simply by the tendency for smaller fish, both within and between groups, to be found in the shallow southern lake. There is a highly significant ($P < 0.001$) difference in the frequency of the *CPK-1* types between the two *LDH-1* groups. There are no significant differences, however, between fish of the same group in different lakes with regard to the frequency of the *CPK-1 (115/115)* genotypes.

A highly interesting observation is the large difference in size between the two *LDH-1* groups as shown in this table. The weight distribution of all of the fish caught in Lake Bunnersjöarna is shown in Fig. 4a. This distribution appears to be bimodal. This bimodality becomes more striking, however, when the weight distributions of the two *LDH-1* groups are plotted separately as in Fig. 4b. The mean difference of these two groups were statistically significant when compared in parametric tests. Because of the skewed distributions, however, these distributions were also compared non-parametrically. Tests of independence, with regard to the distributions around the quartiles revealed significant differences between the two groups ($P < 0.001$).

Additional statistical examination of these data

revealed no differences in body size between the *CPK-1* phenotypes. All of the variation seemed also to be quite independent of sex. The mean ages, as read from the otoliths, were 6.0 and 6.5 years for the *LDH-1(100/100)* and *(240/240)* homozygotes, respectively. Thus, the weight differences can not simply be explained by the group with the larger mean weight being older.

The consistent differences between the two *LDH-1* groups support the interpretation that these two phenotypes correspond to the homozygotes of the two different alleles. Thus, the fish caught in the northern as well as in the southern part of Lake Bunnersjöarna seem to be a mixture of two genetically distinct populations, characterized by an absolute difference with respect to the *LDH-1* alleles and by frequency differences with regard to the *CPK-1* alleles and size distribution.

Two individuals of the *LDH-1(240/240)* type were also found in Lake Lågsjön. As in Lake Bunnersjöarna, no intermediate *LDH-1* phenotypes were seen in this lake. This finding may indicate the existence of two different populations also in Lake Lågsjön. Therefore, in the presentation of the allele frequency estimates in different populations (Table 2) the fish from Lake Lågsjön as well as from Lake Bunnersjöarna were separated with regard to the *LDH-1* pattern. As can be seen from Table 2, however, the two specimens homozygous for the *LDH-1 (240)* allele in Lake Lågsjön were of the same phenotype with regard to *CPK-1* and *AGP* as the rest of the fish in that lake.

The allele frequency estimates for all populations at the loci *LDH-1*, *AGP*, and *CPK-1* are presented in Table 2. These present data no doubt provide a very

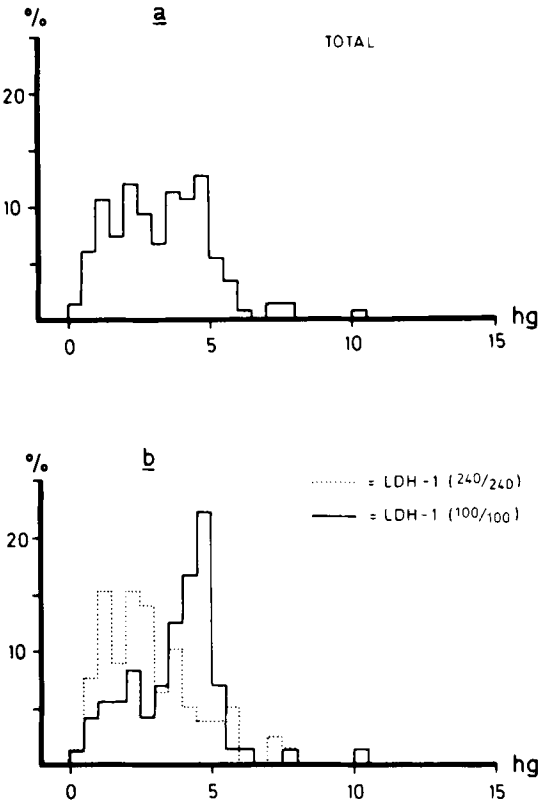

Fig. 4 a and b. Distribution of weights (hg) in the sample from Lake Bunnersjöarna; a: the total material, n = 151; b: separate distributions of the *LDH-1* genotypes.
. . . . . : *LDH-1(240/240)*, n = 79
———: *LDH-1(100/100)*, n = 72

Table 2. Allele frequencies in different populations

The frequencies of the *CPK-1* alleles were estimated from the square root of the frequency of *115/115*-homozygotes while the *AGP*-frequencies were estimated from direct "allele counting". n = number of fish. The populations of Lake Bunnersjöarna and Lake Lågsjön were separated with regard to the *LDH-1* genotypes

Population	n	*LDH-1*		*CPK-1*		*AGP*	
		100	*240*	*100*	*115*	*100*	*50*
Lake Bunnersjöarna	79	–	1	0.297	0.703	1	–
" "	72	1		0.559	0.441	0.993	0.007
Lake Lågsjön	2	–	1	1		1	–
" "	159	1		1		1	
Lake Trollsvattnet	52	1		0.861	0.139	0.750	0.250
Lake Övre Hemsjön	185	1		0.524	0.476	0.997	0.003

limited view of the genetic variation present in the brown trout populations of this overall geographic area. Further studies, including both additional loci and additional lakes, are needed to gain an accurate picture of the amount and patterns of genetic diversity present in these populations. Even these very limited data, however, provide evidence for a great deal of genetic heterogeneity among these populations. The allele frequency for *CPK-1(100)* in the few populations examined varies between 0.52 and 1.00; likewise the allele frequency for *AGP(100)* varies between 0.75 and 1.00. These differences, coupled with the two genetically distinct populations in Lake Bunnersjöarna, reveal the presence of considerable genetic differentiation among these populations.

Discussion

The most important conclusion reached in this paper is the existence of two genetically isolated populations of brown trout in both parts of a twin lake. A large part of the evidence for this conclusion, however, is based on the interpretation of electrophoretic phenotypes confounded by duplicate loci. Controlled inheritance experiments are needed to confirm our genetic interpretations, especially for the LDH phenotypes.

It may be worthwhile to examine these data considering the possibility that our interpretation of the LDH phenotypes is wrong, i.e. the heterozygotes for *LDH-1* are present in Lake Bunnersjöarna but for some reason they are not detectable by our screening procedure. One possible explanation of how this could occur is a polymorphism for a recessive suppressor at a locus involved in the regulation of *LDH-1*. Because of the substantial regulatory differentiation of the duplicated LDH loci in salmonids (see MARKERT et al. 1975), the possibility of polymorphisms at regulatory loci, as well as structural loci, should be kept in mind.

If we ignore the absence of *LDH-1* heterozygotes, and assume the fish sampled from Lake Bunnersjöarna are from a single panmictic population, we still have to consider the significant associations between *LDH-1* phenotype and both weight and *CPK-1* allele frequencies. The weight difference between these two groups is on the order of 30%. Since body size is a polygenic trait it is reasonable to assume that this size difference reflects genetic differences at multiple loci. It is difficult to accept that a single difference at an isozyme locus could have such a large effect on a polygenic trait such as body size. This difference, combined with the nonrandom association of *LDH-1* and *CPK-1* types, leads to the conclusion that these two groups of fish differ at many loci. Therefore, even when we ignore the absence of *LDH-1* heterozygotes, the differences found in these fish appear to indicate the existence of two genetically distinct populations within this lake.

This paper has presented evidence of two genetically distinct populations of brown trout within a single lake on the basis of allele frequencies and size distribution. Although of interest in itself, this observation is more important in terms of possible future studies based on this initial finding. Are these two populations representatives of two widespread sibling species or are they a restricted locally evolved phenomenon? A more extensive analysis of a series of diverse brown trout populations is required to answer this question.

Regardless of whether these two populations represent widespread sibling species or are a local phenomenon, the study of the interactions between these populations is of great interest. What are the mechanisms of genetic isolation? Is there any gene flow between these two populations? If there is, can we gain any information pertaining to the adaptive significance of these allelic variants by a comparison of their relative rates of introgression? If these two isolates represent two widespread sibling species of brown trout, then these questions can be pursued in several different geographical zones of contact.

There is a strong need for a detailed examination of the fish in Lake Bunnersjöarna. We intend that this further examination should include (1) the extension of the number of genetic loci that can be compared between these two groups by electrophoresis, (2) studies detailing possible isolating mechanisms between these two groups, and (3) the identification of any possible ecological specializations that may exist between these groups. The presence of both types of *LDH-1* homozygotes in Lake Lagsjön makes this lake also of interest for further studies.

The understanding of the biological diversity within a species is very important from a fishery management point of view. It is critical to know whether the species in question is separated into different reproductively isolated and ecologically specialized distinct units or not. If the species is separated, how many distinct units are there and in which ways are they different?

A very important consideration is the source of the size difference between the two groups of fish in Lake Bunnersjöarna. Do the fish within these groups have different growth rates or do they have a different

maximum life span? The fishery management implications of these two possibilities are obviously extremely different.

It may be possible that these two groups represent two ecological specializations. These specializations could depend on such factors as food preferences or spawning place (e.g. stream versus lake spawning). If such large differences exist among these brown trout stocks then it is very important to match the genotype of the planted fish with the particular environment in which they are planted.

Another important management consideration is the effect of hatchery-planted fish on existing native stocks. Such plantings can result in the breakdown of naturally existing reproductive isolating mechanisms (BEHNKE 1965) and lead to the loss of this genetic diversity and the possible elimination of locally adapted stocks.

The present study has provided some highly interesting results which have a number of important evolutionary and fishery management implications. We intend that the further study of these populations should provide answers to many of the questions that we have raised in this paper.

Acknowledgments. — We are indebted to Indalsälvens Vattenregleringsföretag, Jämtlands läns Sportfiskeklubb, The Local Board of Agriculture (Jämtland) and especially to fishery consultant Richard Öhman for their guidance and extensive help with the field work. The investigation was supported by a grant from The Swedish Natural Science Research Council (B 3746-002).

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., 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

ALM, G. 1946. Reasons for the occurrence of stunted fish populations. — *Medd. Statens Undersökn. Försöksanst. Sötvattenfisket Stockh. 25*: 1—146

ALM, G. 1949. Influence of heredity and environment on various forms of trout. — *Rep. Swed. State Inst. Fresh-Water Fishery Res. 29*: 29—34

ALM, G. 1959. Connection between maturity age and size in fishes. — *Rep. Swed. State Inst. Fresh-Water Fishery Res. 40*: 6—145

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

BEHNKE, R. J. 1965. A systematic study of the family Salmonidae with special reference to the genus *Salmo*. — *Ph. D. Thesis, Univ. California, Berkeley*

BEHNKE, R. J. 1968. Rare and endangered species: the native trouts of North America. — *Proc. West. Ass. State Game Fish Comm. 48*: 530—533

BEHNKE, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. — *J. Fisheries Res. Board Can. 29*: 639—671

CHERFAS, B. I. 1972. Genetics, selection and hybridization of fish. — *Israel Program for Scientific Translations, Jerusalem, 269*

DONALDSON, L. R. and OLSON, P. R. 1955. Development of rainbow trout brood stock by selective breeding. — *Trans. Am. Fisheries Soc. 85*: 93—101

EPPENBERGER, H. M., SCHOLL, A. and URSPRUNG, H. 1971. Tissue-specific isoenzyme patterns of creatine kinase (2.7.3.2) in trout. — *FEBS Letters 14*: 317—319

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

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

KIRPICHNIKOV, V. S. 1968. Heterosis, inbreeding depression and homeostasis in *Cyprinus carpio* L. populations. — *Proc. 12 Int. Congr. Genet. 1*: 236

LINDSTRÖM, T. 1973. Life in a lake reservoir: Fewer options, decreased production. — *Ambio 2*: 145—153

MARKERT, C. L., SHAKLEE, J. B. and WHITT, G. S. 1975. Evolution of a gene: Multiple genes for LDH isozymes provide a model of the evolution of gene structure, function, and regulation. — *Science 189*: 102—114

MAY, B. M., UTTER, F. M. and ALLENDORF, F. W. 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. Hered. 66*: 227—232

MOAV, R., HULATA, G. and WOHLFARTH, G. 1975. Genetic differences between the Chinese and European races of the common carp. I. Analysis of genotype-environment interactions for growth rate. — *Heredity 34*: 323—340

NENASHEV, G. A. 1966. The determination of heritability of different characters in fishes. — *Genetika 11*: 100—108

NYMAN, L. 1972. A new approach to the taxonomy of the "*Salvelinus alpinus* species complex". — *Rep. Inst. Freshw. Res. Drottningholm 52*: 103—131

OHNO, S. 1974. Animal cytogenetics. — In *Vol. 4: Chordata 1: Protochordata, Cyclostomata and Pisces, Gebrüder Borntraeger, Berlin*

RICHMOND, R. C. 1972. Enzyme variability in the *Drosophila willistoni* group. III. Amounts of variability in the superspecies *D. paulistorum*. — *Genetics 70*: 87—112

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. 1972a. An analysis of growth capability in full sib families of Salmon (*Salmo salar*, L.). — *Hereditas 70*: 119—127

RYMAN, N. 1972b. An attempt to estimate the magnitude of additive genetic variation of body size in the guppy-fish, *Lebistes reticulatus*. — *Hereditas 71*: 237—244

RYMAN, N. 1973. Two-way selection for body weight in the guppy-fish, *Lebistes reticulatus*. *Hereditas 74*: 239 – 246

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

SVÄRDSON, G. 1970. Significance of introgression in Coregonid evolution. – In *Biology of Coregonid Fishes* (*Eds.* C. C. LINDSEY and C. S. WOODS), *Univ. Manitoba Press, Winnipeg*, p. 1 – 59

UTTER, F. M. and ALLENDORF, F. W. 1976. Population genetics of fish. *Fish Physiol. 6* (*in press*)

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. – *Sys. 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 – 138

WOHLFARTH, G., MOAV, R. and HULATA, G. 1975. Genetic differences between the Chinese and European races of the common carp. II. Multicharacter variation - a respons to the diverse methods of fish cultivation in Europe and China. *Heredity 34*: 341 – 350

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