MINNESOTA SEA GRANT

Research Report No. 2. Genetic Identification of Sea Lamprey, *Petromyzon marinus*, Populations From the Lake Superior Basin.

GENETIC IDENTIFICATION OF SEA LAMPREY, PETROMYZON MARINUS, POPULATIONS FROM THE LAKE SUPERIOR BASIN

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University of Minnesota - 1980

This work is the result of research sponsored by the Minnesota Sea Grant Program, supported by the NOAA office of Sea Grant, Department of Commerce under Grant No. NA79AA-D-00134.

ACKNOWLEDGMENTS

We are indebted to the sea lamprey control personnel of the U.S. Fish and Wildlife Service and the Canada Department of Fisheries and Oceans who supplied the numerous ammocoete collections and provided unpublished environmental data on the collection localities. In particular, we wish to acknowledge the excellent cooperation provided by Paul Rugen, Stan Dustin, Robert Braem, James Tibbles, and Harry Purvis. F.W.H. Beamish provided the collections from New Brunswick. This research was coordinated by W.L. Hartman and J. Kutkuhn of the Great Lakes Fishery Laboratory with that of Peter Brussard at Cornell University. James Stewart assisted with the tedium of the laboratory analysis. We were especially appreciative of the constructive comments provided by Fred Allendorf, Kendall Corbin, Bruce Menzel, and David Merrell on the early drafts of this report.

ABSTRACT

Sea lamprey (Petromyzon marinus) ammocoetes collected from eighteen locations within Lake Superior were electrophoretically analyzed for genetic variability at 25 enzyme loci. Enzyme expression did not vary between life stages (ammocoete vs. adult) or collection method (fresh frozen vs. TFM killed). Analysis by F-statistics and chi-square indicated that a significant degree of population structuring occurs among lampreys from Lake Superior. Cluster analysis of genetic distances generally grouped collections that were in geographical proximity to each other. A weak but significant correlation was detected between genetic and geographic distances. It was concluded that multiple populations of sea lamprey occur within Lake Superior and are maintained by homing of spawning adults or restricted dispersal away from natal areas.

CONCLUSIONS/APPLICATIONS

Sea lamprey ammocoetes collected from eighteen locations within Lake Superior were electrophoretically analyzed for genetic variability at 25 enzyme loci. The genetic data presented suggest that multiple populations of sea lampreys exist within localized areas of the Lake Superior basin.

An intensive management program to reduce the sea lamprey populations began in 1953 with the installation of electrical barriers in streams to block spawning runs (Lawrie 1970; Smith 1971; Smith et al. 1974; Smith and Tibbles 1980). Control measures became much more effective when the selective toxicants, TFM (3-trifluoromethyl-4-nitrohpenol) and Bayer 73 (2', 5-dichloro-4'nitrosalicylanilide) were used against the ammocoetes in streams. Application of these lampricides at intervals of three to four years in Lake Superior tributaries continues to be the predominant tool used in sea lamprey control. The control program is organized around a survey system that gathers data on the abundance of ammocoetes in tributary streams. The basic management units for population control are those streams with high densities of ammocoetes nearing the transformation life stage.

Based on the results of this research, the adoption of a stock concept approach (Loftus 1976) to lamprey control is a viable management alternative which may increase control effectiveness. In this case sea lamprey control emphasis would shift from individual streams to population regions in order to minimize the rate of reestablishment subsequent to chemical treatment. It may also be wise to avoid the planting of species with migratory habits so as to prevent the passive transport of adult lampreys out of their population areas.

MATERIALS AND METHODS

Sea lamprey ammocoetes from seventeen tributaries and one bay of Lake Superior were collected during chemical treatment or by electrofishing (Fig. 1). Animals from the Middle and Nebagamon rivers were transported alive and held in aquaria prior to analysis. The other specimens were frozen as soon as possible after collection and stored at -20° C.

Starch gel electrophoresis was conducted on lamprey muscle tissue according to the procedures given by Krueger (1980). The nomenclature for specifying enzymes, loci, and alleles is that proposed by Allendorf and Utter (1979). Gels were stained for sixteen enzymes encoded by 25 gene loci: adenosine deaminase (ADA-1,2), alpha-glycerophosphate dehydrogenase (AGP), aspartate aminotransferase (AAT-1,2), creatine phosphokinase (CPK-1,2), glucose-6-phosphate dehydrogenase (G6PDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH-1,2), lactate dehydrogenase (LDH), malate dehydrogenase (MDH-1,2,3), malic enzyme (ME), peptidase (PEP-1,2), peroxidase (PER-1,2), 6-phosphogluconate dehydrogenase (6PGD), phosphglucose isomerase (PGI-1,2), phosphoglycerate kinase (PGK), phosphoglucomutase (PGM-1), and sorbitol dehydrogenase (SDH). Among these loci, four were demonstrably polymorphic: AGP, MDH-1, PGI-2, and PGM-1. Electrophoretic expression of ME and PER has been reported by Brussard et al. (1981); the remaining enzymes have been described by Krueger (1980). The buffer systems of Ridgway et al. (1970) and Whitt (1970) were used to resolve PER and ME, respectively.

Electrophoretic expressions of enzymes were also compared between fresh-frozen and TFM (3-triflouromethyl-4-nitrophenol) killed lampreys. Fourteen ammocoetes from Nebagamon R. were held in an aquarium and exposed to 12 mg/L TFM, a dosage comparable to that used in lamprey control applications. Death occurred in approximately 4 hours. A single individual, also used in the analysis, was killed by a 1 hour exposure to 40 mg/L TFM. Fresh-frozen ammocoetes from the same river were used as controls.

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., and 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.

The analysis of the genetic data followed the methods outlined by Avise and Felley (1979) and utilized inbreeding coefficients known as F statistics (Wright 1978). The fixation index F_{ij} was calculated for each collection (i) at each locus (j) using Levele's (1949) correction for small sample size. The mean fixation index per locus (F_{is}) was calculated according to the procedures of Kirby (1975). The standardized genetic variance (F_{st}) was calculated as the ratio of the observed variance in allele frequency to the limiting value expected if the localities were completely isolated and fixed. The calculation of F-statistics for the polyallelic PGI-2 locus utilized a "synthetic" allele which represented the data from alleles 92 and 122 (see Eanes and Koehn 1978 for further discussion). For this analysis a locus was considered polymorphic in a population if the frequency of the common allele did not exceed 0.99.

Tests for departures from Hardy-Weinberg expectations at a locus (j) used chi-square and were calculated as:

$$X^2_{ij} = F_{ij}{}^2 N_{ij}$$

where N_{ij} represents the number of lampreys analyzed from collection i and locus j. The overall heterogeneity chi-square test between all collections at a locus (j) was calculated as:

$$X^2_j = 2N_j F_{st}$$

where N_j is the total number of fish analyzed at a locus (j). Other chi-square tests of the genetic data followed that described by Snedecor and Cochran (1967).

The calculations of average heterozygosity per locus and its standard error were determined according to the procedures of Nei and Roychoudhury (1974). Genetic distances (Rogers 1972) were calculated for all pairs of collection locations based on the four polymorphic loci. The matrix of genetic distances was subjected to cluster analysis using the BMDP-1M program (Dixon and Brown 1979). Geographic distances between sample locations were measured as the shortest distances via lake shoreline. Environmental data for the collection locations were provided by the U.S. Fish and Wildlife Service and the Canada Department of Fisheries and Oceans. Spearman's rank correlation coefficient (Conover 1971) was computed to examine the relationships between genetic characteristics, geographical distance and environmental variables.

RESULTS AND DISCUSSION

This report proposes a population structure for sea lampreys in Lake Superior based on differences in allelic frequencies at allozyme loci among lampreys from eighteen localities within the lake basin. The results of this study may have application for developing more effective lamprey control strategies.

The invasion, colonization, and population expansion of sea lampreys in Lake Superior during the 1940s and 1950s temporally corresponded to sharp declines in commercially important fish stocks, notably the lake trout (Salvelinus namaycush) and the lake whitefish (Coregonus clupeaformis) (Smith 1968; Lawrie and Rahrer 1972; Christie 1974; Pycha and King 1975). Much of this decline has been attributed to the predatory nature of sea lamprey adults, which cause mortality of prey fish through a process of attachment and feeding with a circular suctorial mouth. This predatory phase extends for twelve to twenty months until the adult lampreys move into tributary streams, spawn, and die. Larval lampreys (ammocoetes) remain in the tributaries for three to eleven years or more before transformation and migration to the lake.

Electrophoretic studies on lamprey population structure in the Atlantic coastal drainages and the Great Lakes have found the animals to be genetically distinguishable between widespread geographic localities. Brussard et al. (1981) found genetic distinctiveness among lampreys collected from the Finger Lakes of New York, Hudson River, Delaware River, Lake Ontario, and Lake Superior.

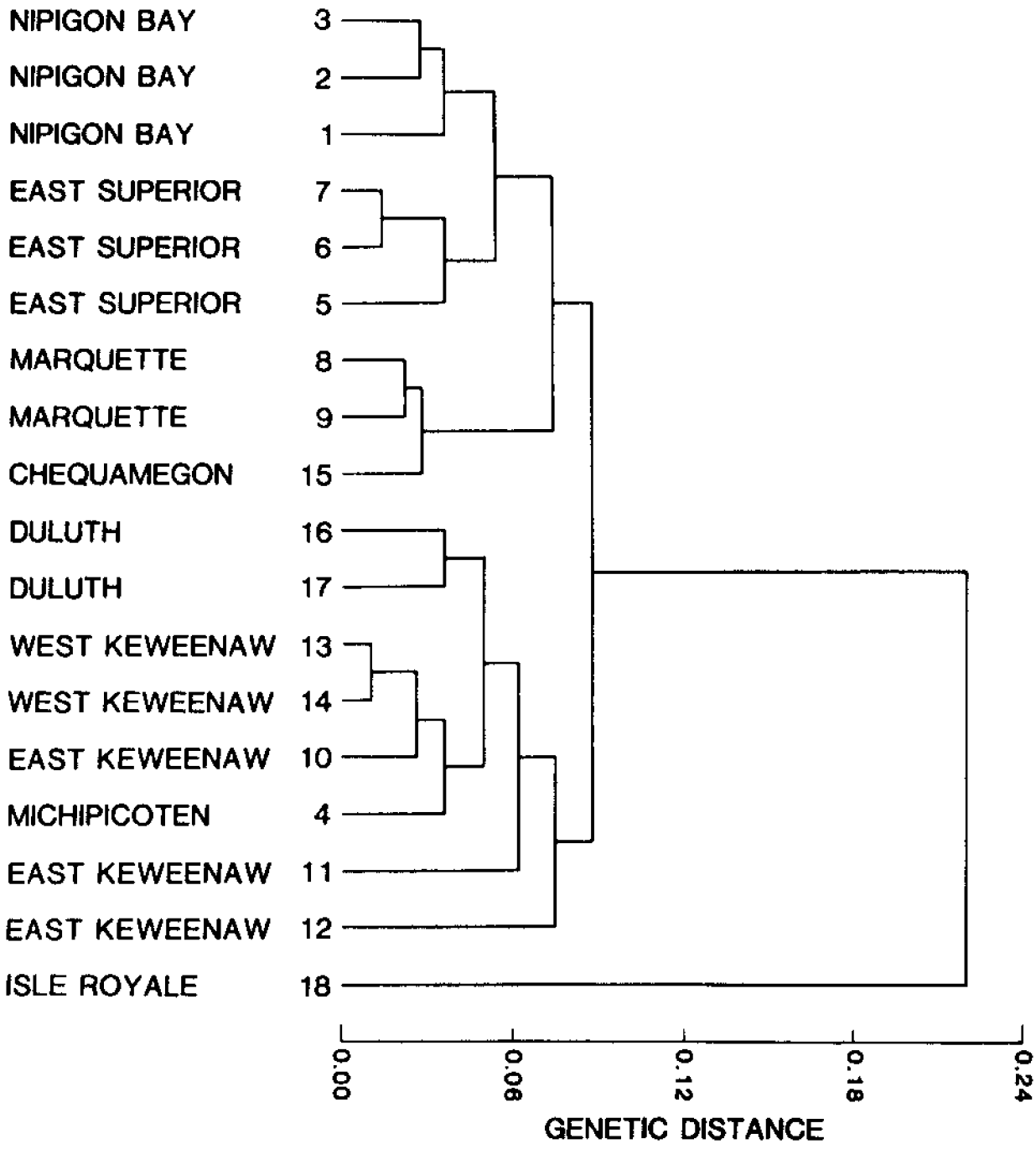
Krueger (1980) determined that statistically significant genetic differences occurred among lampreys from Lake Superior, Oneida Lake, Lake Champlain, and the Bay of Fundy at one or more of four polymorphic allozyme loci. The present study was undertaken to determine whether genetically identifiable populations of sea lamprey have become established in Lake Superior. The geographical scope of this study is narrower than others since collections were taken from the drainages of a single lake basin.

Ammocoete sea lamprey enzyme expressions were compared between animals killed by exposure to TFM and those killed by freezing. This was necessary in order to validate the electrophoresis of ammocoetes collected during normal lamprey control and survey procedures. Gels were stained for ADA, AGP, AAT, G6PDH, LDH, MDH, PEP, 6PGD, PGI, PGM, and SDH. In every case, no differences were observed in enzymatic expression between lamprey subjected to different modes of killing. 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. Uthe and Tsuyuki (1967) found that the transformation process in sea lamprey electrophoretically affected blood proteins but not general muscle proteins.

Genotypic frequencies at the four polymorphic loci (AGP, MDH-1, PGI-2, and PGM-1) within the samples from Lake Superior were close to Hardy-Weinberg expectations; of sixty-four comparisons only three deviated significantly ($P < 0.05$). The deviant comparisons occured at AGP in collections from the Firesteel, Huron, and Nebagamon rivers. The fixation indices (F_{ij}) for these three collections were all positive, indicating heterozygote deficiencies and suggesting that population substructuring may occur at these locations (Wahlund 1928). A trend towards heterozygote deficiency for the collections was indicated by the positive values for the mean fixation index (F_{is}) at each locus (Table 1).

Allelic frequencies among collections were, in general, most similar within regions; the exception was the East Keweenaw region (Table 1). Lamprey from Washington Creek exhibited less genetic variability ($\bar{H} = 0.009$) than the other samples (average $\bar{H} = 0.051$) and were unique in being fixed for the common alleles at AGP and MDH-1 (Table 1).

The amount of genetic differentiation among collections relative to the limiting amount under complete fixation (F_{st}) was computed for each polymorphic locus (Table 1). F_{st} can assume values ranging from 0 (no differentiation) to 1 (maximum differentiation). The F_{st} values at different loci in this study varied between 0.02 to 0.06 with a mean of 0.04, which suggests some genetic differentiation between collections (Wright 1978). The F_{st} values calculated for lampreys are generally higher than those observed for bluegills (Lepomis macrochirus) within lakes in the southeastern United States (Avise Felley 1979; Felley and Avise 1980) and lower than those observed for Atlantic salmon (Salmo salar) from streams in Sweden (Stahl 1980); (see review of F_{st} values for other organisms in Selander and Kaufman 1975). The heterogeneity chi-square values calculated from the F_{st} values indicated that significant genetic heterogeneity ($P < 0.05$) occurred at each locus among collections (Table 1; testing the null hypothesis that samples were drawn from a common population). It may be argued that the Washington Creek collection, with its reduced genetic variability, contributed solely to the large chi-sqare values; however, highly significant differences ($P < 0.005$) remained at MDH-1 and PGM-1 when the heterogeneity tests were recalculated without these data. The above analysis indicates that a significant degree of population structuring occurs among lampreys from Lake Superior.


NIPIGON BAY 3
NIPIGON BAY 2
NIPIGON BAY 1
EAST SUPERIOR 7
EAST SUPERIOR 6
EAST SUPERIOR 5
MARQUETTE 8
MARQUETTE 9
CHEQUAMEGON 15
DULUTH 16
DULUTH 17
WEST KEWEENAW 13
WEST KEWEENAW 14
EAST KEWEENAW 10
MICHIPICOTEN 4
EAST KEWEENAW 11
EAST KEWEENAW 12
ISLE ROYALE 18
0.00
0.06
0.12
0.18
0.24
GENETIC DISTANCE

Genetic distances (Rogers 1972) were calculated between the sea lamprey collections to analyze further the population structure within Lake Superior. Cluster analysis of the genetic distances between the sea lamprey collections suggested a regional localization of lamprey populations (Fig. 2). Samples within regions usually formed discrete clusters with the principal exception being the East Keweenaw collections. At genetic distances less than 0.06, five clusters were formed, representing collections from Nipigon, East Superior, Marquette-Chequamegon, Duluth, and West Keweenaw-East Keweenaw-Michipicoten.

Allelic frequencies and heterozygosities for each collection were compared to several environmental parameters (stream discharge, pH, total alkalinity, average summer water temperatures) and to the sea lamprey control histories of each locality. Using rank correlation coefficients, none of these variables appeared related to the genetics of the lamprey populations. A low but significant rank correlation did exist ($R = 0.18$, d.f. = 134, $p < 0.05$) between genetic and geographic distances.

The genetic data presented suggested that multiple populations of sea lampreys exist within localized areas of the Lake Superior basin. Although significant inter-region variability in allelic frequencies was demonstrated, all alleles were distributed throughout the lake basin and the degree of inter-region differentiation was small.

Deterministic and/or stochastic processes, exclusive of long term genetic differentiation, could have resulted in the establishment of the genetic heterogeneity observed among Lake Superior lampreys. Among the possible mechanisms that may have functioned are founding errors at initial colonization, transitory founding errors after chemical treatments, and selection.

Genetic differences may have become established through founding errors during initial colonization and maintained by homing of spawning adults or restricted dispersal away from natal areas. If this is the case, migration and subsequent mating or genetic exchange between regions is apparently limited since it is unlikely that these differences would persist if lampreys freely interbred within the Lake Superior basin. Tagging studies in the Great Lakes have shown that lampreys are capable of travelling long distances (Applegate and Smith 1951; Smith and Elliot 1953; Moore et al. 1974). These studies, however, have not addressed the question of whether or not spawning runs in streams are comprised of adult lampreys with an admixture of natal origins. Recently, pheromones have been implicated as playing an important role in sea lamprey migration and reproductive behavior (Teeter 1980). The ability to detect trace quantities of chemical substances by spawning adults could facilitate a homing behavior. Irrespective of the mechanism preventing genetic exchange, the reduced genetic variability observed among lamprey from Washington Creek (Table 1) provides evidence that lampreys have not freely intermingled in Lake Superior. The invasion history of Washington Creek is exceptional from that of other localities in that lampreys did not colonize this stream until sometime in the early 1970s (personal communication, P. Rugen and R. Schuldt, Fish and Wildlife Service, Marquette, MI). The probable physical or ecological barriers that occurred in this instance to prevent colonization and genetic exchange with other Lake Superior populations are unknown; however, the invasion history and genetic data suggest that such barriers existed. The weak correlative evidence between genetic and geographic distance implies that isolation by distance (Wright 1943) may be a factor in preventing panmixia in the lake basin.

Alternatively, it might be argued that the genetic differences observed between sample sites are not stable attributes of populations but the transient results of founding errors from a general Lake Superior population repeated as often as each site is subjected to lamprey control treatments and subsequent recolonization. In this case, lampreys in streams geographically proximate could exhibit substantial genetic differences. This would seem to be a less likely explanation since genetic homogeneity occurred within each of the regions studied. Further investigations are planned to study the temporal genetic variability among lampreys within a stream.

Selection is another possible mechanism that could be responsible for the genetic heterogeneity observed among lampreys. In the present study, no correlative evidence was found between genetic variables and the few environmental variables for which we had data. The strength of such correlative analyses for the detection of selection, especially with so few variables, is questionable (Lewontin 1974). We consider this process to be an unlikely origin of the results observed.

We believe that the regional genetic patterns described argue in favor of the existence of several populations in Lake Superior. If this is the case, then the adoption of a stock concept approach to management could increase control effectiveness. Control emphasis might then shift from individual streams to population regions. Each of the streams located within a region, including minor lamprey producers, would be chemically treated simultaneously. This management approach should decrease the probability for recolonization if adult dispersal from adjacent regions is limited. The prevention of passive forms of lamprey movement would reduce such dispersal and enhance population discreteness. It may even be wise to avoid planting fish species (e.g. Pacific salmon) that are known to have extensive migratory habits and which could serve as vectors for lamprey dispersal.

Literature Cited

Allendorf, F.W., and F.W. Utter. 1979. Population genetics. Fish physiology. Hoar, W.S., and D.J. Randall, eds. New York: Academic Press. Vol. VIII: 407-454.

Applegate, V.C., and B.R. Smith. 1951. Movement and dispersion of a blocked Swpaning run of sea lampreys in the Great Lakes. Trans. 16th N. Amer. Wildlife Conf. pp. 743-751.

Avise, J.C., and J. Felley. 1979. Population structure of freshwater fishes I. Genetic variation of bluegill (Lepomis macrochirus) populations in man-made reservoirs. Evolution 33: 15-26.

Brussard, P.F., M.C. Hall, and J.M. Wright. 1981. The origin, structure, and affinities of freshwater sea lamprey populations. Can. J. Fish Aquatic Sc. 37: 000-000.

Christie, W.J. 1974. Changes in the fish species composition of the Great Lakes. J. Fish. Res. Board Can. 31: 827-854.

Conover, W.J. 1971. Practical nonparametric statistics. New York: J. Wiley & Sons Inc.

Dixon, W.J., and M.B. Brown. 1979. BMDP-79 Biomedical computer programs P-series. Los Angeles: Univ. California Press.

Eanes, W.F., and R.K. Koehn. 1978. An analysis of genetic structure in the monarch butterfly, Danaus plexippus, L. Evolution 32: 784-797.

Felley, J.D., and J.C. Avise. 1980. Genetic and morphological variation of bluegill populations in Florida lakes. Trans. Amer. Fish. Soc. 109: 108-115.

Kirby, G.C. 1975. Heterozygote frequencies in small subpopulations. Theoret. Pop. Biol. 8: 31-48.

Krueger, C.C. 1980. Detection of variability of isozyme loci in sea lamprey, Petromyzon marinus. Can. J. Fish. Aquatic Sc. 36: 1630-1634.

Lawrie, A.H. 1970. The sea lamprey in the Great Lakes. Trans. Amer. Fish. Soc. 99: 766-775.

Lawrie, A.H., and J.F. Rahrer. 1972. Lake Superior: effects of exploitation and introductions on the salmonid community. J. Fish. Res. Board Can. 29: 765-776.

Levene, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20: 91-94.

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

Moore, H.H., F.H. Dahl, and A.K. Lamsa. 1974. Movement and recapture of parasitic-phase sea lampreys (Petromyzon marinus) tagged in the St. Marys River and Lakes Huron and Michigan, 1963-1967. Great Lakes Fish. Comm., Tech. Rep. No. 27.

Nei, M., and A.K. Roychoudhury. 1974. Sampling variances ofheterozygosity and genetic distance. Genetics 76: 379-390.

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, 1950-1970. Great Lakes Fish. Comm., Tech. Rep. No. 28.

Ridgway, F.J., S.W. Sherburn, and R.D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Amer. Fish. Soc. 99: 146-151.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Stud. Genetics VII. Univ. Texas Pub. 7214: 145-153.

Selander, R.K., and D.W. Kaufman. 1975. Genetic structure of populations of the brown snail (Helix aspersa). I. Microgeographic variation. Evolution 29: 385-401.

Smith, B.R. 1971. Sea lampreys in the Great Lakes of North America. The biology of lampreys. Hardisty, M.W. and I.C. Potter, New York: Academic Press. Vo. 1: 207-247.

Smith, B.R., and O.R. Elliot. 1952. Movement of parasitic-phase sea lampreys in Lakes Huron and Michigan. Trans. Amer. Fish. Soc. 82: 123-128.

Smith, B.R., and J.J. Tibbles. 1980. The sea lamprey (Petromyzon marinus) in Lakes Huron, Michigan, and Superior - History of invasion and control program. Can. J. Fish. Aquatic Sc. 36: 1780-1801.

Smith, B.R., J.J. Tibbles, and B.G.H. Johnson. 1974. Control of the sea lamprey (Petromyzon marinus) in Lake Superior, 1953-1970. Great Lakes Fish. Comm. Tech. Rep. No. 25.

Smith, S.H. 1968. Species succession and fishery exploitation in the Great Lakes. J. Fish. Res. Board Can. 25: 667-693.

Snedecor, G.W., and W.G. Cochran. 1967. Statistical methods. Ames: Iowa State Univ. Press.

Stahl, G. 1980. Genetic differentiation among natural populations of Atlantic salmon (Salmo salar) in northern Sweden. Ecol. Bull. 00: 000-000.

Teeter, J.H. 1980. Pheromone communication in sea lampreys: implications for population control. Can. J. Fish. Aquatic Sc. 36: 2123-2132.

Uthe, J.F., and H. Tsuyuki. 1967. Comparative zone electropherograms of muscle myogens and blood proteins of adult and ammocoete lamprey. J. Fish. Res. Board Can. 24: 1269-1273.

Wahlund, S. 1928. Zusammensetzung von Populationen un Korrelationscheinungen vom Standpunkt der Vererbungslehre aus betracht. Hereditas 11: 65-106.

Whitt, G.S. 1970. Developmental genetics of the lactate dehydrogenase isozymes of fish. J. Exp. Zool. 175: 1-36.

Wright, S. 1943. Isolation by distance. Genetics 28: 114-138.

Wright, S. 1978. Evolution and the genetics of populations. Variability within and among populations. Chicago: University of Chicago Press. Vol. IV.

Table Allelic frequencies at four enzyme loci, average heterozygosity per locus, and F-statistics observed in sea lamprey collections from the Lake Superior basin.

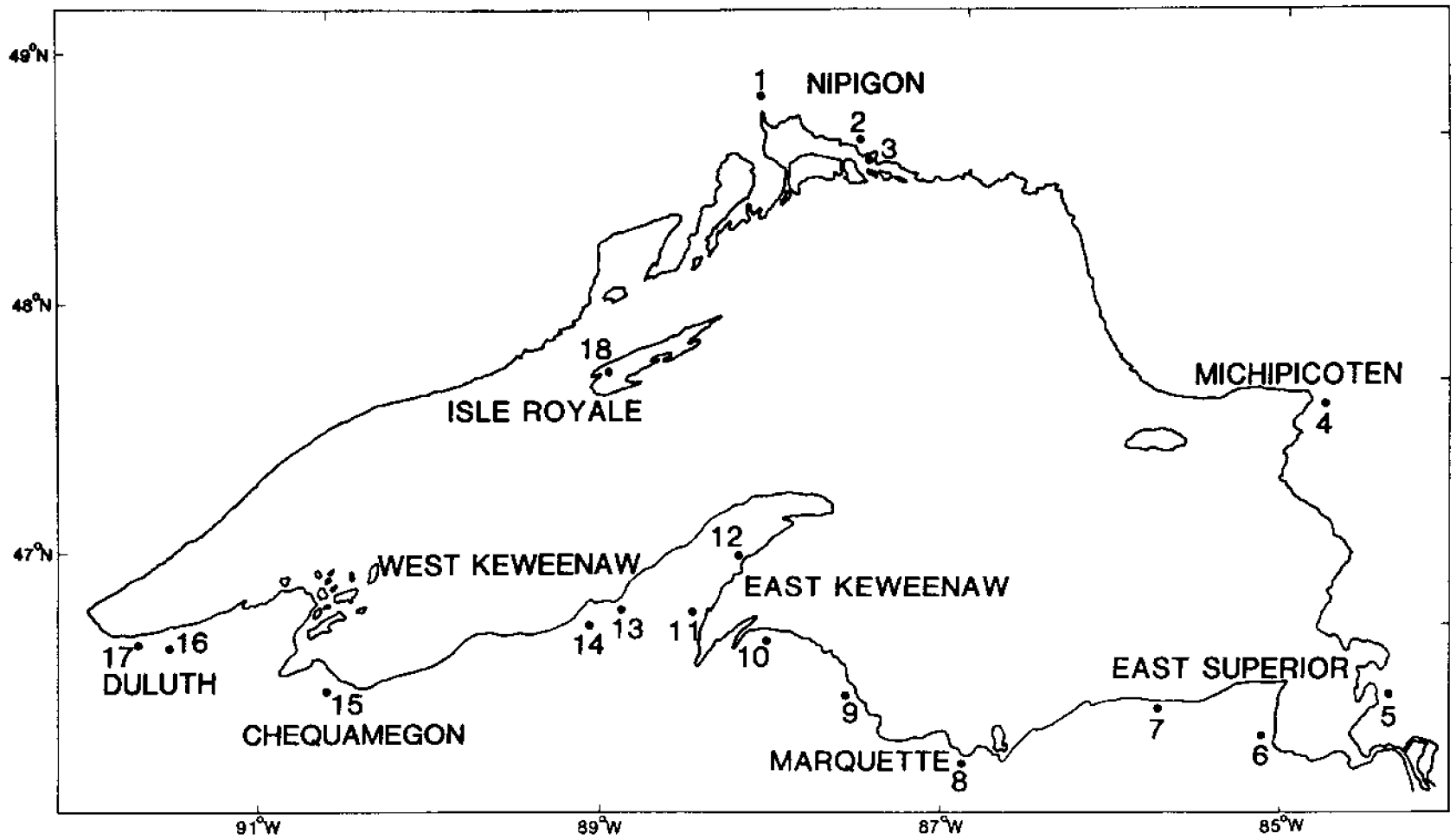
Collection Location	AGP			MDH-1			PGI-2				PGM-1			$\bar{H}$	SE $(\bar{H})$
	100	146	n	-100	-165	n	100	92	122	n	100	148	n		
Nipigon															
1 Nipigon River	0.54	0.46	39	0.79	0.21	39	0.97	0.03	0.0	39	0.56	0.44	39	0.055	0.030
2 Cypress River	0.48	0.52	25	0.76	0.24	25	1.0	0.0	0.0	25	0.50	0.50	25	0.055	0.030
3 Mountain Bay	0.50	0.50	18	0.75	0.25	18	0.97	0.0	0.03	18	0.56	0.44	18	0.057	0.030
Michipicoten															
4 Michipicoten River	0.65	0.35	40	0.81	0.19	40	1.0	0.0	0.0	40	0.70	0.30	40	0.047	0.027
East Superior															
5 Goulais River	0.60	0.40	29	0.87	0.13	40	1.0	0.0	0.0	40	0.50	0.50	29	0.048	0.028
6 Tahquamenon River	0.63	0.37	15	0.78	0.22	27	0.98	0.02	0.0	27	0.54	0.46	26	0.053	0.029
7 Sucker River	0.63	0.37	46	0.80	0.20	63	0.99	0.0	0.01	69	0.51	0.49	69	0.052	0.029
Marquette															
8 Au Train River	0.48	0.52	46	0.89	0.11	47	0.98	0.02	0.0	47	0.57	0.43	47	0.049	0.028
9 Big Garlic River	0.51	0.49	40	0.87	0.12	40	0.95	0.02	0.03	40	0.59	0.41	40	0.052	0.028
East Keweemaw															
10 Huron River	0.61	0.39	40	0.84	0.15	40	0.96	0.03	0.01	40	0.62	0.38	40	0.052	0.028
11 Sturgeon River	0.66	0.34	34	0.69	0.31	51	0.98	0.01	0.01	49	0.65	0.35	49	0.055	0.029
12 Traverse River	0.76	0.24	17	0.87	0.13	44	1.0	0.0	0.0	44	0.61	0.39	44	0.043	0.025
West Keweenaw															
13 Misery River	0.60	0.40	25	0.80	0.20	25	0.98	0.02	0.0	25	0.66	0.34	25	0.052	0.028
14 Firesteel River	0.60	0.40	81	0.78	0.22	81	0.96	0.04	0.0	81	0.67	0.33	81	0.052	0.028

Table Continued

Collection Location	AGP 100	AGP 146	n	MDH-1 -100	MDH-1 -165	n	PGI-2 100	PGI-2 92	PGI-2 122	n	PGM-1 100	PGM-1 148	n	$\bar{H}$	$\bar{(H)}$
Chequamegon															
15 Bad River	0.53	0.47	44	0.90	0.10	73	0.90	0.03	0.05	74	0.61	0.39	79	0.052	0.028
Duluth															
16 Nebagamon River[a]	0.67	0.33	41	0.86	0.14	62	0.98	0.02	0.0	70	0.77	0.23	42	0.043	0.024
17 Middle River	0.57	0.43	74	0.85	0.15	79	0.99	0.01	0.0	79	0.75	0.25	79	0.046	0.026
Isle Royale															
18 Washington Creek	1.0	0.0	44	1.0	0.0	69	1.0	0.0	0.0	60	0.87	0.13	63	0.009	0.009
F_{is}		0.036			0.018			0.135				0.025			
F_{st}		0.059			0.037			0.019				0.042			
Heterogeneity															
Chi-Square[b]		82.7***			63.1***			32.7*				69.7***			

[a]Data from Krueger (1980).

[b]Testing the null hypothesis that all samples were drawn from a common population. Single asterisk indicates $P < 0.05$; tripple asterisk indicates $P < 0.005$.


49°N
48°N
47°N
91°W
89°W
87°W
85°W
1 NIPIGON
2
3
18
ISLE ROYALE
MICHIPICOTEN
4
12
WEST KEWEENAW
EAST KEWEENAW
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16
DULUTH
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CHEQUAMEGON
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MARQUETTE
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EAST SUPERIOR