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Detection of Variability at Isozyme Loci in Sea Lamprey, (*petromyzon marinus)*

by:

Charles C. Krueger[2] and George R. Spangler[2]

[2]Department of Entomology, Fisheries and Wildlife
University of Minnesota
St. Paul, Minnesota 55108

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Charles C. Krueger and George R. Spangler
Department of Entomology, Fisheries, and Wildlife
University of Minnesota
St. Paul, Minnesota 55108

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ABSTRACT

Electrophoretic expression of 15 enzymes coded by a minimum of 20 gene loci are reported for sea lamprey. Five loci, *AGP*, *MDH*, *PGI-1*, *PGI-2*, and *PGM-1* were polymorphic. ADH, AGP, AAT, G6PDH, LDH, MDH, PEP, 6PGDH, PGI, PGM, PK, AND SDH yielded gel resolution satisfactory for use in future population studies. Enzyme expression did not vary between life stages (ammocoete vs. adult) or collection method (fresh frozen vs. TFM killed). Statistically significant genetic differences occurred between lamprey from Lake Superior, Oneida Lake, Lake Champlain, and Bay of Fundy drainages at one or more of the polymorphic loci.

INTRODUCTION

The invasion and subsequent control of sea lampreys (Petromyzon marinus) in the Great Lakes has been well documented (S. H. Smith 1968, B. R. Smith 1971, Christie 1974, Smith et al. 1974 and others). Fundamental fisheries management theory stresses the importance of knowledge of the stocks within geographical management areas (Cushing 1968, Royce 1972). Although much research has been initiated and completed with respect to the biology and ecology of sea lamprey (see Sea Lamprey International Symposium[a]), little is known about the genetic organization of lamprey populations within the Great Lakes. This information would be particularly valuable as it would provide the temporal and spatial basis on which to formulate lamprey management policies.

The applicability of electrophoretic techniques to population identification of fishes has been well established (de Ligny 1969, Utter et al. 1974, Smith et al. 1975, Allendorf and Utter 1979). The success of these techniques in the discrimination of fish populations has encompassed a diverse array of fish species in a variety of habitats, for example: anadromous Pacific salmon (Utter et al. 1976), marine striped bass (Morone saxatilis, Morgan et al. 1973), bluegill sunfish (Lepomis macrochirus, Avise and Smith 1974), brook trout (Salvelinus fontinalis, Krueger and Menzel 1979) and lake whitefish (Coregonus clupeaformis, Kirkpatrick and Selander 1979).

This is the first report of a continuing study of the genetic population structure of sea lampreys within the Great Lakes. The

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specific objectives of the research reported herein were:

1. Determination of the best electrophoretic methodology for the detection of enzyme variability in lampreys.

2. Genetic description of the electrophoretic expressions of enzyme loci.

3. Comparison of enzyme expressions between lamprey ammocoetes and adults.

4. Comparison of enzyme expressions between freshly killed and TFM killed ammocoetes.

5. Genetic and statistical analysis for detection of significant differences between geographically discrete populations of sea lamprey.

MATERIALS AND METHODS

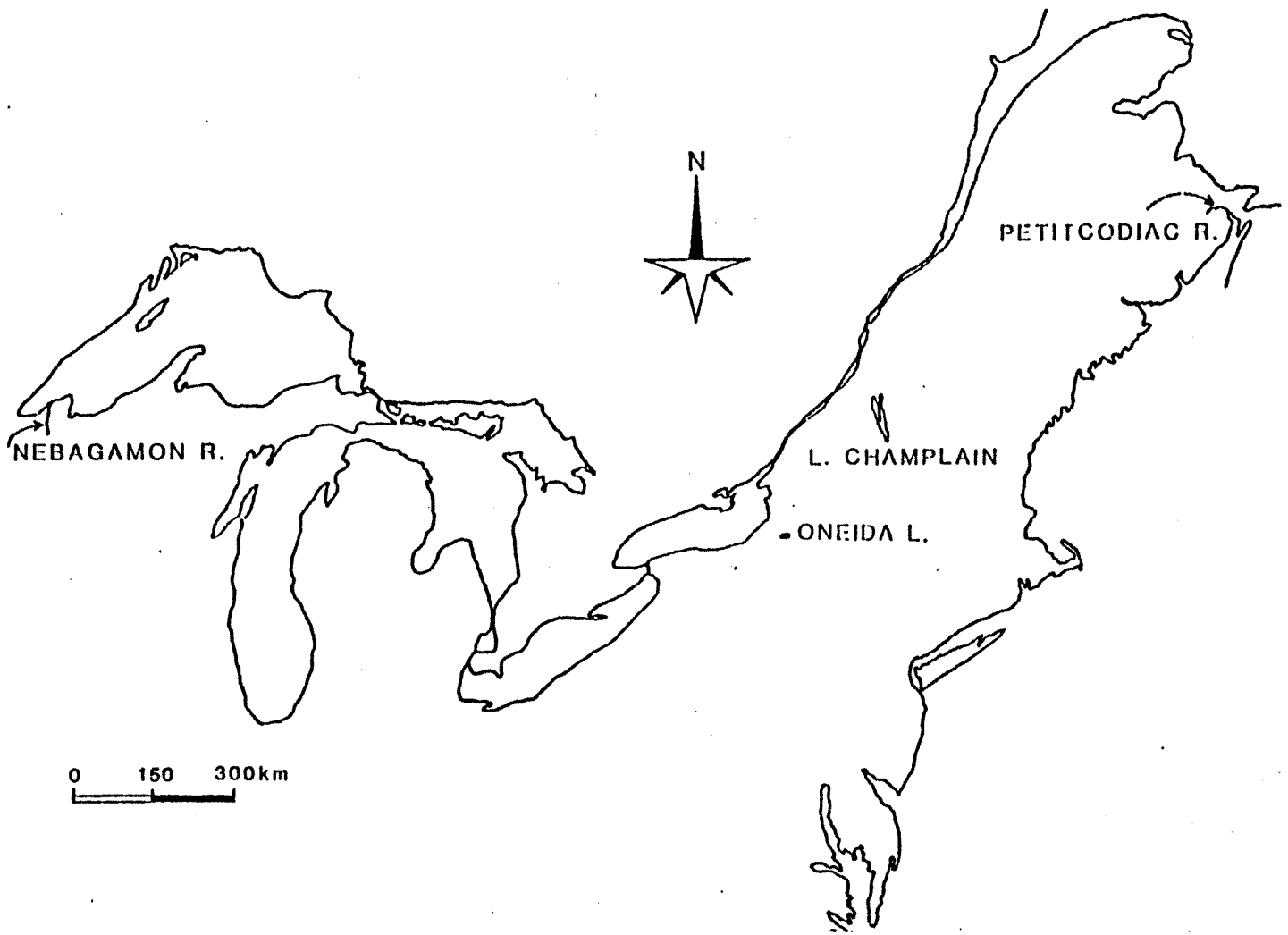
An enzyme survey was conducted among sea lamprey collected by electrofishing from four localities: Nebagamon R., Lake Superior drainage; Fish Cr., Oneida Lake drainage; Lake Champlain drainage; and Petitcodiac R., Bay of Fundy drainage (Fig. 1). Animals from Nebagamon R. were transported alive and held in aquaria at the University of Minnesota for approximately one month before analysis. The other specimens were frozen as soon as possible after collection and stored at -20C.

Electrophoretic expressions of enzymes were also compared between fresh-frozen and TFM (3-trifluoromethyl-4-nitrophenol) killed lampreys. Fourteen ammocoetes from Nebagamon R. were held in an aquarium and exposed to 12 mg/l TFM. Death occurred in approximately 4 hours. A single individual, also used in the analysis, was killed by a 1 hr exposure to 40 mg/l TFM. Fresh-frozen ammocoetes from the same river were used as controls in the gels. Chemical analysis of the well water used in the aquaria for these experiments has been given by Adelman and Smith (1972).

Electrophoretic enzyme patterns were also compared between ammocoetes (40-100 mm), newly transformed adults (110-145 mm) and spawning adults (375-445 mm). Ammocoetes were from the Nebagamon R. while the newly transformed adults were from the St. John's River, New Brunswick. Spawning adults used in the analysis were captured from the Brule R., Wisconsin.

Lampreys were prepared for electrophoresis by the removal of viscera and skin. Usually, muscle located caudally from the gill pouches was used as a tissue sample. When ammocoetes were less than 45 mm in length

Figure 1. Sea lamprey ammocoete collection locations.


N
PETITCODIAC R.
NEBAGAMON R.
L. CHAMPLAIN
ONEIDA L.
0 150 300km

the entire animal was used. Muscle tissues were homogenized by a motor powered Teflon pestle in approximately one-half the tissue volume of distilled water. Homogenized tissues were then centrifuged at approximately 4000 RPM for one minute. Supernatant fluid was then applied to the gels using 3 x 11 mm filter paper wicks (Whatman No. 3). Up to 40 wicks were used per gel. Throughout the sample preparation process, the muscle homogenates were cooled with crushed ice baths.

Gels, 20.5 x 15.2 x 0.9 cm, were prepared with 58.8 g of hydrolyzed potato starch (Electrostarch Co., Madison, Wis.) in 420 ml of gel buffer. Electrophoretic techniques followed those detailed by May et al. (1979). Four different buffer systems were used.

A. 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 with 99% gel buffer and 1% electrode buffer. Potential: 200v for 5 hours.

B. Clayton and Tretiak (1972). Gel: 0.002 M citric acid, pH 6.0. Electrode: 0.04 M citric acid, pH 6.1. Both buffers were pH adjusted with N-(3-Aminopropyl)-morpholine. Potential: 185v for 5 hours.

C. Selander et al. (1971). Gel: 1:9 dilution of electrode buffer. Electrode: 0.10 M maleic acid - 0.01 M EDTA (ethylene diamene tetraacetic acid) - 0.01 M magnesium chloride, pH 7.4. Potential: 100v for 5 hours.

D. Whitt (1970). Stock solution: 0.75 M Tris - 0.25 M citric acid, pH 6.9. Gel: 1:60 dilution of stock. Electrode: 1:20 dilution of stock. Potential: 200v for 5 hours.

Staining procedures generally followed those given by Allendorf et al. (1977). An exception was aspartate aminotransferase staining which

followed Selander et al. (1971). Genic nomenclature used here is that proposed by Allendorf and Utter (1979) for salmonids (see also May et al. 1979). Tests for statistical homogeneity between sets of phenotypic frequencies at the polymorphic loci utilized chi-square in contingency tables (Snedecor and Cochran 1967:250).

RESULTS AND DISCUSSION

Electrophoretic Expression of Enzymes

Most of the results of the sea lamprey enzyme survey are displayed in Table 1. The number of loci coding for a particular enzyme were estimated conservatively. For example, single invariant bands were treated as if they were expressions from one gene locus. Such bands may represent the products of a number of loci that share the same common allele for a particular enzyme. The exact number of loci, therefore, cannot be determined.

Enzymes yielding resolution satisfactory for use in future population studies are ADH, AGP, AAT, G6PDH, LDH, MDH, PEP, 6PGDH, PGI, PGM, PK, and SDH. These represent at least 15 loci. Among these loci, five were polymorphic within the four populations studied: *AGP*, *MDH*, *PGI-1*, *PGI-2*, and *PGM-1* (Table 1).

A relatively strong staining intensity with four generally monomorphic bands was observed for IDH. Two individuals from the Petitcodiac R., however, exhibited a seven banded phenotype. No genetic model was proposed because a lack of muscle tissue from these individuals prevented further study of these variants. PMI demonstrated a strong single invariant band on Selander gels. On Clayton-Tretiak gels, PMI stained very faintly and appeared to be polymorphic. Adequate resolution has not been achieved with this gel buffer. Research is being continued in studying IDH, PMI and other enzymes.

Ammocoete sea lamprey enzyme expressions were compared between animals killed by exposure to TFM and those killed by freezing. Gels

Table 1. Results of screening for electrophoretically detectable loci in sea lamprey.

Enzyme	Loci	Alleles[a]	Preferred Buffer System[b]
Adenosine deaminase	*ADA-1*	M	S
	ADA-2	M	
Alcohol dehydrogenase	*ADH*	M	CT
Alpha-glycerophosphate dehydrogenase	*AGP*	*100*	CT
		146	
Aspartate aminotransferase	*AAT-1*	M	CT
	AAT-2	M	
Diaphorase	*DIA*[c]	---	--
Fructose-1,6-diphosphate	*FDP*	M	S
Glutamate dehydrogenase	*GDH*[c]	---	--
Glucose-6-phosphate dehydrogenase	*G6PDH*	M	W or S
Hexokinase	*HK*[c]	---	--
Isocitrate dehydrogenase	*IDH*[d]	---	CT
Lactate dehydrogenase	*LDH*	M	R
Malate dehydrogenase	*MDH*	*-100*	CT
		-165	
Peptidase	*PEP-1*	M	S
	PEP-2	M	
6-phosphogluconate dehydrogenase	*6PGDH*	M	W or S
Phosphoglucose isomerase	*PGI-1*	*100*	R
		130	
	PGI-2	*100*	
		92	
		122	

Table 1 continued.

Phosphoglycerate kinase	*PGK*[c]	---	--
Phosphoglucomutase	*PGM-1*	*100*	R
	PGM-2[d]	*14 8* ---	--
Pyruvate kinase	*PK*	M	W or S
Phosphomannose isomerase	*PMI*[d]	M	W or S
Sorbitol dehydrogenase	*SDH*	M	R
Succinate dehydrogenase	*SUCDH*[c]	---	--
Xanthine dehydrogenase	*XDH*[c]	---	--

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[a] M = monomorphic

[b] R = Ridgway et al. (1970)
CT = Clayton and Tretiak (1972)
S = Selander et al. (1971)
W = Whitt (1970)

[c] Insufficient staining activity to designate loci

[d] See text for further details

were stained for ADA, ADH, AGP, AAT, FDP, G6PDH, IDH, LDH, MDH, PEP, 6PGDH, PGI, PGM, PMI, PK, and SDH. In every case no differences were observed in enzymatic expression between lamprey with different death origins. TFM does not appear to alter these enzymes in an electrophoretically detectable way. Ammocoetes, newly transformed adults, and spawning adults were also compared for the same enzymes listed above. Similarly no difference in enzymatic expression was observed.

Genetic Variability Observed in Sea Lamprey From Different Localities

AGP was highly variable in allelic frequencies between Nebagamon R., Fish Cr., and L. Champlain (Table 2). The three phenotypes observed at this locus fit a two allele dimeric model. Phenotypic frequencies observed for *AGP* in the three streams were in good conformity with Hardy-Weinberg (H-W) expectations ($p > 0.20$, Table 2). Gels with tissue samples from the Petitcodiac R. stained for AGP were not scorable due to a lack of enzymatic activity.

MDH (supernatant) allelic frequencies were fixed for the common allele in ammocoetes from Fish Cr. and L. Champlain but variable in collections from Nebagamon R. and the Petitcodiac R. (Table 3). The phenotypes for this dimeric enzyme fit a two allele model; however, the *MDH -165/-165* phenotype was not observed. *MDH* phenotypes from Nebagamon R. and Petitcodiac R. were in H-W equilibrium ($p > 0.45$, Table 3). Two anodal bands, possibly mitochondrial forms, were also observed on gels stained for MDH.

Table 2. ∝ - Gycerophosphate dehydrogenase phenotypes, allelic frequencies, and p values values for H-W equilibrium tests.

Sample Location	Phenotypes[a]			Total	P of a larger X^2	Allelic Frequency	
	100/100	*100/146*	*146/146*			*100*	*146*
Nebagamon R.	21	13	7	41	> 0.20	0.67	0.33
Fish Cr.	5	16	10	31	> 0.90	0.42	0.58
L. Champlain	24	11	0	35	> 0.75	0.84	0.16

[a] Numeric values refer to allelic designations (see Table 1)

Table 3. Malate dehydrogenase phenotypes, allelic frequencies, and p values for H-W equilibrium tests.

Sample Location	Phenotypes[a]			Total	P of a larger X^2	Allelic Frequency	
	-100/-100	*-100/-165*	*-165/-165*			*-100*	*-165*
Nebagamon R.	45	17	0	62	> 0.45	0.86	0.14
Fish Cr.	31	0	0	31	---	1.0	0.0
L. Champlain	30	0	0	30	---	1.0	0.0
Petitcodiac R.	18	11	0	29	> 0.45	0.81	0.19

[a] Numeric values refer to allelic designations (see Table 1)

PGI activity in sea lamprey was typically a three banded phenotype. The two outer bands represent homodimeric products from separate loci. The middle band is the heterodimer interaction between the two loci. Similar control of PGI banding has been observed in other fishes (Avise and Kitto 1973, Dando 1974, Schmidtke et al. 1975). At the *PGI-1* locus, variability was only observed in ammocoetes from the Petitcodiac R. (Table 4). At the *PGI-2* locus, expressions of three alleles with different electrophoretic mobilities were observed (Table 5). Nebagamon R. had a unique alternate allele not observed in the other three collections. Fish Cr. was the most variable at the *PGI-2* locus of the four collections. Conformity with H-W expectations was observed at both loci ($p > 0.50$, Tables 4 and 5).

PGM staining revealed two zones of activity with Whitt gels. The slower zone (*PGM-1*) could be reliably stained and scored using a Ridgway gel type. The most anodal zone (tentatively *PGM-2*) stained only faintly regardless of gel type and was not scorable. The three phenotypes observed at the *PGM-1* locus followed a two allele monomeric model (Table 6). Greatest variability at this locus was observed in lamprey from Nebagamon R. Each collection followed H-W predictions ($p > 0.15$, Table 6).

Paired comparisons of phenotypic frequencies by locus were made between collections (Table 7). Statistically significant differences ($p < 0.05$) were demonstrated between collection localities for one or more loci. Ammocoetes from Nebagamon R. appeared most genetically distinctive with significant differences found in 11 of 14 possible comparisons. This analysis (Table 7) demonstrates the capabilities of electrophoretic techniques in population identification of sea lampreys.

Table 4. *PGI-1* (phosphoglucose isomerase) phenotypes, allelic frequencies, and p values for H-W equilibrium tests.

Sample Location	Phenotypes[a]				P of a	Allelic Frequency	
	100/100	*100/130*	*130/130*	Total	larger X^2	*100*	*130*
Nebagamon R.	70	0	0	70	---	1.0	0.0
Fish Cr.	31	0	0	31	---	1.0	0.0
L. Champlain	30	0	0	30	---	1.0	0.0
Petitcodiac R.	27	2	0	29	> 0.95	0.97	0.03

[a] Numeric values refer to allelic designations (see Table 1)

Table 5. *PGI-2* (phosphoglucose isomerase) phenotypes, allelic frequencies, and p values for H-W equilibrium tests.

Sample	Phenotypes[a]						P of a	Allelic Frequency		
Location	*100/100*	*100/92*	*92/92*	*100/122*	*122/122*	Total	larger X^2	*100*	*92*	*122*
Nebagamon R.	67	3	0	0	0	70	> 0.97	0.98	0.02	0.0
Fish Cr.	19	0	0	9	3	31	> 0.50	0.76	0.0	0.24
L. Champlain	26	0	0	4	0	30	> 0.90	0.93	0.0	0.07
Petitcodiac	25	0	0	4	0	29	> 0.90	0.93	0.0	0.07

[a]Numeric values refer to allelic designations (see Table 1).

Table 6. *PGM-1* (phosphoglucose mutase) phenotypes, allelic frequencies, and p values for H-W equilibrium tests.

Sample Location	Phenotypes[a]			Total	P of a larger X^2	Allelic Frequency	
	100/100	*100/148*	*148/148*			*100*	*148*
Nebagamon R.	23	19	0	42	> 0.15	0.77	0.23
Fish Cr.	29	2	0	31	> 0.95	0.97	0.03
L. Champlain	28	2	0	30	> 0.95	0.97	0.03
Petitcodiac R.	27	1	1	29	> 0.60	0.95	0.05

[a]Numeric values refer to allelic designations (see Table 1).

Table 7. Probability levels for X^2 tests of homogeneity at five loci of sea lamprey.

	Nebagamon River					Fish Creek					Lake Champlain				
	MDH	*PGI-1*	*PGI-2*	*PGM-1*	*AGP*	*MDH*	*PGI-1*	*PGI-2*	*PGM-1*	*AGP*	*MDH*	*PGI-1*	*PGI-2*	*PGM-1*	*AGP*
Fish Cr.	***	1.0	***	***	*	-	-	-	-	-	-	-	-	-	-
L. Champlain	***	1.0	***	***	*	1.0	1.0	*	1.0	***	-	-	-	-	-
Petitcodiac R.	>0.30	*	***	***	NA	***	>0.05	>0.05	>0.45	NA	***	>0.10	1.0	>0.45	NA

[a]Single asterisk indicates P<0.05, triple asterisks indicate P<0.005, NA indicates Not Available.

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