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Genetic variability among lake whitefish from Isle Royale and the Upper Great Lakes

Wendylee Stott[1], Thomas N. Todd[1] & Larry Kallemeyn[2]

[1)] *US Geological Survey, Great Lakes Science Center, 1451 Green Rd., Ann Arbor, MI 48105, USA*
[2)] *US Geological Survey, Columbia Environmental Research Center, International Falls Biological Station, 3131 Highway 53, International Falls, MN 56649, USA*

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The coregonine fishes from Isle Royale National Park represent a unique group that has escaped the successional changes observed elsewhere in North America. Analysis of microsatellite DNA loci revealed significant genetic differences among samples of lake whitefish (*Coregonus clupeaformis*) from Isle Royale, Lake Superior, and Lake Huron. The amount of genetic variation observed is consistent with that seen in other studies of whitefishes from North America. The lake whitefish from Isle Royale had previously been assigned sub-species status, but no evidence was found to support this. The effects of common ancestry and demographics both play a role in determining the relatedness of the populations. As with other fish species from Isle Royale and the upper Great Lakes, the lake whitefish have their origins in the Mississippi refugium.

Introduction

Fisheries biologists throughout the world have questions about the taxonomy and population differentiation of coregonine fishes. Previous and ongoing surveys of whitefish and cisco in North America, Asia, and Europe are helping to determine their origins and population structure (e.g., Smith & Todd 1984, Bodaly *et al*. 1992, Snyder *et al*. 1992, Vuorinen *et al*. 1993, Sajdak & Phillips 1997, Douglas *et al*. 1999, Lu & Bernatchez 1999, Lu *et al*. 2001, Turgeon & Bernatchez 2001). Morphological plasticity among and within populations is observed, making it difficult to reach agreement on species assignment and stock assignment, which in turn creates problems for the management of the resource.

The whitefishes are important forage fish for economically important species such as lake trout and are sometimes also the target of commercial fisheries. The lake whitefish (*Coregonus clupeaformis*) shows a wide range of life history and morphometric variation. Genetic analyses (e.g., Bernatchez & Dodson 1991, Bodaly *et al*. 1992, Bernatchez *et al*. 1996) indicate that five different races of lake whitefish evolved in separate glacial refugia in North America. Their origins may be the result of a complex series of events involving allopatric divergence, sympatric divergence, and secondary contact events (Pigeon *et al*. 1997, Lu *et al*. 2001).

Isle Royale National Park (Fig. 1) provides an opportunity to study relationships between geological events and genetic relatedness. The

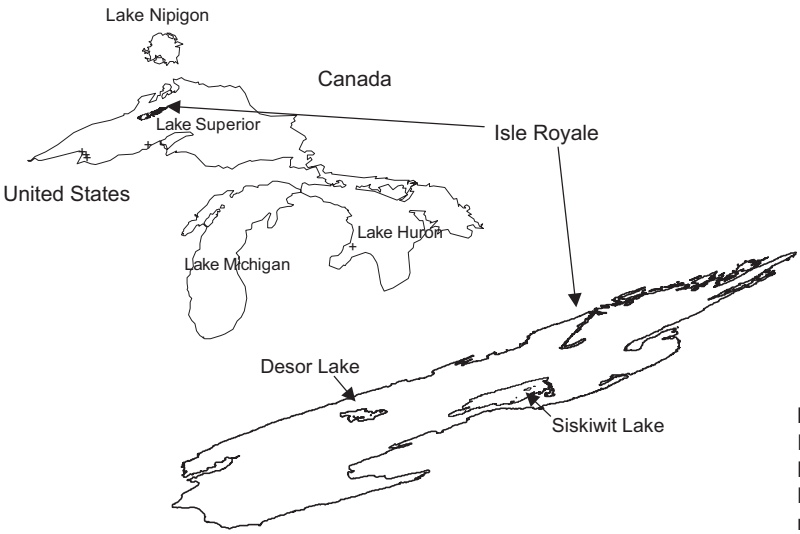

Fig. 1. Sample lakes on Isle Royale and the upper Great Lakes. Sample sites on Lake Huron and Lake Superior are marked with a '+'.

fish species found in lakes on the island represent a unique group that has seemingly escaped some of the successional changes that characterize the Great Lakes basin. The glacial history of Isle Royale is fairly well known. Most lakes on Isle Royale were created by glacial quarrying from the post-glacial lakes Minong and Nipissing (Hutchinson 1957). The lakes on Isle Royale are in the Great Lakes basin but are isolated from other Great Lakes populations. It is possible that a one-way migration of fish may occur from Siskiwit Lake to Lake Superior via the Siskiwit River during high water conditions (L. Kallemeyn pers. obs.). In addition, the lakes on Isle Royale are isolated from each other by elevational differences; therefore there is little or no gene flow among the lakes on the island. There is no record of any stocking events into any of the lakes on the island, although there is some anecdotal evidence that walleye were stocked into Ritchie Lake in 1925 (Kallemeyn 2000).

Four lakes on Isle Royale have populations of coregonines (Kallemeyn 2000). Lake whitefish are found in Desor and Siskiwit lakes and cisco (*C. artedi*) are found in Desor, Siskiwit, Sargent, and Ritchie lakes. A unique cisco from Siskiwit Lake was classified as a separate species in earlier work (*Coregonus bartletti*: Koelz 1931, Hubbs & Lagler 1949). Fish populations on Isle Royale do not mix and are probably derived from Lake Superior populations. However, the genetic variability and relatedness of whitefishes from the island are unknown. In addition, taxonomic issues exist among these small populations. The lake whitefish in both Siskiwit and Desor lakes were classified as separate sub-species of *C. clupeaformis*. The fish from Siskiwit Lake were classified as *C. clupeaformis neo-hantoniensis*, a form characterized by longer pectoral fins (Koelz 1931). The fish from Desor Lake were called *C. c. dustini*, a new sub-species that was characterized by having more lateral line scales, longer head, maxillary bone, and pectoral fins (Koelz 1931).

Recent surveys of the fish communities of Isle Royale provided an opportunity to re-examine the coregonines on the island. The object of this study is to determine whether lake whitefish from Isle Royale are genetically different from those found in Lake Superior. It is expected that samples from Desor Lake should be most distinct from Lake Superior and the samples from Siskiwit Lake should be less distinct since Desor Lake has been isolated from Lake Superior for a longer period of time. We also expect that samples from the island would be more closely related to each other than to other Great Lakes populations and will exhibit less diversity than Great Lakes samples.

Methods

Fin clips from lake whitefish were collected from Isle Royale in the summer of 1996 and

2002 during lake surveys performed by park staff using gill nets. Samples of lake whitefish from Lake Superior and Lake Huron were collected in 1999 and 2002 and analyzed to place the genetic diversity and genetic relatedness of the Isle Royale samples in context of the diversity in the upper Great Lakes. Samples from Lake Huron and Lake Superior were collected during routine trawl surveys performed by the Great Lakes Science Center (GLSC) during the spring and early summer. Lake Huron samples came from the western shore of the lake near the port of Alpena. Lake Superior samples were taken from four locations at the western end of the lake (Fig. 1). We wished to place the genetic diversity of lake whitefish from the Great Lakes region in a broader geographic context, so data from a study of lake whitefish from six lakes in the St. John River basin (eastern Canada and northern Maine; (Lu & Bernatchez 1999)) were also included in the analyses. Therefore, ten DNA samples of lake whitefish previously analyzed by other authors (e.g. Lu *et al*. 2001) were obtained to allow calibration of allele sizes between laboratories.

DNA was extracted from the tissue samples using the Puregene® protocol and reagents (Gentra Systems). The extracted DNA was quantified using a Hoefer Dynaquant 200 fluorometer and then amplified using the polymerase chain reaction (PCR). PCR primers for six microsatellite DNA loci known to amplify lake whitefish DNA were used following previously established methods (Bwf1, Bwf2, Cocl22, Cocl23, C2-157, C4-157; Turgeon 2000, Douglas *et al*. 1999). Two loci were amplified alone (C4-157 and Cocl22) and the others were co-amplified in two multiplex reactions (Bwf1 with C2-157 and Bwf2 with Cocl23). Each multiplex polymerase chain reaction (PCR) was carried out in a 15ul volume using the manufacturer's (Promega) buffer at 1× concentration, 0.2 mM each dNTP, 0.35 μM of each primer, 3.75 mM $MgCl_2$, 1 Unit *Taq* DNA polymerase, and 120 ng template DNA. The single primer polymerase chain reactions were carried out in a 15 μl volume using the manufacturer's buffer at 1× concentration, 0.2 mM each dNTP, 0.4 μM of each primer, 1.5 mM $MgCl_2$, 1 Unit *Taq* DNA polymerase, and 120 ng template DNA. The PCR thermal profile was similar for all loci; only the annealing temperature was altered. An initial denaturation step of 2 min. at 94 °C was performed, followed by 35 cycles of 1 min. at 94 °C, 1 min. at the annealing temperature, and a 1 min. extension at 72 °C. Annealing temperatures were the same as those reported by Lu and Bernatchez (1999) for all loci except Cocl22 (58 °C instead of 52 °C).

PCR products were prepared according to manufacturer's guidelines (Applied Biosystems) for capillary electrophoresis. Fragment size data were collected using the ABI Prism 310 Genetic Analyzer. The manufacturer's (Applied Biosystems) software (Genescan V1.14) was used to generate genotype data and Genotyper V2.3 was used to score and bin genotypes.

Standard measures of genetic diversity (expected and observed heterozygosity, allele number, and F_{ST}; Weir & Cockerham 1984) were calculated using the GENEPOP (Raymond & Rousset 1995) software package. Sample sites and loci were tested for deviation from Hardy-Weinberg equilibrium using the Markov chain iteration in GENEPOP. The significance level for multiple tests was adjusted using the sequential Bonferroni technique (Rice 1989). GENEPOP was also used to test the homogeneity of allele frequencies between all pairs of populations and the *P* values were also adjusted with the Bonferroni technique (Rice 1989).

The Cavalli-Sforza Edwards' chord distance measure (Cavalli-Sforza & Edwards 1967) was used to compare the lake whitefish from the Great Lakes basin with lake whitefish from six lakes in the St. John River basin (eastern Canada and northern Maine) analyzed in an earlier study (Lu & Bernatchez 1999). Allele sizes reported by Lu and Bernatchez (1999) were calibrated to those observed in the present study using DNA samples obtained from the authors. The Neighbor-joining method was used to construct a dendrogram of the genetic relationships among samples. The software package PHYLIP (Felsenstein 1989) was used to perform the analyses. The data set was re-sampled using the bootstrapping procedures available in PHYLIP and 10 000 replicates were used to create a consensus tree. The tree of genetic relationships created using PHYLIP was visualized using TreeView (Page 1996).

Two measures of geographic distance were used to examine the effect of geography and time since separation on genetic differentiation. Elevation and distance between sampling sites were used as surrogates for time since separation of the lakes in the Great Lakes basin. Elevation and distance were compared to the values of divergence as suggested by Rousset (1997; ln $[F_{ST}/(1 - F_{ST})]$). Elevation was measured as difference in height above sea level between pairs of lakes. The minimum distance among sample sites on the Great Lakes and the visual centroid of each of the Isle Royale lakes was used to measure Great Lake to island distances and the minimum distance on the water was used to measure the distance between the Great Lakes samples.

Results

Forty lake whitefish were analyzed from each of lakes Huron and Superior, 28 and 38 fish were analyzed from Desor and Siskiwit lakes, respectively. No PCR product was consistently obtained from the primers for C4-157, therefore it was not included in the analysis. Moderate to high levels of genetic diversity were observed at the five remaining loci. The number of alleles ranged from three to 15 and diversity (as measured by observed heterozygosity) ranged from 0.30 to 0.88 (Table 1). Heterozygosity over all loci for each sample was similar for all four lakes (Table 1), the number of alleles observed in the Isle Royale populations was slightly smaller than the number observed in the Great Lakes samples and there was overlap in the size range of alleles observed at each locus in all four sample sets (Table 1).

After adjustment for multiple tests, allele frequencies at three different loci in three different samples did not meet the expectations for Hardy-Weinberg equilibrium (Siskiwit Lake at Bwf1, Lake Superior at Bwf2, and Lake Huron at Cocl22). In all cases there was a significant deficiency of heterozygotes ($P = 0.001$ for Bwf1, $P = 0.004$ for Bwf2, and $P = 0.014$ for Cocl22). Tests of allelic homogeneity between pairs of populations indicated that there were significant differences ($P < 0.001$ for all tests with Bonferroni correction) in allele frequencies between all pairs of populations.

Genetic distances between lakes ranged from 0.015 to 0.184 (Table 2). Samples from the Great Lakes and St. John River drainage are on distinct, well-supported clusters (Fig. 2). Among the Great Lakes samples, the genetic distance between the Great Lakes samples is the smallest and the largest is between the two Isle Royale

Table 1. Allelic variability at five microsatellite DNA loci from lake whitefish from the upper Great Lakes and Isle Royale. Number of alleles at each locus (A), range of allele sizes (A_R, in base pairs), observed heterozygosity (H_O), expected heterozygosity (H_E), and average heterozygosity (H_M) for each sample.

Lake		Bwf1	Bwf2	C2-157	Cocl22	Cocl23	H_M
Desor Lake	A	6	3	11	5	3	
$N = 28$	A_R	200–224	149–159	143–177	117–133	254–266	
	H_O	0.74	0.71	0.80	0.54	0.32	0.62
	H_E	0.74	0.53	0.85	0.55	0.60	0.65
Lake Huron	A	7	6	14	10	9	
$N = 40$	A_R	202–216	145–159	137–167	111–135	252–270	
	H_O	0.72	0.50	0.80	0.40	0.81	0.65
	H_E	0.76	0.55	0.90	0.54	0.85	0.72
Lake Superior	A	10	7	15	9	7	
$N = 40$	A_R	202–224	145–165	141–175	113–133	252–268	
	H_O	0.56	0.30	0.88	0.80	0.77	0.66
	H_E	0.78	0.41	0.91	0.73	0.79	0.72
Siskiwit Lake	A	6	5	11	7	4	
$N = 38$	A_R	204–226	145–163	141–183	117–133	252–270	
	H_O	0.32	0.68	0.72	0.76	0.67	0.63
	H_E	0.69	0.66	0.72	0.79	0.52	0.68

samples. The plot of the two distance measures versus divergence does not produce a significant trend for either measure (Fig. 3).

Discussion

The number of alleles and diversity observed in the lake whitefish samples collected for this study are within the range reported in previous studies of lake whitefish from eastern North America (Lu & Bernatchez 1999, Lu *et al*. 2001). The primer set for the locus C4-157 is an exception, however. In previous studies, this primer was characterized by a null allele and 19 other alleles (Lu & Bernatchez 1999, Lu *et al*. 2001). However, we could not resolve C4-157 at the reported conditions or with any modification that was attempted. This also included the samples obtained for calibration purposes. Some PCR product was observed in some samples at a size that was smaller (about 239bp) than that previously reported. There are several possible explanations for the difficulty: the precise conditions required to visualize this locus were never found, the DNA quality was not sufficient to allow amplification of C4-157, or the null allele is very common in this region and was observed in the majority of the samples. The alleles previously reported at C4-157 ranged in size from 273 to 305bp (Lu & Bernatchez 1999), which is rather large for microsatellite DNA. Highly sheared or poor quality DNA may not contain enough template DNA to amplify larger DNA fragments.

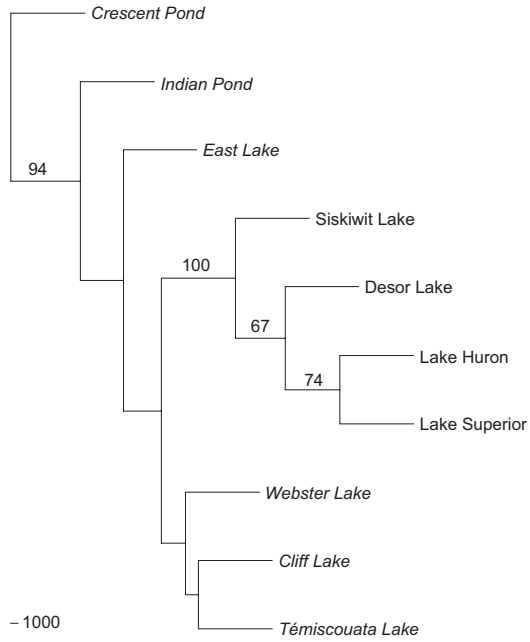

Fig. 2. Neighbour-joining dendrogram of Cavelli-Sforza and Edwards' chord distance among lakes. Numbers at branches indicate percent bootstrap support over 10 000 replicates, only values greater than 60 are reported. Data for the lakes in italics are from Lu and Bernatchez (1999).

However, a test of the DNA quality of the samples used in the current study revealed that most of the samples contained adequate quantities of intact DNA and another locus with large alleles (Cocl23) did amplify with little difficulty. If null allele(s) were present in the current sample set, they would occur at a combined frequency of

Table 2. Pairwise genetic divergence among lakes as measured by Cavalli-Sforza and Edwards chord distance (lower triangular matrix) and F_{ST} (upper triangular matrix; NA means not available). Data for the lakes in italics are taken from Lu and Bernatchez (1999).

	Desor	Huron	Superior	Siskiwit	*Témiscouata*	*Cliff*	*Webster*	*East*	*Indian*	*Crescent*
Desor		0.074	0.134	0.188	NA	NA	NA	NA	NA	NA
Huron	0.074		0.031	0.103	NA	NA	NA	NA	NA	NA
Superior	0.086	0.026		0.076	NA	NA	NA	NA	NA	NA
Siskiwit	0.097	0.062	0.053		NA	NA	NA	NA	NA	NA
Témiscouata	0.172	0.138	0.121	0.139		NA	NA	NA	NA	NA
Cliff	0.178	0.150	0.145	0.146	0.029		NA	NA	NA	NA
Webster	0.168	0.143	0.125	0.137	0.015	0.025		NA	NA	NA
East	0.178	0.154	0.142	0.148	0.028	0.043	0.023		NA	NA
Indian	0.174	0.160	0.146	0.155	0.018	0.025	0.017	0.023		NA
Crescent	0.184	0.168	0.155	0.171	0.051	0.058	0.043	0.040	0.029	

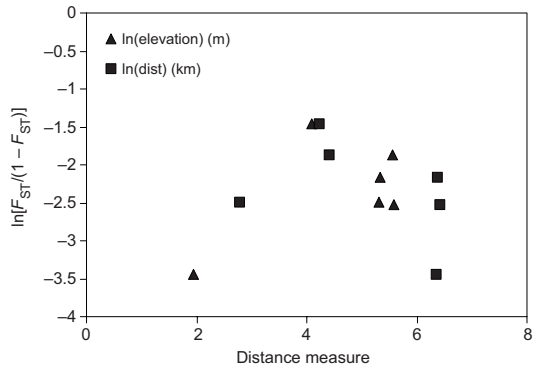

Fig. 3. Plot of transformed distance measures (ln(elevation) and ln(distance)) versus genetic diversity measured as ln [F_{ST}/(1 – F_{ST})].

almost 0.400 and only one other additional allele was observed. The estimated frequencies of the null allele(s) at C4-157 were not reported in previous studies, (Lu & Bernatchez 1999, Lu *et al*. 2001), but the other alleles occurred at much higher frequencies than observed here, if null allele(s) were present.

Three of the 20 comparisons did not conform to Hardy-Weinberg equilibrium. Two of the three sample–locus combinations were samples from the Great Lakes and the third was from Siskiwit Lake. The sample locations used by the GLSC are part of long-term studies of species abundance in the Great Lakes. The sites reflect areas of the lakes that differ in jurisdiction, abundance of certain species (Fabrizio *et al*. 1996), environment, and differences in life history parameters (Fabrizio *et al*. 2000). Therefore, it is possible that the departures from Hardy-Weinberg equilibrium are an indication that the Great Lakes samples are composed of two or more genetic populations. Other studies of population parameters and genetic characters have indicated that more than one stock of lake whitefish are found in Lakes Huron and Superior (Casselman *et al*. 1981, Hill 1982). Departures from Hardy-Weinberg equilibrium caused by admixtures of different populations have been observed in sympatric populations of brook trout (Dynes *et al*. 1999) and lake whitefish (Lu & Bernatchez 1999, Lu *et al*. 2001). However, since the deviations were not consistently observed over all loci, it is possible that other factors such as sample size or the presence of null alleles caused the deviations from Hardy-Weinberg equilibrium. Null alleles have been found in other loci used to analyze lake whitefish (Lu & Bernatchez 1999).

Due to differences in methodologies in different laboratories, scoring of the same samples can sometimes differ by one base pair or more (Wright & Bentzen 1995). Therefore, previously analyzed lake whitefish samples were run to calibrate the allele sizes and allow the comparison of data sets. Allele sizes reported previously for lake whitefish (Lu & Bernatchez 1999, Lu *et al*. 2001) were also observed in this study, once allele size calibrations were performed. The number of alleles observed at each locus was similar to that reported previously while the average observed heterozygosity was slightly higher in the current study. For example, average observed heterozygosity ranged from 0.42 to 0.63 in the six lakes studied by Lu and Bernatchez (1999) and in the current study they ranged from 0.62 to 0.66. This may be due to the presence of samples from the Great Lakes. The majority of samples from previous studies were taken from small to medium-sized inland lakes that have much smaller populations than either Lake Huron or Lake Superior. Since genetic diversity is correlated with effective population size, the Great Lakes should be expected to have higher diversity due to the great abundance of fish (Wright 1938). It is also possible that the Great Lakes samples are made up of several stocks that exchange genes at a fairly high rate. It has been shown that a sub-divided population that has some gene exchange will have higher diversity than a single population of similar size (Castric *et al*. 2001).

The analyses indicate that all sample collections are genetically distinct. F_{ST} values did not exceed those generally associated with species or sub-species boundaries. If the value of 0.2 suggested by Avise (1994) is used as a benchmark (values less than 0.2 are indicative of gene flow) then the F_{ST} values indicated that some gene flow was occurring between pairs of populations. Although the Isle Royale samples form a group with the Lake Superior samples (Fig. 2), the smallest genetic distances and F_{ST} values are observed between Lake Superior and Lake Huron (0.031, Table 2) suggesting that there is some gene flow between them. The two

Great Lakes are connected by the St. Mary's River, which allows a one-way migration from Lake Superior to Lake Huron. However, periodic high water levels may allow migration from Lake Huron into Lake Superior. The Isle Royale lakes sampled for this study have been isolated from the Great Lakes for 8700 to 10 000 years. Desor Lake was first exposed during the Lake Minong phase about 10 000 years ago (Raymond *et al*. 1975). Siskiwit Lake was first exposed by the formation of Houghton Lake during a low water phase that followed the formation of Lake Minong about 8700 years ago (Raymond *et al*. 1975, Bailey & Smith 1981, Flakne 1997). This is reflected by the fact that the largest genetic distances are observed between Desor Lake and the other lakes and that the genetic distance between Desor Lake and Lake Superior is greater than that between Lake Superior and Siskiwit Lake. Similar trends were also observed with the F_{ST} estimates. Trends in genetic divergence could be related to differences in elevation, however no relationship was observed in the current data set. Elevation was also related to genetic differentiation of brook trout in the St. John River drainage in Maine, but not in another proximate drainage (Castric *et al*. 2001), therefore the authors concluded that landscape could shape genetic diversity within a drainage system.

Duration of common ancestry, contemporary landscape, and population demographics will have an effect on genetic relatedness and it appears that all elements are affecting the population structure of lake whitefish on Isle Royale and from the Great Lakes. It is possible that the effects of common ancestry may be observed at a broader geographic scale than covered in the current study. On a smaller scale, patterns of divergence may be more affected by demographic factors such as genetic drift, which is reflected in the reduced number of alleles in Desor and Siskiwit Lakes as compared to the Great Lakes. A similar result has been observed in comparisons of lake trout from Siskiwit Lake and Lake Superior (Burnham-Curtis *et al*. 1997). In their study, mitochondrial DNA diversity in Siskiwit Lake samples was about half that observed in samples from Lake Superior.

Five glacial lineages have been identified in lake whitefish from North America using allozyme and mitochondrial (mtDNA) variation (Bodaly *et al*. 1992, Bernatchez & Dodson 1991). No samples from Isle Royale were examined in either study, but they did analyze samples from lakes Superior, Huron, Michigan, and Ontario. The ten samples from Lake Superior all had the same mtDNA haplotype. Genetic diversity estimates were also very low (nucleotide diversity < 0.015, Bernatchez & Dodson 1991) in the remaining samples. Lake whitefish from all of these lakes were concluded to have Mississippian origins. Fish from the Mississippi lineage are found in the Northwest Territories, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, and Labrador (Bodaly *et al*. 1992). The presence of three lake whitefish glacial lineages in eastern North America was also confirmed by microsatellite DNA data (Lu *et al*. 2001). Furthermore, Lu *et al*. (2001) were able to identify lineage-specific allelic size groupings at three loci for the Acadian and Atlantic lineages. The Acadian and Atlantic alleles were not observed at high frequencies in any of the samples from the Great Lakes or Isle Royale. The alleles that were observed were more consistent with Mississippian origins. Previous studies of Isle Royale have indicated that other fish species also came from the Mississippi refugium (e.g., Bailey & Smith 1981). An analysis of lake trout (Kallemeyn 2000) revealed that all three mtDNA lineages observed in North America (Wilson & Hebert 1996) were found in Siskiwit Lake, but the fish most likely had Mississippian origins. A similar result was observed in a study of brook trout (Burnham-Curtis 1996) and northern pike from Isle Royale (Senanan & Kapuscinski 2000).

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