GREAT LAKES FISHERY COMMISSION

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Biochemical Genetic Evidence For Fish Stocks In Lake Trout, (Salvelinus namaycush), Bloater (Coregonus hoyi), and Brook Trout (Salvelinus fontinalis) In The Great Lakes Region

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May 1983

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BIOCHEMICAL GENETIC EVIDENCE FOR FISH STOCKS IN LAKE TROUT (*Salvelinus namaycush*), BLOATER (*Coregonus hoyi*), AND BROOK TROUT (*Salvelinus fontinalis*) IN THE GREAT LAKES REGION

by

Brian M. DuVall

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ABSTRACT

Electrophoretic analysis of polymorphic isoenzyme systems in lake trout (Salvelinus namaycush) from the Apostle Islands, Lake Superior, in the bloater (Coregonus hoyi) from the western region of Lake Michigan, and in brook trout (Salvelinus fontinalis) from a central Wisconsin stream and from a state hatchery, was carried out to determine if intraspecific genetic differences could be found as evidence of the existence of genetically discrete stocks in these three species. Lake trout liver tetrazolium oxidase allele frequencies were homogenous among three samples from different areas in the Apostle Islands region, but liver NADP dependent isocitrate dehydrogenase gamete frequencies were significantly different among these groups of lake trout. Bloater muscle glycerol-3-phosphate dehydrogenase allele frequencies were homogenous among five samples collected over the range from Algoma to Kenosha, Wisconsin. Bloater muscle isocitrate dehydrogenase allele frequencies differed among all sample pairs on the basis of a best-fit genetic model. The latter differences support data of the Wisconsin Department of Natural Resources on the possibility of separate stocks of bloaters based on different age composition data of samples from various areas of western Lake Michigan. Samples of brook trout from Brewer Creek, Juneau County, Wisconsin collected in the year before and after stocking was discontinued were compared genetically with hatchery fish from Langlade Hatchery, Langlade County, Wisconsin on the basis of malate dehydrogenase, glycerol-3-phosphate dehydrogenase, and lactate dehydrogenase. Allele frequencies were significantly different in at least one system among all three samples. Nei similarity indices indicated that the hatchery sample was most similar to the stream sample collected before stocking was discontinued and that the two stream samples,

collected before and after stocking was discontinued, were most dissimilar. These data indicate a genetic effect of stocking on stream populations.

ACKNOWLEDGEMENTS

I wish to thank Dr. Henry Booke and Ann DuVall for their patience, advise and encouragement throughout this study. I also wish to thank Dr. Daniel Coble, Dr. Fred Copes, Dr. Robert Weaver, and Dr. Douglas Post for their guidance and review of the maunscript. The Great Lakes Fishery Commission provided financial support for this study.

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INTRODUCTION

The objective of this study was to determine if intraspecific genetic differences could be found among samples of lake trout (Salvelinus namaycush) from Lake Superior, bloaters (Coregonus hoyi) from Lake Michigan, and hatchery and wild brook trout (Salvelinus fontinalis) from central Wisconsin as evidence of the existence of genetically discrete stocks in these three species. A stock, or population, is here defined as a group of individuals of the same species in which reproduction occurs at random. In other words, any mature individual has an equal probability of reproducing with any other mature individual of the opposite sex.

Until recently, attempts to characterize discrete stocks of fish involved mainly comparisons of morphological and meristic characteristics. Thurston (1962) and Kahn and Qadri (1970) described differences of this sort between several forms of lake trout. However, there is no way of knowing the extent to which these characteristics are affected by environmental conditions; hence, the identification of genetically discrete stocks cannot be reliably accomplished by this method (Gottlieb 1971). Tagging fish remains valuable for stock identification studies but requires intensive sampling and large numbers of fish (Mourant 1970). Tagging also does not yield information about genetic uniqueness among stocks.

Electrophoresis of polymorphic isoenzyme systems has become a useful tool in the investigation of salmonid stock structures of, among others, lake whitefish (Coregonus clupeaformis) (Imhof et al. 1980), brook trout (Krueger and Menzel 1979), and pink salmon (Oncorhynchus gorbuscha) (Aspinwall 1973). Electrophoresis differentiates between stocks on the basis of the frequency of occurence of distinct alleles through their products (enzymes) and minimizes the influence of environmental conditions. Because reproduction within a stock

occurs at random, the stock behaves evolutionally as a discrete unit. Allele frequencies at a given genetic locus obtained from subsamples of a stock should be the same, whereas those from separate stocks may show differences. Significant departure from homogeneity between samples is taken as evidence that they did not originate from the same stock. Homogeneity between samples is, however, not taken as complete evidence that they originated from the same stock because a small portion of the genome is being assayed. In the absence of complete knowledge of the factors that could potentially serve to isolate stocks from one another, it is important to sample fish during the time they reproduce. If the individual stocks exist within a species, then they must be reproductively isolated from one another by spatial, temporal, or behavioral isolating mechanisms. Collecting fish during the spawning season maximizes the possibility of obtaining fish from individual stocks rather than a random mixture.

Lake trout in Lake Superior have a number of characteristics indicating a complex structure and a high degree of reproductive isolation. Fishermen have recognized at least four distinct forms of lake trout from Lake Superior: lean, fat (siscowet), humper, and halfbreed. These appear to differ from one another in morphology, habitat selection, reproductive characteristics, and physiology (Eschmeyer and Phillips 1965; Pycha and King 1975). Further evidence in support of the existence of various stocks of lake trout in Lake Superior is their tendency to home to a spawning site after wide dispersal for most of the year (Martin 1960; Lawrie and Rahrer 1973; Loftus 1977; Bruce Swanson, Wisconsin Department of Natural Resources, Personal Communication).

Collections of bloaters from various areas of western Lake Michigan have been found to have different age structures among samples suggesting

the possibility of discrete stocks in the species. Schultz (1978) found that bloaters taken from the northern part of Lake Michigan near Door and Kewaunee Counties, Wisconsin were dominated by older fish than samples of bloaters from the southern nearshore waters which had a more even age distribution dominated by 3- to 6- year olds. Bloaters taken from the Milwaukee-Sheboygan reef complex further offshore had an age composition dominated by 6- and 7- year olds with a significant number of 4- year olds.

Concern has been expressed for the effect of stocking on the genetic composition of natural fish stocks (Miller 1957; Calaprice 1969). Genetic change might come as a result of interbreeding, creation of new selective pressures through undefined interactions, and displacement of all or part of a resident stock by hatchery fish of a different genetic character. Brook trout in Wisconsin have supported an extensive sport hatchery for many years and stocking of cultured trout to supplement natural populations has been a common practice since the early part of this century (Krueger and Menzel 1979). In Brewer Creek, Juneau County, Wisconsin, brook trout stocking began in 1966 and, except for a three year break from 1969 through 1971, continued every year until 1978. Hatchery fish were supplied by eight different hatcheries during this time and originated from the Langlade Hatchery, Langlade County, Wisconsin in 1978. Brook trout from this hatchery had a more pronounced tail fork than did those in the stream in the fall of 1978, suggesting that the hatchery and resident brook trout in the stream might be genetically different.

METHODS

The lake trout were collected by personnel of the United States Fish and Wildlife Service or were bought from commercial fishermen (Figure 1). Bloaters were obtained from commercial fishermen by personnel of the Wisconsin Department of Natural Resources (Figure 2). All samples of these two species were collected as close to their respective spawning times as was possible (Table 1). The two samples of wild brook trout from Brewer Creek were selected on the basis of tail fork characteristics and only fish smaller than those being stocked in the stream were taken. The localities and sample data are given in Table 1.

Fish were either iced or frozen immediately after capture, and after transportation to the University of Wisconsin, Stevens Point, they were stored at -20° C. White muscle, liver, and whole eye samples were obtained by excising one to two grams of tissue from partially thawed specimens. Tissue samples were placed in chilled 15- ml ground glass homogenizers to which was added 5 ml of distilled water containing nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) at a concentration of approximately 15 mg of each per 200 ml of water. After grinding, the homogenates were poured into 5- ml sealable plastic tubes for storage at 20° C. Liver homogenates were centrifuged at 14,500 g for one hour before storage.

Lake trout liver NADP-dependent isocitrate dehydrogenase (IDH) and tetrazolium oxidase (TO), bloater white muscle IDH and glycerol-3-phosphate dehydrogenase (G-3-PDH), and brook trout white muscle malate dehydrogenase (MDH), G-3-PDH, and whole eye lactate dehydrogenase (LDH) isoenzymes were separated with vertical, starch-gel electrophoresis in a Buchler apparatus at 20-25 mA/gel for 16 to 18 hours in an environmental chamber at 4° C. All enzyme systems used were chosen on the basis of repeatable electrophoretic

Figure 1. Map of Apostle Islands region of Lake Superior showing collecting locations for 3 lake trout samples used for electrophoresis. See Table 1 for description of areas.

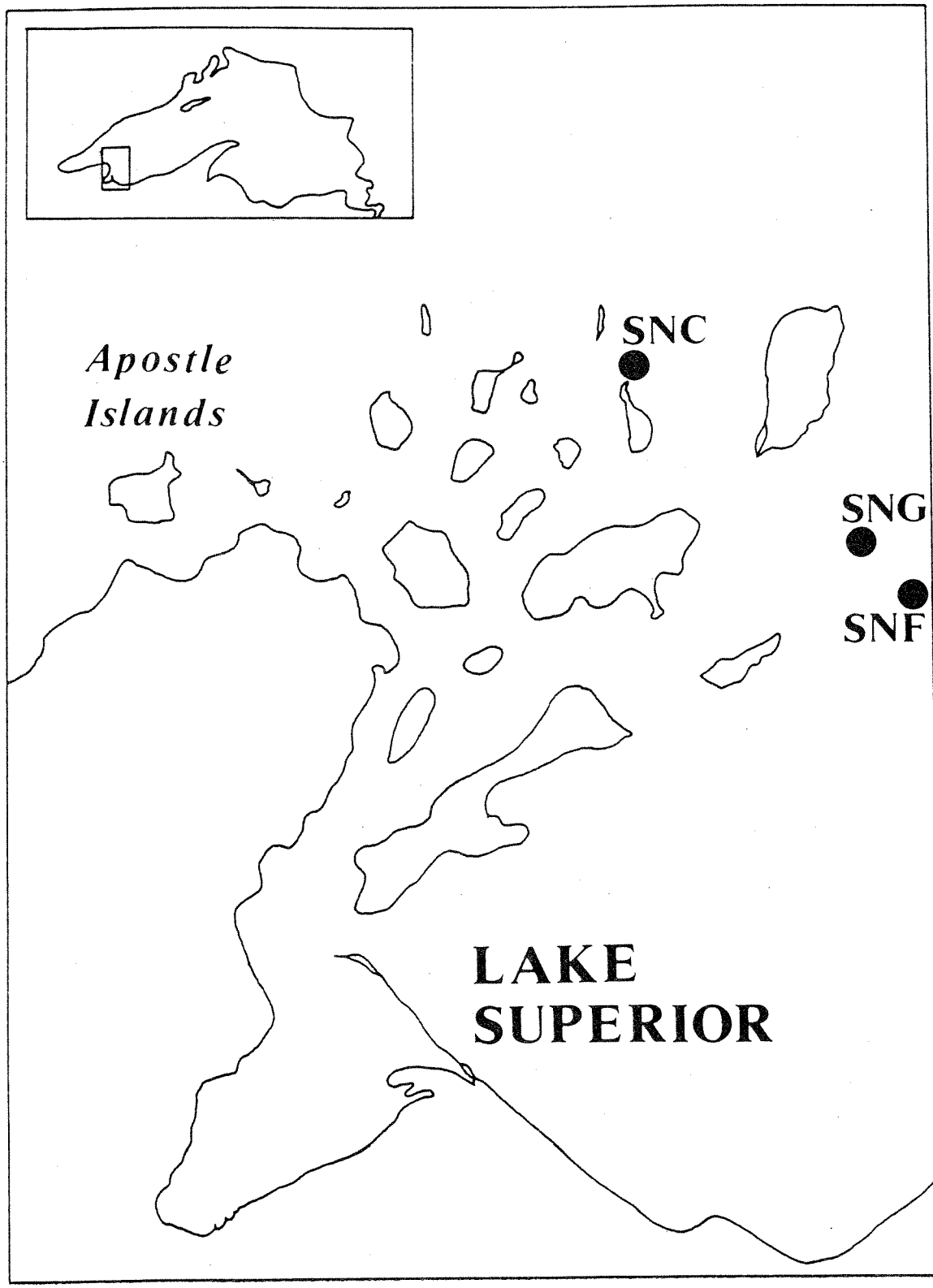

Apostle
Islands
SNC
SNG
SNF
LAKE
SUPERIOR

Figure 2. Map of Lake Michigan showing collecting locations for 5 bloater samples used for electrophoresis. See Table 1 for description of areas.

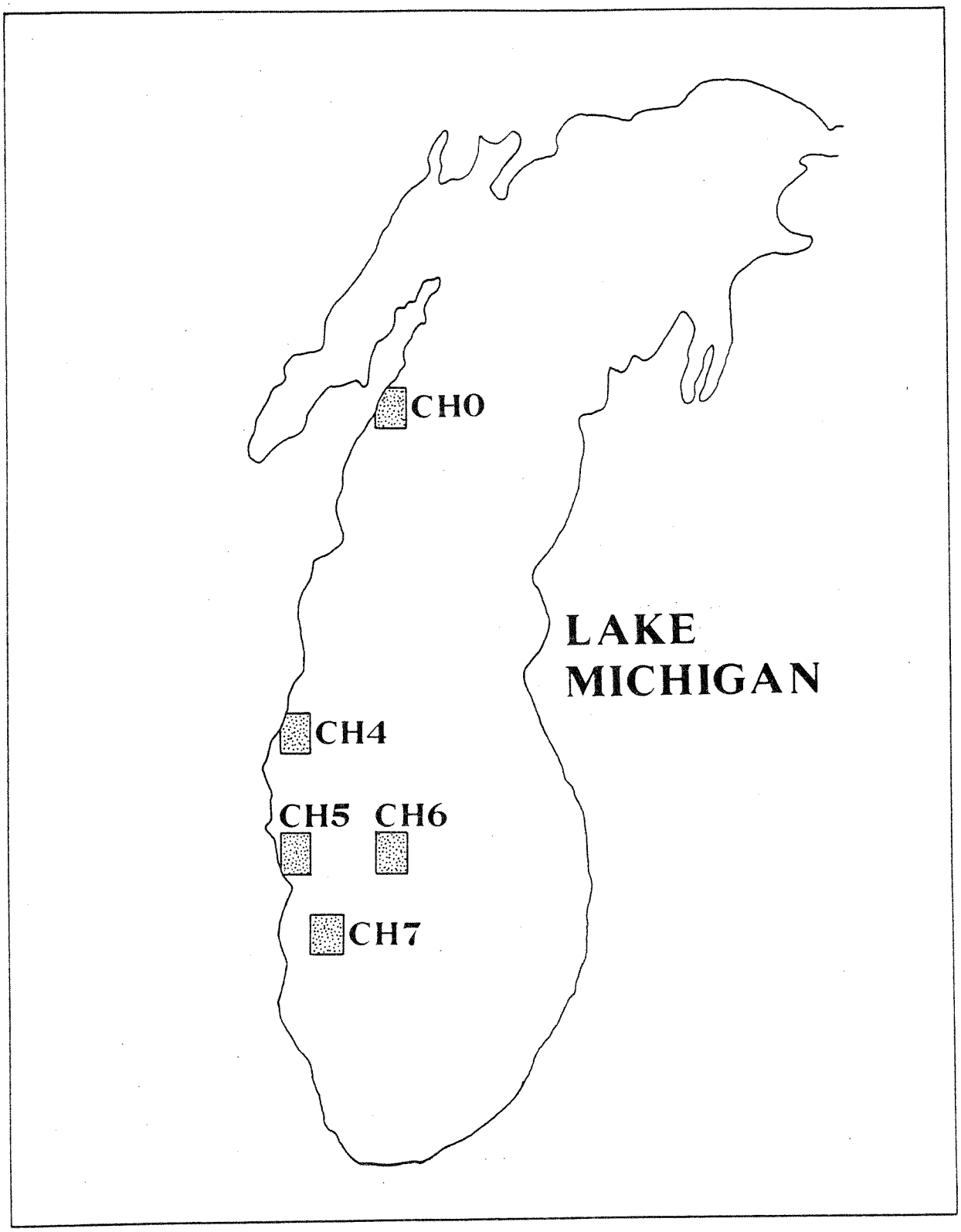

CH0
LAKE
MICHIGAN
CH4
CH5
CH6
CH7

Table 1. Sources of lake trout, bloaters, and brook trout used in electrophoretic analysis.

Species	Sample	Sample[a] Size	Date of Collection	Location[b]	Depth m
Lake trout					
lean	SNG	76	10:X:78	Apostle Islands Gull Island Shoal	5-7
lean	SNC	85	14:X:79	Apostle Islands Cat Island, north end	5-7
siscowet	SNF	66	17:X:79	Apostle Islands 6 mil. NE of Michigan Island	74-92
Bloater					
	CH0	98	17:XII:79	grid 905	64-73
	CH4	100	12:XII:89	grid 1702	79-86
	CH5	99	07:XII:78	grid 2002	82-90
	CH6	106	05:XII:78	grid 2005	69-90
	CH7	89	11:XII:78	grid 2203	71-77
Brook trout					
	SFL	78	24:X:78	Langlade Hatchery Langlade County	-
	SFB1	75	14:XI:78	Brewer Creek Juneau County	-
	SFB2	37	15:IV:80	Brewer Creek Juneau County	-

a All samples of lake trout and bloaters were collected with gillnets. Brook trout samples were collected with a 110 v DC electroshocker.

b Lake trout samples were from the Apostle Islands region of Lake Superior (Figure 1). Bloater samples were from western Lake Michigan (Figure 2). Brook trout samples were obtained from Langlade Hatchery, Langlade County, Wisconsin and collected from Brewer Creek, Juneau County, Wisconsin. Grid numbers refer to the Wisconsin Department of Natural Resources grid reporting system, Fish Management Handbook, November, 1973.

patterns, the presence of polymorphism, and the potential for logical genetic interpretation. A summary of enzyme systems not used for statistical analysis is presented in Appendix 2. Gel preparation and buffer systems were the same as those described by Clayton and Gee (1969), Clayton et al. (1971), and Clayton and Tretiak (1972). Electrophoresis gel buffers were adjusted to pH 8.2 for lake trout liver IDH and bloater muscle G-3-PDH, pH 7.8 for brook trout muscle MDH, and pH 5.5 for lake trout liver TO. Buffer systems at pH 6.9 and above consisted of citric acid and tris-hydroxymethylaminomethane combination and those below pH 6.9 consisted of a citric acid and N-3(amino-propyl)morpholine (Clayton and Tretiak 1972). Electrostarch from the Electro-starch Company of Madison, Wisconsin was used; lot #307 was used for bloaters and lot #392 for lake trout and brook trout. Lot #392 had substantially reduced resolution capabilities compared to lot #307 in all isoenzymes that were separated.

After electrophoresis was terminated, gels were trimmed, sliced, and placed in the appropriate staining solution for 4 hours in an incubator at 37^{o} C, after which, stain was poured off and replaced with a 5:5:1 preserving solution of water: methanol: and acetic acid. After 24 hours, the gels were stored in plastic bags at room temperature. The basic stain buffer consisted of 6.25 g of diethanolamine in 500 ml of water (pH 9.0 HCl) per gel. Stain ingredients for the 5 enzyme systems used in this study are given in Table 2.

The electrophoretic phenotypes were scored on the basis of genetic models. In the absence of breeding data, the genetic models used were assumed correct if the observed phenotypes complied with the requirements developed by Utter et al. (1974):

1. Band patterns must be repeatable with subsampling of the same

Table 2. Starch gel stain ingredients[a].

System[b]	Substrate	Cofactor	Coenzyme	Stain
IDH	DL-isocitric acid trisodium salt 1g	$MgCl_2$ 0.1 g	B-NADP 20 mg	nitro blue tetrazolium 15 mg phenazine methosulfate 5 mg
G-3-PDH	B-glycerophosphate disodium salt 9g	-	B-NAD 20 mg	nitro blue tetrazolium 15 mg phenazine methosulfate 5 mg
LDH	DL-lactic acid 3g	-	B-NAD 20 mg	nitro blue tetrazolium 15 mg phenazine methosulfate 5 mg
MDH	DL-malic acid 5g	-	B-NAD 20 mg	nitro blue tetrazolium 15 mg phenazine methosulfate 5 mg
TO	-	-	-	nitro blue tetrazolium 30 mg phenazine methosulfate 10 mg

a All quantities are on a per gel basis. Basic stain buffer consisted of 6.25 g of diethanolamine in 500 ml of distilled water (pH 9.0 HCl).

b IDH = isocitrate dehydrogenase, G-3-PDH = glycerol-3-phosphate dehydrogenase, LDH = lactate dehydrogenase, MDH = malate dehydrogenase, TO = tetrazolium oxidase.

tissue of an individual.

2. The bands resolved must fit the interpretable patterns based on simple genetic hypotheses.

In order to compare allele frequencies statistically among samples of fish, a chi-square contingency table was prepared for all possible sample pairs for each enzyme system from each species. A normalized Nei index of genetic similarity, which estimates the proportion of alleles that are identical between two samples, was calculated for intraspecific sample pairs (Nei 1972, 1975). Nei indices for bloaters were clustered into genetically similar groups (average linkage-merged groups method; Fred Hilpert, University of Wisconsin Computer Services, Stevens Point, Wisconsin, Personal Communication).

RESULTS AND DISCUSSION

Lake Trout

Chi-square analysis of the lake trout IDH gamete numbers indicated that all three samples originated from different stocks. Lake trout IDH electropherograms contained five phenotypes, three 3-banded patterns and two 1-banded patterns (Figure 3). Assuming dimeric molecular structure (Henderson 1965), I interpreted these patterns to represent two loci each with two alleles. The slower moving alleles of both loci overlapped in electrophoretic mobility, as did the faster moving alleles, making it impossible to assign a genotype to all observed band patterns. For example, an S2F2 pattern can be produced by a fish possessing the genotype SS FF or SF SF. Similarly, the SF3 and S3F patterns can each be produced by two different genotypes. The chi-square statistic can only deal with one locus at a time, and the overlapping phenotypes in this system make it impossible to separate the variation at the two loci into its component parts. A maximum liklihood estimation of gamete frequencies from the observed phenotypic distribution allowed the two loci to be treated as one (Crow and Kimura 1970; Imhof et al. 1980). Gamete frequencies were used for statistical comparisons rather than allele frequencies (Table 1 Appendix). Chi-square analysis of the lake trout IDH gamete numbers indicated significant departure from homogeneity at the 5% significance level among all three samples (Table 3).

Lake trout liver tetrazolium oxidase phenotypes consisted of two 3-banded patterns and one 5-banded pattern (Figure 4). I have interpreted these patterns to represent two loci, one, A, with two alleles, A^1 and A^2, and the other, B, monomorphic. In rainbow trout (Salmo gairdneri) and Pacific salmon (Oncophynchus spp.), TO exhibits similar patterns, indicative

Figure 3. Lake trout liver isocitrate dehydrogenase (IDH) phenotypes and hypothesized sets of possible genotypes.

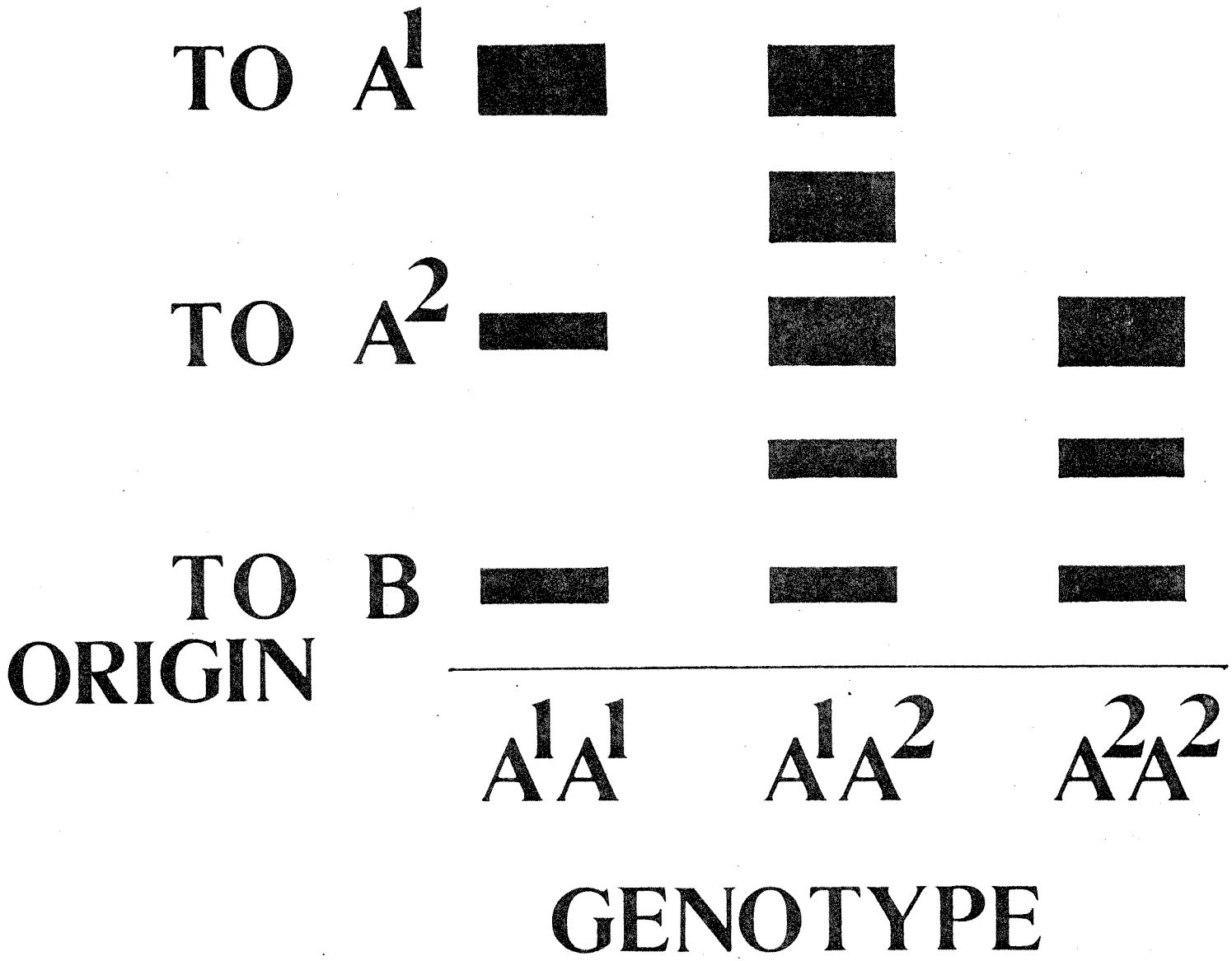
ORIGIN
FFFF
SFFF
SSFF
SSSF
SSSS
FFSF
SFSF
SFSS
GENOTYPES

Table 3. Paired X^2 and Nei Index of Similarity for TO and IDH among three lake trout samples. (Refer to Table 1 for sample designations; * significant at P = 0.05, ** significant at P = 0.01; IDH d.f. = 2, TO d.f. = 1).

Sample		SNG	SNC	SNF
			X^2	
SNG	TO		3.13	3.05
	IDH		27.81**	30.17**
SNC	TO			0.00
	IDH	.98721		28.47**
SNF	TO			
	IDH	.97719	.96385	

Nei Index of Similarity (I)

Figure 4. Lake trout tetrazolium oxidase (TO) phenotypes and hypothesized genotypes.


TO A^1
TO A^2
TO B
ORIGIN
A^1A^1
A^1A^2
A^2A^2
GENOTYPE

of a dimeric molecular structure (Utter 1971; Utter and Hodgins 1972: Cedarbaum and Yoshida 1972). The polymorphic A locus was significantly correlated with Hardy-Weinberg equilibrium supporting the proposed genetic model. No A^2A^2 homozygotes were present in the 227 fish assayed for TO and I assume that had more fish been available this genotype would have been found (allele numbers and frequencies given in Table 2 Appendix).

A chi-square analysis of the TO A-locus allele numbers indicated no significant departure from homogeneity at the 5% significance level in a 2 by 3 contingency table or in individual analysis of all possible sample pairs (Table 3). Therefore, the TO A-locus did not provide evidence that the three lake trout samples originated from distinct stocks. However, there was the suggestion of a difference in that the Cat Island sample (SNC) contained the A^2 allele at a frequency of .09 compared to the other two samples in which it was present at a frequency of .04. Comparisons between the sample pairs SNC/SNG and SNC/SNF were close to being statistically significant but the overall low frequency of the A^2 allele tended to mask its effect in the chi-square analysis.

Nei similarity indices calculated with the TO A and B loci and IDH gamete frequencies ranged from .96385 to .98721 and indicated that the siscowet sample (SNF) was less similar to the two lean trout samples than they were to each other (Table 3). These differences were characterized by variations in allele frequencies and did not involve unique alleles or altered mobilities in the siscowet sample. The Nei similarity index, in this case, cannot be used as a systematic indicator of the subspecific taxonomic status of the siscowet (Scott and Crossman 1973) because of the small number of genetic loci used to calculate it. Selection of enzyme systems used in this study was on the basis of the presence of polymorphism.

The group of enzyme loci assayed was neither random nor large, two criteria that are necessary for the Nei index to take on systematic value (Avise 1976). However, the index can be used as a relative measure of similarity among these samples and enzyme systems.

Bloaters

The polymorphic G-3-PDH B allele provided no evidence of genetic differences among the five bloater samples because chi-square analysis indicated no significant departure from homogeneity (Table 4). G-3-PDH phenotypes were similar to those found in lake whitefish consisting of two loci, A and B (Clayton et al. 1973). The A locus was monomorphic and the B locus had four alleles, B_1 through B_4 (Figure 5). Staining of bands resulting from the B locus was much darker than A-locus staining making it possible to distinguish the B_1B_1 genotype from the very similar B_1B_4 genotype (allele numbers and frequencies are given in Table 3 Appendix).

Bloater white muscle IDH comparisons indicated there may be discrete stocks in Lake Michigan. Electropherograms of this system consisted of 3-banded patterns with variations in staining intenstiy producing seven different phenotypes. A genetic model developed by James Clayton (Freshwater Institute, Winnepeg, Manitoba) fits the observed phenotypes with two exceptions. The model (Figure 6) consists of two loci, 1 and 2, each with two alleles which overlap electrophoretically. Locus 2 stained less intensely than locus 1 thereby distinguishing all genotypes. Two single banded patterns produced by both double homozygotes, $\frac{BB}{AA}$ and $\frac{CC}{BB}$, are present in the model but were not found in the 492 bloaters assayed for IDH. The lack of these two phenotypes among the samples brings into question the validity of the proposed model; however, in the absence of a better model, I assumed it to be correct and treated the data accordingly

Table 4. Paired X^2 and Nei Index of Similarity (I) for G-3-PDH locus B and IDH locus 1 and 2 among five Lake Michigan bloater samples. (Refer to Table 1 for sample designations; * significant at P = 0.05, ** significant at P = 0.01; G-3-PDH d.f. = 3, IDH d.f. = 2).

Sample		CHO	CH4	CH5	CH6	CH7
				X^2		
	G-3-PDH		1.71	2.65	4.46	6.55
CHO	IDH locus 1		36.43**	8.04**	10.97**	27.16**
	IDH locus 2		15.59**	2.38	1.67	2.21
	G-3-PDH			4.15	5.78	5.18
CH4	IDH locus 1	.92938		10.64**	8.01**	0.29
	IDH locus 2			5.85*	7.26**	5.29*
	G-3-PDH				3.00	5.59
CH5	IDH locus 1	.98368	.98123		0.21	6.57*
	IDH locus 2				0.07	0.00
	G-3-PDH					1.89
CH6	IDH locus 1	.97951	.99903	.99150		4.61*
	IDH locus 2					0.07
	G-3-PDH					
CH7	IDH locus 1	.98018	.95187	.98960	.99331	
	IDH locus 2					
				Nei Index of Similarity (I)		

Figure 5. Bloater white muscle glycerol-3-phosphate dehydrogenase (G-3-PDH) phenotypes and hypothesized genotypes.

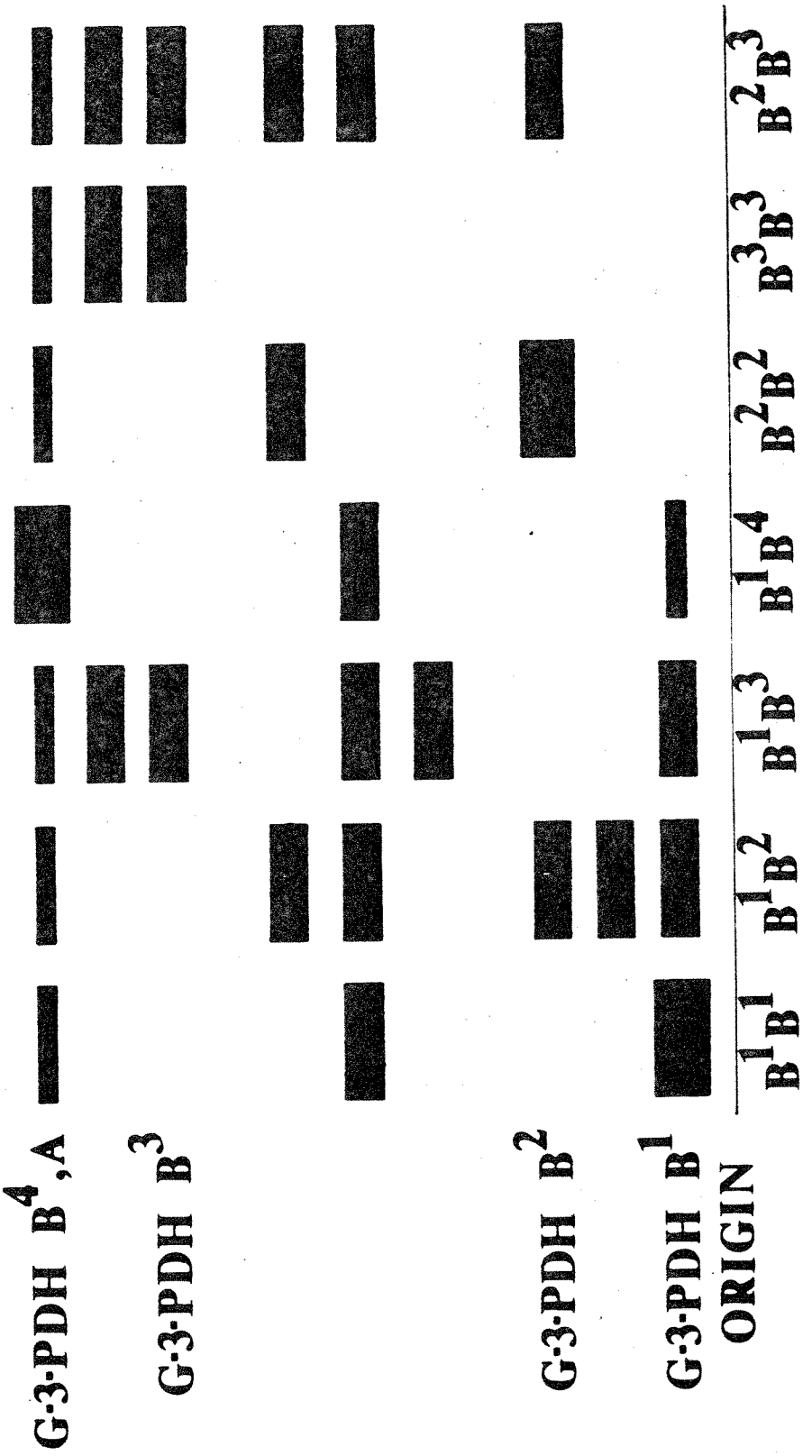
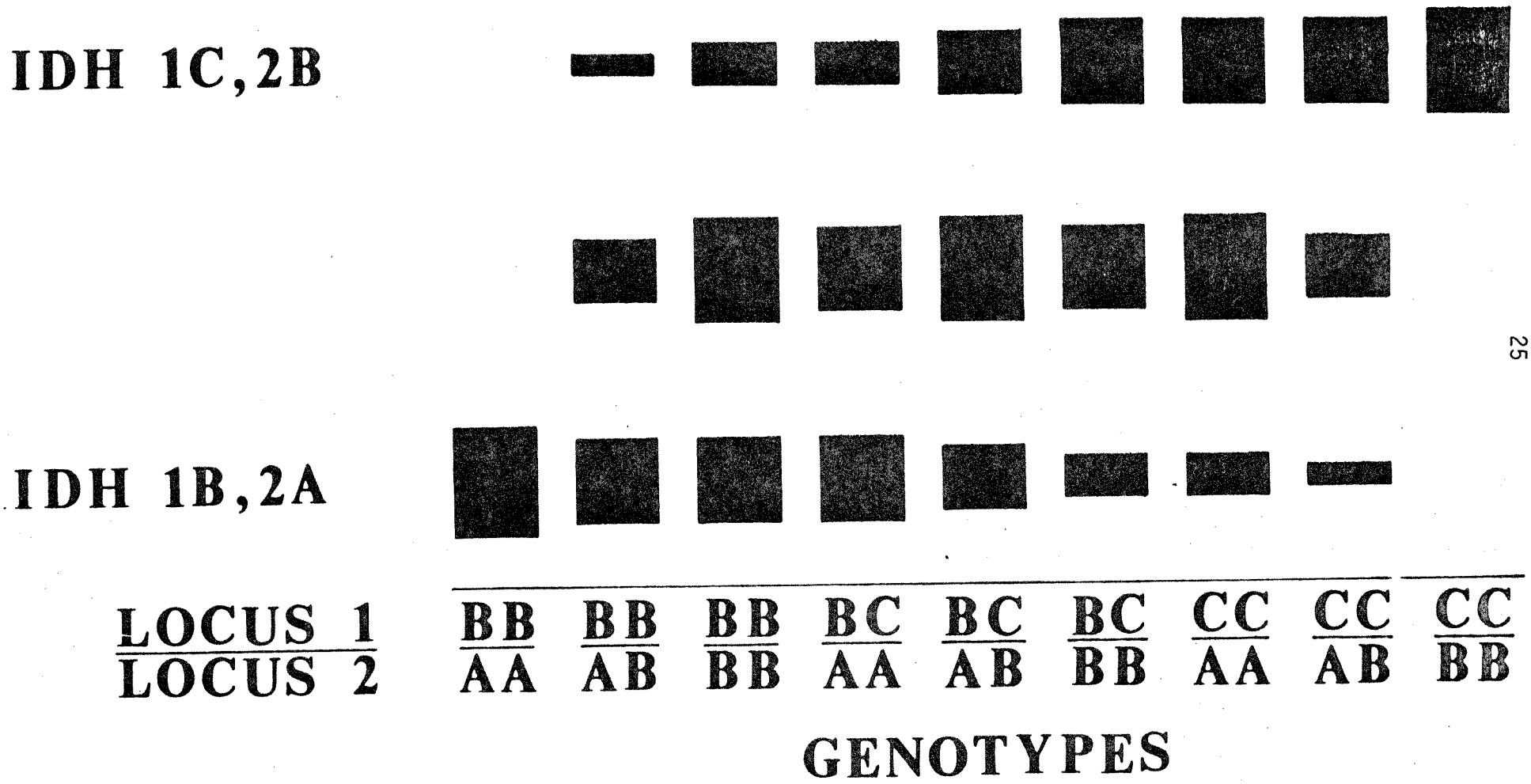

G-3-PDH B4,A
G-3-PDH B3
G-3-PDH B2
G-3-PDH B1
ORIGIN
B1B1 B1B2 B1B3 B1B4 B2B2 B3B3 B2B3
GENOTYPES

Figure 6. Bloater white muscle isocitrate dehydrogenase (IDH) phenotypes and hypothesized genotypes. Model contributed by James Clayton, Freshwater Institute, Winnepeg, Manitoba.


IDH 1C,2B
IDH 1B,2A
LOCUS 1
LOCUS 2
BB AA
BB AB
BB BB
BC AA
BC AB
BC BB
CC AA
CC AB
CC BB
GENOTYPES

(Table 4 Appendix). Chi-square analysis indicated 8 out of 10 sample pairs differed at locus one and 4 out of 10 differed at locus 2. CH5/CH6 was the only sample pair that showed no significant difference at either locus. CHO/CH4, CH4/CH5 and CH4/CH6 were the only sample pairs that differed at both loci. The fact that the genetic model for this system is tenuous makes it difficult to draw a conclusion, but I believe my data should encourage further study.

Nei indices of similarity for each sample pair were calculated from the G-3-PDH B locus and both IDH loci and ranged from .92938 to .99903 (Table 4). A dendrograph of the clustered Nei indices indicates two clusters, CH5/CH7 and CH4/CH6, with one outlying single sample, CHO (Figure 7). The sample designated CHO originated from the waters off Algoma, Wisconsin, 190 km from the center of the other four samples. CHO was also collected one year after the other four samples. It is impossible to determine whether the differences between northern and southern samples reflect temporal or spatial isolating mechanisms. The stability of allele frequencies with time is unknown; however, age composition differences between groups of bloaters (Schultz 1978) from various geographically separated areas of Lake Michigan tend to support these findings of potential genetic differences.

Brook trout

Chi-square analysis of three brook trout loci indicated significant genetic differences among both Brewer Creek samples and the Langlade Hatchery sample (Table 5). The 1978 Brewer Creek sample and the Langlade Hatchery sample were significantly different at LDH and MDH. The two Brewer Creek samples differed at MDH and the 1980 Brewer Creek sample and the Langlade Hatchery sample differed at LDH. No significant departure from homogeneity was found among

Figure 7. Dendrograph depicting cluster analysis of Nei Similarity indices from bloater sample comparisons. See Table 1 for sample designation, date, and location of collection.

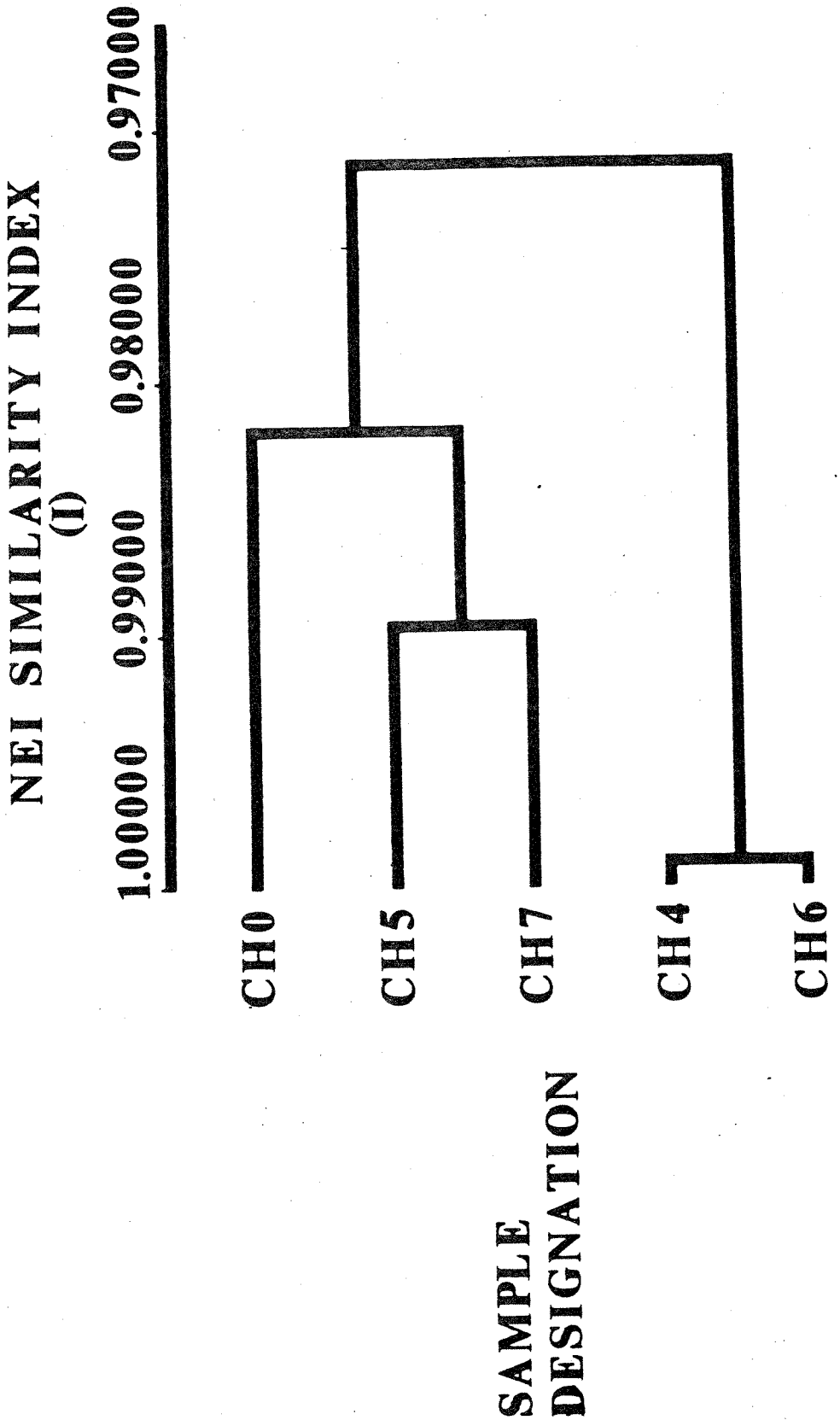

NEI SIMILARITY INDEX
(I)
1.00000
0.99000
0.98000
0.97000
CH0
CH5
CH7
CH4
CH6
SAMPLE
DESIGNATION

Table 5. Paired X^2 and Nei Index of Similarity (I) for G-3-PDH, LDH and MDH among three samples of brook trout.(Refer to Table 1 for sample designation; * significant at P = 0.05, ** significant at P = 0.01; G-3-PDH d.f. = 1, LDH d.f. = 1, MDH d.f. = 1).

Sample		SFL	SFB1	SFB2
			X^2	
	G-3-PDH		0.11	0.13
SFL	LDH		4.31*	7.25*
	MDH		3.94*	1.51
	G-3-PDH			0.37
SFB1	LDH	.99855		0.64
	MDH			6.02*
	G-3-PDH			
SFB2	LDH	.99757	.99677	
	MDH			

Nei Index of Similarity (I)

the three samples at G-3-PDH.

Band patterns of brook trout MDH and G-3-PDH both corresponded to a genetic model consisting of two loci, one with two alleles, the other monomorphic (Figures 8 and 9). Whole eye LDH produced electrophoretic patterns identical to those found by Wright and Atherton (1970). Their study indicated through breeding experiments the validity of the model consisting of three loci, A, B, and C, with three alleles at the B locus (B, B', B''). The 196 brook trout assayed for LDH in this study did not contain an individual carrying the B'' allele. Variant allele frequencies for all systems in all samples fell below .11 and in all cases except one, the homozygote was not found (allele and genotype frequency data given in Tables 5,6 and 7 Appendix). All loci in all samples were significantly correlated with a Hardy-Weinberg equilibrium which tends to support the proposed genetic models.

Nei similarity indices calculated from the three polymorphic loci indicate that the 1978 and 1980 Brewer Creek samples were the most dissimilar whereas the 1979 Brewer Creek sample and the hatchery sample were the most similar (Table 5). 1978 was the last year brook trout were stocked in Brewer Creek indicating the possibility of a genetic effect of stocking on the stream gene pool through interbreeding, creation of new selective pressures, or displacement of part of the existing population by hatchery fish of a different genetic character. After curtailment of stocking, the gene pool may have changed again in the absence of the hatchery fish. The observed genetic differences between the 1978 and 1980 Brewer Creek samples may also have been due to the instability of allele frequencies with time or the possible existence of more than one stock of brook trout in the stream.

Figure 8. Brook trout white muscle malate dehydrogenase (MDH) phenotypes and hypothesized genotypes.

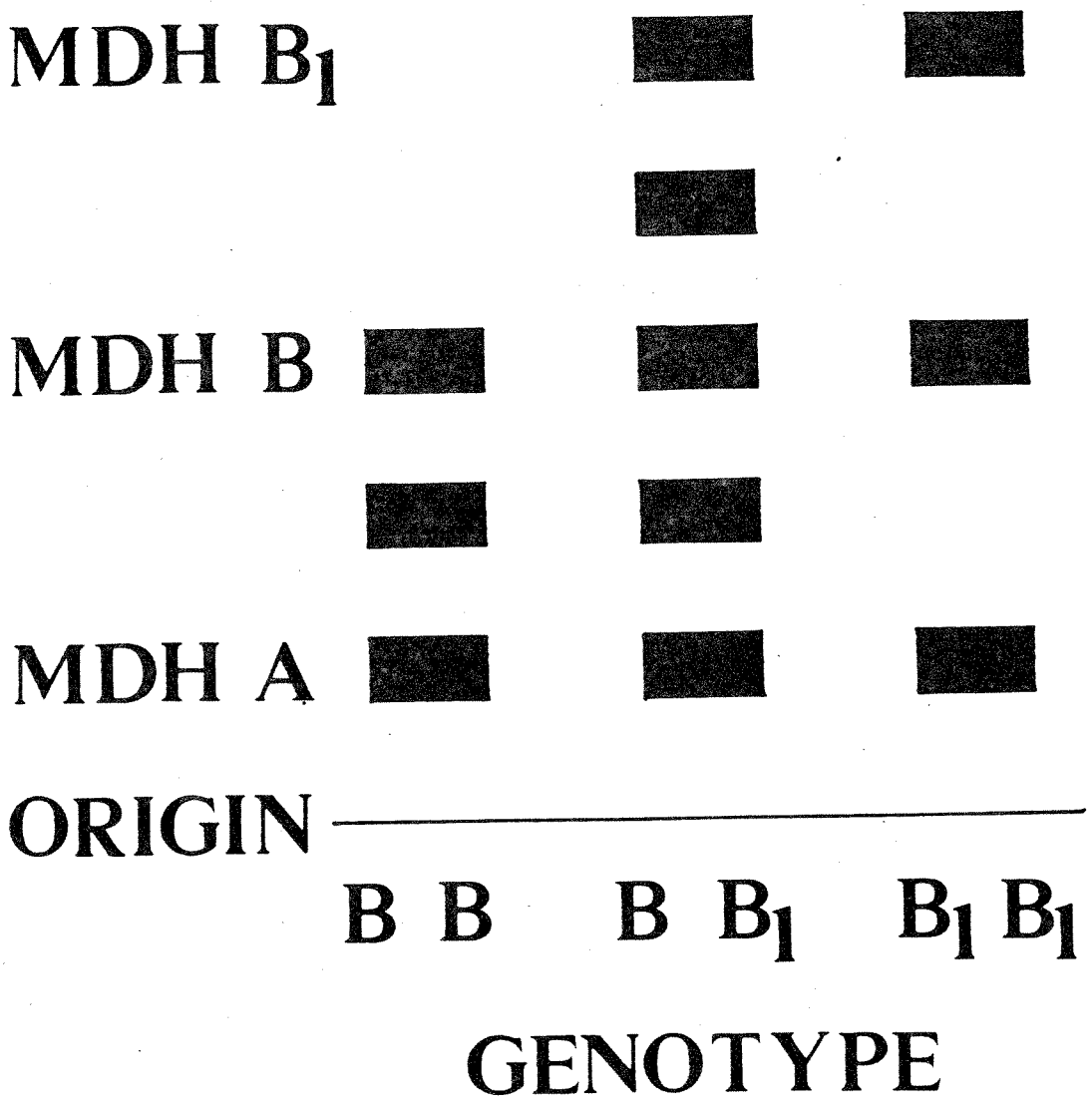
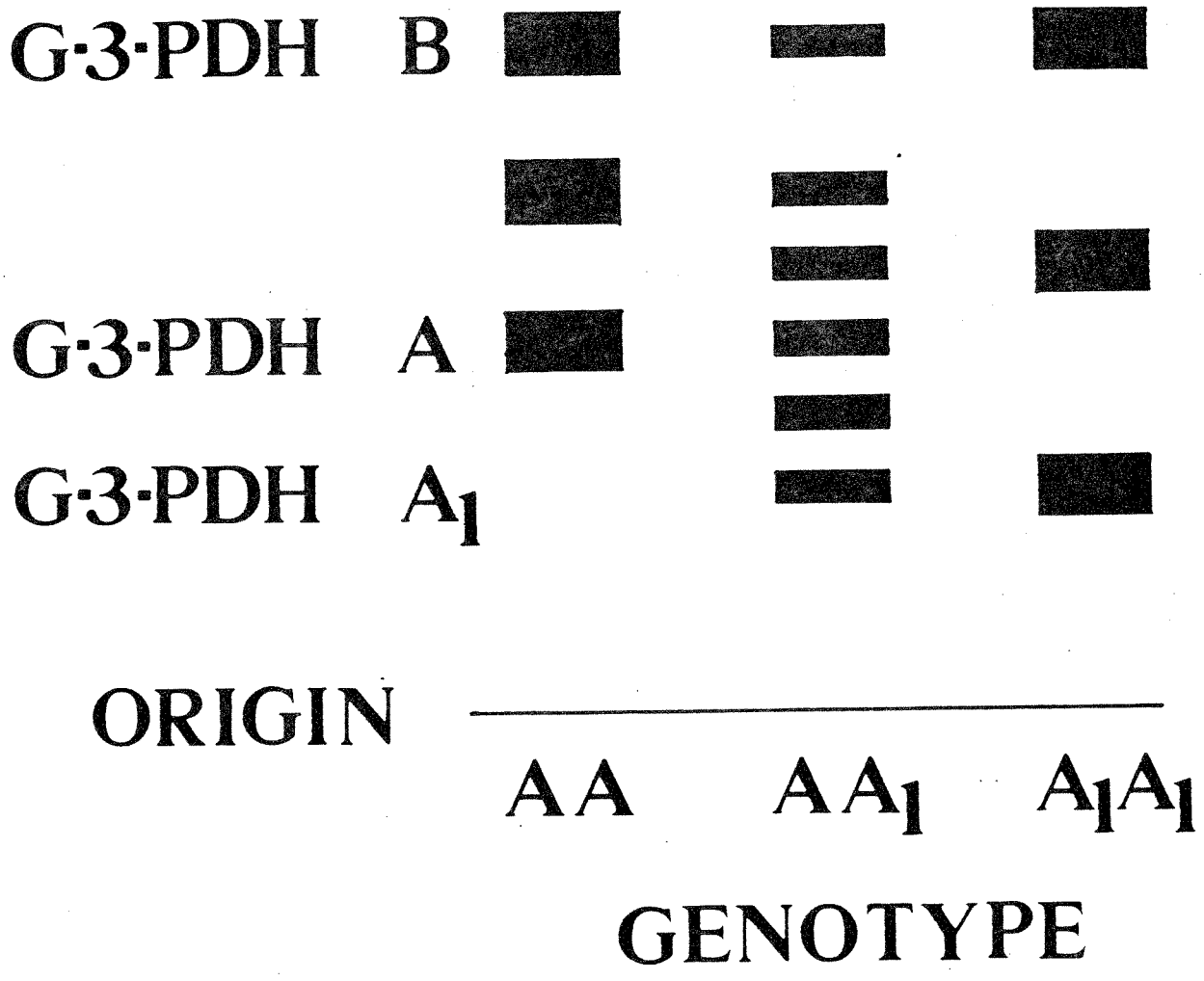

MDH B_1
MDH B
MDH A
ORIGIN
B B
B B_1
B_1 B_1
GENOTYPE

Figure 9. Brook trout white muscle glycerol-3-phosphate dehydrogenase (G-3-PDH) phenotypes and hypothesized genotypes.


G-3-PDH B
G-3-PDH A
G-3-PDH A1
ORIGIN
AA
AA1
A1A1
GENOTYPE

MANAGEMENT IMPLICATIONS

Preservation of genetic diversity is an important aspect of managing fish species. Maintaining genetic diversity may enable fish species to adapt more easily to changing environmental stresses. Commercial and sport harvest, stocking, habitat alteration, and climatic variation may affect the survival of individual stocks. For this reason, I believe it is important to identify the presence of discrete stocks of fish and to determine population dynamics and seasonal movements. Local stocks of fish may be fished out or displaced by hatchery fish unless a multi-stock management policy is adopted. Delineation of geographic ranges and susceptibility to harvest pressures and hatchery influences of individual stocks will require specific investigation. I have demonstrated the possible existence of discrete stocks of lake trout, bloaters, and brook trout as a preliminary step towards greater understanding of stock structures within these species.

LITERATURE CITED

Aspinwall, N. 1974, Genetic analysis of duplicate MDH loci in the pink salmon. Genetics 76:65-72.

Avise, J.C. 1976. Genetic differentiation during speciation, p. 106-122. In Ayala, F.J. (ed.) Molecular Evolution. Sinauer Associates Inc. Massachusettes.

Calaprice, J.R. 1969. Production and genetic factors in managed salmonid populations, p. 377-388. In J.G. Northcote (ed.) Symposium on salmon and trout fisheries. H.R. MacMillan lectures in fisheries, University of British Columbia, Vancouver.

Cederbaum, S.D., and A. Yoshida. 1972. Tetrazolium oxidase polymorphism in rainbow trout. Genetics 72:363-367.

Clayton, J.W., and J.H. Gee. 1969. LDH isozymes of longnose and blacknose dace and their hybrid. J. Fish. Res. Bd. Can. 26:3049-3053

Clayton, J.W., D.N. Tretiak, and A.H. Koozman. 1971. Genetics of multiple MDH isozymes in skeletal muscle of walleye. J. Fish. Res. Bd. Can. 28:1005-1008.

Clayton, J.W., and D.N. Tretiak. 1972. Amine citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Can. 29:1169-1172.

Clayton, J.W., W.G. Franzin, and D.N. Tretiak. 1973. Genetics of G-3-PDH isozymes in white muscle of lake whitefish. J. Fish. Res. Bd. Can. 30:187-193.

Crow, J.F., and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row, N.Y. p. 509-514.

Eschmeyer, P., and A. Phillips. 1965. Fat content of the flesh of siscowets and lake trout from Lake Superior. Trans. Am. Fish. Soc. 94:62-74.

Gottlieb, L.D. 1971. Gel electrophoresis; new approach to the study of evolution. Bioscience 21:939-944.

Henderson, N. 1965. Isozymes of IDH; subunit structure and intracellular location. J. Exp. Zool. 158:263-274.

Imhof, M., R. Leary, and H.E. Booke. 1980. Population or stock structure of lake whitefish in northern Lake Michigan as assessed by isozyme electrophoresis. Can. J. Fish. Aquatic Sci. 37:783-793.

Kahn, N.Y., and S.U. Qadri. 1970. Morphological differences in Lake Superior lake char. J. Fish. Res. Bd. Can. 27:161-167.

Krueger, C.C., and B.W. Menzel. 1979. Effects of stocking on genetics of wild brook trout populations. Trans. Am. Fish. Soc. 108:277-287.

Lawrie, A.H., and J.F. Rahrer. 1973. Lake Superior; a case history of the lake and its fisheries. Great Lakes Fishery Commission Tech. Rep. 19.

Loftus, K.H. 1976. Science for Canada's fisheries rehabilitation needs. J. Fish. Res. Bd. Can. 33:1822-1857.

Martin, N.V. 1960. Homing behavior in spawning lake trout. Can. Fish. Cult. 26:3-6.

Miller, R. B. 1957. Have the genetic patterns of fishes been altered by introduction or by selective fishing. J. Fish. Res. Bd. Can. 14:797-806.

Mourant, A.E. 1970. Fish stocks: biochemical and serological identification. Science 167:1760-1761.

Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106:283-292.

Nei, M. 1975. Molecular population genetics and evolution. North Holland Publ. Co., Amsterdam. p. 175-178.

Pycha, R.L., and G.R. King. 1975. Changes in the lake trout population of southern Lake Superior in relation to the fishery, the sea lamprey, and stocking. Great Lakes Fishery Commission Tech. Rep. 28.

Schultz, P. 1978. Lake Michigan chub assessment. Project #3-245R-3 Wis. DNR.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fishery Research Board of Canada Bulletin 184.

Thruston, C.E. 1962. Physical characteristic and chemical composition of two subspecies of lake trout. J. Fish. Res. Bd. Can. 19:39-44.

Utter, F.M. 1971. Tetrazolium oxidase phenotypes of rainbow trout and Pacific salmon. Comp. Biochem. Physiol. 39:891-895.

Utter, F.M., and H.O. Hodgins. 1972. Biochemical genetic variation at six loci in four stocks of rainbow trout. Trans. Am. Fish. Soc. 3:494-502.

Utter, F.M., and H.O. Hodgins, and F.W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations, p. 213-237. In D.C. Malins and J.R. Sargent (ed.) Biochemical and biophysical perspectives in marine biology. Vol. 1 Academic Press, New York, N.Y.

Table 1. Postulated lake trout liver IDH isoenzyme gamete frequencies.

Sample[a]		Sample Size	Phenotypes S4	S3F	S2F2	SF3	F4		Gametes SS	SF	FF
SNG	obs.[b]	65	0	0	10	42	13	#[c]	0.0	62.0	68.0
	exp.		(0.0)	(0.0)	(14.78)	(32.43)	(17.78)	F	(0.0)	(.477)	(.523)
SNC	obs.	76	6	9	10	32	19	#	27.44	48.11	76.44
	exp.		(2.48)	(8.69)	(21.42)	(24.20)	(19.22)	F	(.181)	(.317)	(.503)
SNF	obs.	66	0	12	26	26	2	#	15.34	83.32	33.34
	exp.		(0.89)	(9.68)	(30.17)	(21.04)	(4.21)	F	(.116)	(.631)	(.253)

a Refer to Table 1 for sample designations.
b obs. = observed, exp. = expected.
c # = number, F = frequency.

APPENDIX 1

Table 2. Lake trout liver TO A-locus isoenzyme frequencies.

Sample[a]		Sample Size	Genotypes			Alleles		
			A^1A^1	A^1A^2	A^2A^2		A^1	A^2
SNG	obs.[b]	76	70	6	0	#[c]	146	6
	exp.		(70.22)	(5.70)	(.114)	F	(.961)	(.039)
SNC	obs.	85	70	15	0	#	155	15
	exp.		(70.72)	(13.69)	(.655)	F	(.912)	(.088)
SNF	obs.	66	61	5	0	#	127	5
	exp.		(61.05)	(4.82)	(.092)	F	(.962)	(.038)

a Refer to Table 1 for sample designations.
b obs. = observed, exp. = expected.
c # = number, F = frequency.

Table 3. Bloater white muscle G-3-PDH isoenzyme frequencies.

Sample	Sample Size		Genotypes										Alleles[c]			
			B_1B_1	B_1B_2	B_1B_3	B_1B_4	B_2B_2	B_2B_3	B_2B_4	B_3B_3	B_3B_4	B_4B_4	B_1	B_2	B_3	B_4
CH0	96	obs.[b]	62	5	21	3	3	0	0	2	0	0	153	11	25	3
		exp.	(60.98)	(8.72)	(19.89)	(2.45)	(0.31)	(1.42)	(0.18)	(1.62)	(0.40)	(0.02)	(0.797)	(0.057)	(0.130)	(0.016)
CH4	100	obs.	71	10	14	1	0	1	0	3	0	0	165	11	21	1
		exp.	(68.06)	(9.08)	(17.33)	(0.83)	(0.32)	(1.16)	(0.06)	(1.10)	(0.11)	(0.003)	(0.825)	(0.055)	(0.105)	(0.005)
CH5	99	obs.	70	6	14	5	0	1	1	2	0	0	165	8	19	6
		exp.	(68.70)	(6.60)	(15.83)	(4.95)	(0.16)	(0.76)	(0.24)	(0.91)	(0.57)	(0.09)	(0.833)	(0.040)	(0.096)	(0.030)
CH6	105	obs.	65	17	15	7	0	0	0	1	0	0	173	17	17	7
		exp.	(71.12)	(14.00)	(14.00)	(5.70)	(0.69)	(1.38)	(0.56)	(0.69)	(0.56)	(0.11)	(0.0823)	(0.081)	(0.081)	(0.033)
CH7	88	obs.	64	10	8	2	2	0	1	1	0	0	150	15	10	3
		exp.	(63.88)	(12.75)	(8.55)	(2.55)	(0.64)	(0.85)	(0.25)	(0.29)	(0.17)	(0.03)	(0.852)	(0.085)	(0.057)	(0.017)

a Refer to Table 1 for sample designations.
b obs. = observed, exp. = expected.
c Upper row is number of alleles, lower row is frequency of occurance.

Table 4. Bloater white muscle IDH isoenzyme frequencies, locus 1 and 2.

Sample[a]	Sample Size		Genotypes						Alleles			
									locus 1		locus 2	
			BB	BC	CC	AA	AB	BB	B	C	A	B
CH0	98	obs.[b]	28	28	42	42	10	46	#[c] 84	112	94	102
		exp.	(18.00)	(48.00)	(32.00)	(22.54)	(48.92)	(26.54)	F (0.4286)	(0.5714)	(0.4796)	(0.5204)
CH4	98	obs.	62	19	17	20	16	62	# 143	53	56	140
		exp.	(52.17)	(38.67)	(7.16)	(8.00)	(40.00)	(50.00)	F (0.7296)	(0.2704)	(0.2857)	(0.7143)
CH5	97	obs.	42	27	28	31	16	50	# 111	83	78	116
		exp.	(31.76)	(47.49)	(17.75)	(15.68)	(46.64)	(34.68)	F (0.5722)	(0.4278)	(0.4021)	(0.5979)
CH6	100	obs.	44	31	25	31	21	48	# 119	81	83	117
		exp.	(35.40)	(48.20)	(16.40)	(17.22)	(48.56)	(16.40)	F (0.5950)	(0.4050)	(0.4150)	(0.5850)
CH7	81	obs.	41	32	8	26	13	42	# 114	48	65	97
		exp.	(40.11)	(33.78)	(7.11)	(13.04)	(38.92)	(29.05)	F (0.7037)	(0.2963)	(0.4012)	(0.5988)

a See Table 1 for sample designations.
b obs. = observed, exp. = expected.
c # = number, F = frequency.

APPENDIX 1

Table 5. Brook trout LDH isoenzyme frequencies.

Sample[a]	Sample Size		Genotypes				Alleles	
			BB	BB_1	B_1B_1		B	B_1
SFL	81	obs.[b]	78	3	0	#[c]	159	3
		exp.	(77.95)	(3.02)	(0.029)	F	0.981	0.041
SFB1	77	obs.	67	10	0	#	144	3
		exp.	(67.32)	(9.85)	(0.342)	F	0.935	0.065
SFB2	37	obs.	30	7	0	#	67	7
		exp.	(30.30)	(6.36)	(0.334)	F	0.905	0.095

a Refer to Table 1 for sample designation.
b obs. = observed, exp. = expected.
c # = number, F = frequency.

APPENDIX 1

Table 6. Brook trout MDH isoenzyme frequencies.

Sample[a]	Sample Size		Genotypes			Alleles	
			BB	BB_1	B_1B_1	B	B_1
SFL	77	obs.[b]	70	7	0	#[c] 147	7
		exp.	(70.23)	(6.62)	(0.156)	F 0.955	0.045
SFB1	76	obs.	62	14	1	# 136	16
		exp.	(60.88)	(14.28)	(0.838)	F 0.895	0.105
SFB2	37	obs.	36	1	0	# 73	1
		exp.	(35.97)	(1.02)	(0.007)	F 0.986	0.014

a Refer to Table 1 for sample designations.
b obs.= observed, exp. = expected.
c # = number, F = frequency.

APPENDIX 1

Table 7. Brook trout G-3-PDH isoenzyme frequencies.

Sample[a]	Sample Size	Genotypes			Alleles		
		BB	BB_1	B_1B_1		B	B_1
SFL	78	obs.[b] 70	8	0	#[c]	148	8
		exp. (70.25)	(7.55)	(0.203)	F	0.949	0.051
SFB1	75	obs. 66	9	0	#	141	9
		exp. (66.27)	(8.46)	(0.270)	F	0.940	0.060
SFB2	37	obs. 34	3	0	#	71	3
		exp. (34.03)	(2.91)	(0.062)	F	0.959	0.041

a Refer to Table 1 for sample designations.
b obs. = observed, exp. = expected.
c # = number, F = frequency.

APPENDIX 2

Isoenzyme systems investigated but not used for genetic analysis.

Fish Species	Isoenzyme System	Tissue	Comments
bloater	IDH	liver	apparently polymorphic but electrophoretic resolution poor and unreliable
bloater	LDH	muscle	monomorphic 2 loci
lake trout	AAT	muscle	electrophoretic resolution extremely poor