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Stability of Allozyme and Mitochondrial DNA Markers among Three Year-Classes of Lake Trout Propagated from Seneca Lake, New York

PETER M. GREWE[1] AND CHARLES C. KRUEGER[2]

Department of Natural Resources, College of Agriculture and Life Sciences
Fernow Hall, Cornell University, Ithaca, New York 14853, USA

CHARLES F. AQUADRO

Section of Genetics and Development
Biotechnology Building, Cornell University, Ithaca, New York 14853, USA

BERNIE MAY

Cornell Laboratory for Ecological and Evolutionary Genetics, Department of Natural Resources
Fernow Hall, Cornell University, Ithaca, New York 14853, USA

Abstract.—Lake trout *Salvelinus namaycush* representing three year-classes (1988, 1989, and 1990) of gametes collected from adults captured in Seneca Lake, New York, were examined for allelic variation at 18 polymorphic loci and for mitochondrial DNA (mtDNA) haplotype variation; mtDNA was digested with the four restriction endonucleases *Ava* I, *Bam*H I, *Hinf* I, and *Taq* I. Analysis of allelic frequencies among these three year-classes of the Seneca strain and a previously analyzed collection (1983 year-class, also a wild egg taken from Seneca Lake) indicated temporal heterogeneity ($P < 0.01$) at three loci: two for proline dipeptidase (*PEPD-1,2**) and one for malic enzyme ($NADP^+$) (*mMEP-1**). However, cluster analysis of genetic distances demonstrated that all four Seneca Lake samples exhibited a much lower level of differentiation than observed among other strains stocked into Lake Ontario. Mitochondrial DNA haplotype frequencies were not significantly different among the 1988, 1989, and 1990 year-class samples, which supported the conclusion of genetic similarity among Seneca strain samples. Allozyme differences among Seneca collections did not significantly affect estimates by mixed-stock analysis (MSA) of parental strain contributions to a mixture of wild fry from Lake Ontario. Based on these results, data from the four year-classes could be pooled and used as Seneca strain baseline data for future MSA estimates.

Mixed-stock analysis (MSA), or genetic stock identification (GSI), has become a standard analytic technique used to determine the proportions of different stocks that contribute to fishery harvests of Pacific salmonids *Oncorhynchus* spp. (Shaklee et al. 1990b; Utter and Ryman 1993). Mixed-stock analysis has also been used to determine the parental origins of fry of lake trout *Salvelinus namaycush* captured in Lake Ontario (Marsden et al. 1989). In the Lake Ontario appli-

[1] Present address: Commonwealth Scientific and Industrial Research Organization, Division of Fisheries, GPO Box 1538, Hobart, Tasmania 7001, Australia.

[2] Author to whom correspondence should be addressed.

cation of MSA, allozymes were used as genetic characters to discriminate strains of lake trout stocked into Lake Ontario that could have contributed gametes to the wild fry. Examination of these characters in a mixture of wild fry permitted estimates of the reproductive contributions made by each of the stocked strains. The purpose of both the stocking and the stocking evaluations is to facilitate rehabilitation of naturally reproducing populations of lake trout in Lake Ontario.

A critical assumption of the baseline data used in MSA is the temporal genetic stability of hatchery strains stocked into Lake Ontario. Several year-classes of any strain stocked into Lake Ontario could contribute, by now, to a mixture of wild fry. However, the baseline data used in the past MSA study of lake trout fry from Lake Ontario came from a single year-class, because it was logistically difficult and expensive to obtain and analyze collections from each year-class of potential contributor strains stocked 5–10 years previously (Krueger et al. 1989). Different year-classes of a strain were assumed to possess similar frequencies of the genetic characters (allozymes) used to discriminate strains.

Techniques for rapidly processing individuals for mitochondrial DNA (mtDNA) analysis have made practical the use of mtDNA haplotype frequencies as an additional baseline data type in the MSA of lake trout fry. However, because of the strictly maternal inheritance of the mitochondrial genome (Moritz et al. 1987), the effective population size (N_e) of the next generation of mitochondria is one-fourth that of biparentally inherited nuclear genes (e.g., genes that encode allozymes; Birky et al. 1983). Thus, frequencies of mtDNA haplotypes are more susceptible to random stochastic variation or genetic drift than allelic frequencies in the same population (Carson and Templeton 1984), and they should be more prone to instability among year-classes of lake trout stocked into Lake Ontario. Such temporal instability would weaken conclusions drawn from MSA if mtDNA characters were used for baseline data. Temporal variation of allelic and mtDNA haplotypes has been examined in natural populations of *Drosophila mercatorum* (DeSalle et al. 1987) and bluegill *Lepomis macrochirus* (Chapman 1989). In both studies, allozyme allelic frequencies were temporally stable, but mtDNA haplotype frequencies fluctuated between sampling periods (2–5 years). Small effective population sizes were suspected as the cause of the observed instability of mtDNA haplotypes. Small numbers of adults collected from wild populations and used as a source of gametes may also promote drift among temporally spaced samples of fish propagated in hatcheries (Waples and Teel 1990). Thus, we had to determine the magnitude of mtDNA haplotype variation among year-classes of hatchery lake trout before we could incorporate haplotype frequencies into baseline data used for MSA of wild Lake Ontario fry.

The Seneca strain of lake trout, from Seneca Lake, New York, was used to evaluate mtDNA stability because of its apparent contribution to natural reproduction in Lake Ontario. Mixed-stock analysis of wild fry (1986 year-class) and data from the 1988–1990 year-classes captured near Stony Island, Lake Ontario, indicated that the Seneca strain accounted for 70% or more of the parentage (Marsden et al. 1989; Grewe 1991; Grewe et al. 1994). Stability of allelic frequencies in the Seneca strain was demonstrated between two year-classes propagated from a hatchery broodstock (Krueger et al. 1989). No genetic comparison has been made among year-classes derived directly from the Seneca Lake population, which currently serves as the source of hatchery broodstocks for Lake Ontario stocking. Most year-classes of mature Seneca-strain lake trout now in Lake Ontario (ages 8–15) were propagated from gametes collected from adults captured in Seneca Lake and not from recently developed hatchery broodstocks. Examination of the temporal stability of allozyme allelic and mtDNA haplotype frequencies permits assessment of the genetic variation that may have occurred in past stocking.

The purpose of this study was to describe the temporal variation in allozyme allelic and mtDNA haplotype frequencies among year-classes of lake trout derived from the population in Seneca Lake. Similarity of both allelic and mtDNA haplotype frequencies among year-classes would permit year-class data to be pooled to represent the Seneca Lake strain as baseline data for MSA.

Methods

Collections.—A collection of 80–90 individuals was obtained from each of the 1988, 1989, and 1990 year-classes of lake trout propagated from gametes collected from adults caught in Seneca Lake (Table 1). Individuals of the 1988 and 1989 year-class were samples from a rearing pond that contained progeny from all adults used for the gamete collection for the year. The sample of the 1990 year-class was obtained from two raceways

containing progeny from a subsample of adults used for the entire gamete collection. Each year-class represented gametes collected from 800–1,000 adults (typically 150–250 females) netted over 3 weeks from the third week of September through the first week of October. Nets were set near Glenora and Peach Orchard points on the eastern and western sides, respectively, of Seneca Lake (M. Babenzien, New York State Department of Environmental Conservation, personal communication). These gamete collections were made in an identical manner as those in the 1970s and early 1980s that were used as the source of Seneca-strain fish stocked into Lake Ontario. Samples were frozen on dry ice or in liquid nitrogen at the hatchery and stored at −80°C in the laboratory until analyzed. Additional allozyme data used in this study came from the Clearwater Lake, Jenny Lake, Killala Lake, Manitou, Seneca Lake (three samples), and Lake Superior hatchery-propagated strains and from the Seneca wild strain (MN-CWL, GL-JL, GL-KL, GL-LM, FL-SEN81, FL-SEN83, FL-SEN84, GL-SUP, FL-SEN-W, respectively; data and abbreviations are from Krueger et al. 1989).

Genetic analysis.—Allozyme variation at 18 polymorphic loci was examined by horizontal starch gel electrophoresis and histochemical staining (May et al. 1979; Krueger et al. 1989). Polymorphic loci were defined as loci that had at least two alleles present at frequencies exceeding 0.05, as described by Krueger et al. (1989). Abbreviations used for protein-coding loci and their alleles follow the nomenclature presented by Shaklee et al. (1990a). An exception was the proline dipeptidase locus (*PEPD**), which was observed with a stain containing phenyl-alanyl-proline substrate (*PEP-PAP** of Krueger et al. 1989). Allozyme data that characterize Clearwater, Jenny, Killala, Manitou, and Superior hatchery-propagated strains were from Krueger et al. (1989).

Mitochondrial DNA was extracted from white muscle tissue by a modified hexadecyltrimethylammonium bromide (CTAB) protocol (Grewe et al. 1993). Samples were digested with the restriction enzymes *Ava* I, *Bam*H I, *Hin*f I, and *Taq* I. Restriction fragments were separated and visualized with the methods described by Grewe et al. (1993). Mitochondrial haplotypes were designated according to restriction profiles described by Grewe et al. (1993). Haplotype designations were limited to profiles resolved with fragments that were larger than 400 base pairs.

Statistical procedures.—Allelic counts at five isoloci (*sAAT-1,2**, *FH-1,2**, *MDH-B1,2**, *PEPD-1,2**, and *PGM-3,4**; enzymes coded by these and other loci are listed in Table 2) were combined for each isolocus pair because assignment of allelic variation to a specific isolocus was impossible to determine from electrophoretic banding patterns (i.e., each isolocus had an allele count of four per individual for a total count of 4n). Waples (1989) provided a method to test whether observed statistical differences of temporally spaced samples resulted from genetic drift and sampling error or from other causes. However, because of our "two-tiered" sampling design (examined progeny represented a sample of adults that, in turn, represented the adult population of Seneca Lake), we could not use that method to analyze the data (R. S. Waples, National Marine Fisheries Service, personal communication). Thus, the heterogeneity of allelic and haplotype frequencies among year-classes was tested by contingency table analysis and the *G*-statistic (Sokal and Rohlf 1981). A significance level of $P \leq 0.05$ was used to reject the null hypothesis of genetic homogeneity of allele and haplotype frequencies among year-classes for individual locus tests. The critical values used were adjusted to account for the increase in type I error when multiple tests of the same hypothesis were made (see Cooper 1968).

Genetic distances (Nei 1972) were calculated over 18 polymorphic loci and subjected to the unweighted pair-group method cluster analysis (Sneath and Sokal 1973). A microcomputer program designed by B. May and C. C. Krueger and written in "C" by W. Eng, Cornell University, was used to analyze data (unsupported versions of "Genes in Populations" are available from Bernie May, upon request).

TABLE 1.—Year-class and origin of lake trout used in this study. All fish were raised from gametes produced by Seneca Lake adults captured during the New York State Department of Environmental Conservation's annual egg take.

Year-class	Sample size	Abbreviation	Hatchery from which sample was obtained
1983	80	FL-Sen-W83	Bath State Fish Hatchery, Bath, New York (from Krueger et al. 1989)
1988	80	SenW88	Allegheny National Fish Hatchery, Warren, Pennsylvania, Mar 1988
1989	90	SenW89	Bath State Fish Hatchery, Dec 1989
1990	90	SenW90	Bath State Fish Hatchery, Feb 1990

TABLE 2.—Allozyme allelic frequencies at the 18 loci examined among year-classes of the Seneca Lake strain of lake trout. Double asterisks on *G*-statistics denote $P < 0.01$**; for values without asterisks, $P > 0.05$. See Table 1 for explanation of sample abbreviations. Values for the 1983 year-class sample were obtained from Krueger et al. (1989); *MUP-1** of Krueger et al. (1989) has been renamed *ACP-1** as per Morizot and Schmidt (1990) to reflect the nomenclature of Shaklee et al. (1990a).

Locus[a]	Allele	Allelic frequencies and sample sizes (*N*) for Seneca-strain year-class: FL-Sen-W83	SenW88	SenW89	SenW90	Total *G*	df
*sAAT-1,2**	*85	0.34	0.28	0.30	0.32	2.82	3
	*100	0.66	0.72	0.70	0.68		
		N = 160	*N* = 154	*N* = 178	*N* = 176		
*ACP-1**	*–100	0.98	0.98	0.98	0.99	1.08	3
	*–140	0.02	0.02	0.02	0.01		
		N = 80	*N* = 78	*N* = 90	*N* = 90		
*FH-1,2**	*100	0.88	0.88	0.89	0.88	0.66	3
	*90	0.12	0.12	0.11	0.12		
		N = 160	*N* = 154	*N* = 174	*N* = 180		
*G3PDH-1**	*100	0.93	0.93	0.93	0.95	1.10	3
	*35	0.07	0.07	0.07	0.05		
		N = 80	*N* = 78	*N* = 90	*N* = 90		
*GPI-B1**	*100	1.00	0.99	1.00	1.00	2.94	3
	*200	0.00	0.01	0.00	0.00		
		N = 80	*N* = 78	*N* = 90	*N* = 90		
*LDH-B1**	*100	1.00	1.00	1.00	0.99	2.66	3
	*78	0.00	0.00	0.00	0.01		
		N = 80	*N* = 80	*N* = 90	*N* = 89		
*MDH-B1,2**	*100	1.00	1.00	0.99	0.99	4.15	3
	*144	0.00	0.00	0.01	0.01		
		N = 80	*N* = 79	*N* = 90	*N* = 89		
*mMEP-2**	*100	0.93	0.96	0.96	0.87	19.07**	3
	*115	0.07	0.04	0.04	0.17		
		N = 80	*N* = 78	*N* = 90	*N* = 89		
*PEPD-1,2**	*100	0.08	0.10	0.11	0.10	30.48**	6
	*179	0.89	0.88	0.82	0.89		
	*138	0.03	0.02	0.07	0.01		
		N = 160	*N* = 160	*N* = 174	*N* = 172		
*PGK-1**	*–100	1.00	1.00	1.00	1.00	0.00	
		N = 80	*N* = 78	*N* = 90	*N* = 90		
*PGM-2**	*100	0.99	0.98	1.00	1.00	7.17	3
	*150	0.01	0.02	0.00	0.00		
		N = 80	*N* = 78	*N* = 90	*N* = 90		
*PGM-3,4**	*100	0.61	0.51	0.66	0.57	8.68	3
	*94	0.39	0.49	0.34	0.43		
		N = 160	*N* = 160	*N* = 176	*N* = 180		
*sSOD-1**	*100	1.00	1.00	1.00	1.00	0.00	
		N = 80	*N* = 78	*N* = 90	*N* = 90		

[a]Enzymes (with their international numbers: IUBNC 1984) coded by these loci are:

*sAAT-1,2**: aspartate aminotransferase (2.6.1.1)
*ACP-1**: acid phosphatase (3.1.3.2)
*FH-1,2**: fumarate hydratase (4.2.1.2)
*G3PDH-1**: glycerol-3-phosphate dehydrogenase (1.1.1.8)
*GPI-B1**: glucose-6-phosphate isomerase (5.3.1.9)
*LDH-B1**: L-lactate dehydrogenase (1.1.1.27)
*MDH-B1,2**: malate dehydrogenase (1.1.1.37)
*mMEP-2**: malic enzyme (1.1.1.40)
*PEPD-1,2**: proline dipeptidase (3.4.13.9)
*PGK-1**: phosphoglycerate kinase (2.7.2.3)
*PGM-2**; *PGM-3,4**: phosphoglucomutase (5.4.2.2)
*sSOD-1**: superoxide dismutase (1.15.1.1)

Mixed-stock analysis.—The mixed-stock analysis procedure described by Marsden et al. (1989), as modified by Grewe et al. (1994), was used to calculate estimates of parental strain contributions that would result from using the four baseline files created with each of the four year-class samples of the Seneca strain. Baseline data sets were established with each Seneca data file that included

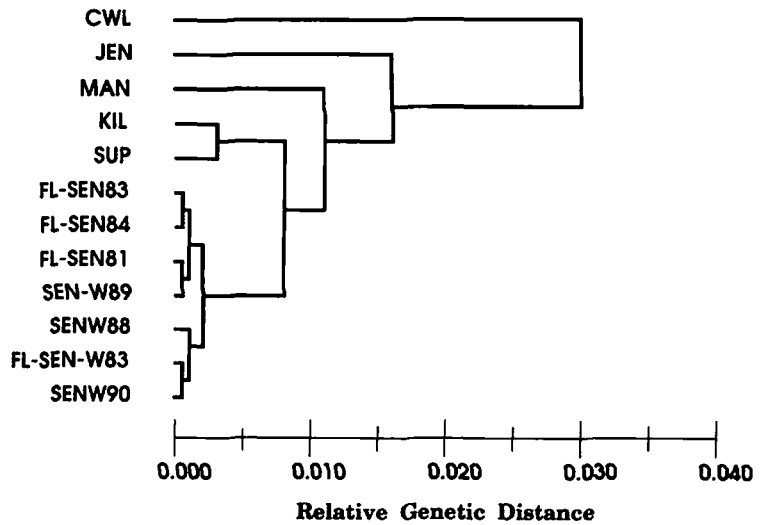

FIGURE 1.—Dendrogram of Nei's genetic distance among several year-classes of lake trout originating in Seneca Lake and other areas of the Great Lakes. Relative genetic distances were calculated for the 18 polymorphic loci described by Krueger et al. (1989). Abbreviations: CWL = Clearwater, JEN = Jenny, KIL = Killala, MAN = Manitou, and SUP = Superior strains of Krueger et al. (1989); FL-SEN81 = 1981 Seneca Lake broodstock, FL-SEN83 = 1983 offspring from the 1978 Seneca Lake broodstock, and FL-SEN84 = 1984 offspring from the 1978 Seneca Lake broodstock; FL-SEN-W83, SENW88, SENW89, and SENW90 are, respectively, the 1983, 1988, 1989, and 1990 progeny of wild Seneca Lake parents.

interstrain crosses between the Clearwater, Jenny, Killala, Manitou, Seneca, and Superior strains. These baseline files were created with the methods and programs described by Grewe et al. (1994) to test the effect of using the individual Seneca strain year-classes as the source of Seneca strain allelic data. Mitochondrial DNA data used for the 1983 year-class were the pooled data from the 1988, 1989, and 1990 year-classes. The 1989 year-class of wild fry collected from Lake Ontario was chosen as the test mixture for this analysis.

Results

Allozyme Variation

Significant allelic frequency differences were detected among the 1983, 1988, 1989, and 1990 year-classes of lake trout propagated from Seneca Lake, based on a total G-statistic summed over all loci ($G = 89.2$, df = 36, $P < 0.01$; Table 2). Individual locus tests indicated that only *PEPD-1,2** and *mMEP-2** exhibited differences among year-classes ($P < 0.01$). These three loci accounted for more than 55% of the total G-value summed over all loci among the four year-classes. Differences observed for *PEPD-1,2** ($G = 30.5$, df = 6, $P < 0.01$) were primarily a result of the high frequency of allele **138* and low frequency of allele **179* in the 1989 year-class sample (Table 2). No significant differences were observed among the 1983, 1988, and 1990 year-classes at *PEPD-1,2** (Table 2). The significant G-value observed for *mMEP-2** was primarily a result of the relatively low frequency of the **100* allele for the 1990 year-class relative to the other samples ($G = 30.5$, df = 3, $P < 0.01$; Table 2). No significant differences occurred among the 1983, 1988, and 1989 year-classes at *mMEP-2**.

Cluster analysis of relative genetic distances between the four Seneca Lake year-classes, Seneca hatchery broodstocks, and the Clearwater, Jenny, Killala, Manitou, and Superior-strain collections (data from Krueger et al. 1989) placed all Seneca fish in a group with a maximum observed genetic distance of 0.002 (Figure 1). Genetic distance of the next closest group of samples to the Seneca samples was 0.008. The maximum genetic distance (0.030) was between Clearwater Lake and all other strains.

Mitochondrial DNA Haplotype Variation

Three mtDNA haplotypes were observed in each of the 1988, 1989, and 1990 Seneca Lake year-classes (Table 3). Frequencies of these haplotypes were not significantly different among the three

TABLE 3.—Numbers and frequencies of mtDNA haplotypes among three year-classes of lake trout propagated from gametes collected from wild Seneca Lake adults. Values in parentheses are 95% confidence intervals (CI) of the proportion estimates. Four-letter haplotype designations AAAA, AADD, and BBAA correspond to fragment patterns observed for *Ava* I, *Bam*H I, *Hin*f I, and *Taq* I restriction enzymes as described by Grewe et al. (1993).

Year-class and statistic	Haplotype designation			Total sample size
	AAAA	AADD	BBAA	
1988				
N	17	10	48	75
Frequency	0.227	0.133	0.640	
95% CI	(0.13–0.32)	(0.06–0.21)	(0.53–0.75)	
1989				
N	24	18	48	90
Frequency	0.267	0.200	0.533	
95% CI	(0.18–0.36)	(0.12–0.28)	(0.43–0.64)	
1990				
N	35	16	38	89
Frequency	0.393	0.180	0.427	
95% CI	(0.29–0.50)	(0.10–0.26)	(0.32–0.53)	

year-classes ($G = 8.8$, df = 4, $0.10 > P > 0.05$; Table 3).

Mixed-Stock Analysis

Similar parental strain estimates were obtained with each of the four baseline files for MSA of the 1989 wild fry collected from Stony Island (Table 4). Each analysis indicated that the Seneca strain contributed the majority of gametes to the fry mixture (62–74%). The Killala and Superior strains were estimated to have contributed gametes to the fry mixture at proportions that ranged from 12 to 24% (Table 4).

TABLE 4.—Calculated contributions of lake trout strains to fry production when different year-classes of the Seneca strain were used to create the baseline data. Mixed-stock analysis results are for a 1989 collection of wild fry from Lake Ontario near Stony Island. Mitochondrial DNA frequencies used for the 1983 year-class of Seneca fish were derived from average frequencies in the 1988, 1989, and 1990 year-class samples.

Contributing strain[a]	Year-class of Seneca strain used as allozyme baseline data				
	1983	1988	1989	1990	Seneca pooled
Killala	0.15	0.16	0.24	0.13	0.15
Seneca	0.73	0.69	0.62	0.74	0.72
Superior	0.12	0.15	0.14	0.14	0.13

[a] No contributions from the Clearwater, Jenny, or Manitou strains in any analysis.

Discussion

Genetic Variability among Year-Classes

The heterogeneity of allelic frequencies detected among the 1983, 1988, 1989, and 1990 year-classes of Seneca Lake samples may have been an artifact due to sampling error (i.e., type I error), or it could represent real differences among year-classes. One rejection of the null hypothesis (homogeneity of frequencies) out of the 13 individual-locus *G*-tests could be explained in part by random sampling error at the alpha level of 0.05. The significant *G*-value for *mMEP-2** in the 1990 year-class may have been due to random sampling error of adults from Seneca Lake, but examination of the hatchery records indicates this result was more likely due to nonrandom distribution of fish in the hatchery raceways. Fish sampled from the 1990 year-class were taken from raceways that represented the progeny from only 100 fish. These progeny represented approximately 12% of the adults (over 800 fish) that were collected from Seneca Lake and used as the gamete source for the 1990 year-class. Therefore, this sample may not have accurately represented the allelic frequencies of the 1990 hatchery year-class. Alternatively, the observed differences may indicate real changes among the temporally separated samples that could have been caused by genetic drift, migration, mutation, or selection within the Seneca Lake population. Regardless of whether the differences in allelic frequencies are statistical artifacts or reflect real changes in the Seneca Lake population, all four Seneca year-class samples exhibited a much lower level of differentiation than that observed among the other strains stocked into Lake Ontario (Figure 1).

The homogeneity of the mtDNA haplotypes observed among the 1988, 1989, and 1990 year-classes provides evidence that the year-classes of Seneca-strain lake trout stocked into Lake Ontario were genetically similar. Theoretical predictions suggest that mtDNA haplotype frequencies could fluctuate due to the effects of drift under conditions that leave allelic frequencies unaffected (Carson and Templeton 1984); by extension, the conditions that promote drift of allelic frequencies should also influence mtDNA haplotype drift. These theoretical predictions have been supported by studies of *Drosophila* and bluegill populations, which revealed temporal stability of allelic frequencies but heterogeneity of mtDNA haplotype frequencies (DeSalle et al. 1987; Chapman 1989).

Implications for Mixed-Stock Analysis

The assumption of temporal stability among year-classes for both allelic and mtDNA haplotype frequencies is critical when more than one year-class of a lake trout strain contributes to a second-generation mixture subjected to MSA. This assumption is especially important when data cannot be obtained from each year-class that could contribute to a mixture. For example, several year-classes of fish propagated from gametes collected from Seneca Lake fish and stocked into Lake Ontario during the 1970s were not available for sampling. Thus, the ability to use a single collection to represent several year-classes of a hatchery strain simplifies development of baseline data.

The allelic frequency differences among the four Seneca samples tested could warrant that the samples be considered separate sources of baseline data for MSA. However, Waples (1990) argued that temporally spaced samples should be pooled to increase the precision of MSA estimates unless dramatic frequency differences are observed among collections. Dramatic differences were not present among the four year-class samples of the Seneca strain. Close grouping of all Seneca-strain samples relative to other strains occurred in the cluster analysis of genetic distances calculated from allozyme frequencies (Figure 1). Furthermore, observed differences among the Seneca samples did not substantially affect MSA estimates (Table 4). Thus, all four year-classes were similar enough that the genetic data may be pooled among year-classes to provide the Seneca-strain baseline data for MSA of lake trout fry mixtures in Lake Ontario.

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