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PHYLOGEOGRAPHIC STRUCTURE IN MITOCHONDRIAL DNA OF THE LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS*) AND ITS RELATION TO PLEISTOCENE GLACIATIONS

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Abstract.—Restriction-fragment length polymorphisms were employed to evaluate the phylogenetic relationships, the genetic diversity and the geographic structure in mitochondrial DNA (mtDNA) lineages of the lake whitefish, *Coregonus clupeaformis*. Thirteen restriction enzymes that produced 148 restriction fragments were used to assay mtDNAs of 525 specimens collected among 41 populations. The sampling covered the entire range of the species, from Alaska to Labrador. Four distinct phylogeographic assemblages were identified. The Beringian assemblage, confined to Yukon and Alaska, was phylogenetically distinct from other assemblages and exhibited the highest level of nucleotide diversity. The Acadian assemblage was confined to southeastern North America and composed of a unique mtDNA clade. The Atlantic assemblage was confined to southern Québec and the northeastern United States and was also observed among anadromous populations of northern Hudson Bay. This group was highly polymorphic and responsible for most of the mtDNA diversity observed outside Beringia. The Mississippian assemblage occupied most of the actual range of lake whitefish, from the Mackenzie delta to Labrador. Ninety-two percent of all whitefish of this proposed origin belonged to a single mtDNA haplotype. Overall, the diversity, the geographic structure and the times of divergence of mtDNA phylogenetic assemblages correlate with the Pleistocene glaciations classically assumed to have dramatically altered the genetic diversity of northern fishes in recent evolutionary times. Our results emphasize the dominant role of these catastrophic events in shaping the population genetic structure of lake whitefish.

Key words.—Coregoninae, glacial refugia, haplotype distribution, haplotype diversity, North America, population differentiation.

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In recent years, mitochondrial DNA (mtDNA) restriction analysis has been widely used to study the geographic and genetic structure of animal populations (Wilson et al., 1985; Avise 1986, 1989; Avise et al., 1987*b*; Moritz et al., 1987). Major findings provided by these studies include: a) most species exhibit a high level of diversity in mtDNA haplotypes whose relationships can be interpreted phylogenetically; b) geographically separated populations most often occupy different branches of an intraspecific evolutionary tree; and c) historical biogeographic and demographic events are largely responsible for the magnitude and pattern of genetic population structure existing today. Jointly, these observations gave rise to the discipline of intraspecific phylogeography (Avise et al., 1987*a*). Phylogeographic studies in mtDNA have reinforced the view that any attempt to interpret population differentiation in terms of evolutionary factors may lead to erroneous conclusions in the absence of historical considerations (Selander and Whittam, 1983; Bermingham and Avise, 1986; Slatkin, 1987).

Lake whitefish, *Coregonus clupeaformis*, is a member of the salmonid family endemic to North America and is widely distributed from western Alaska to eastern Labrador (Fig. 1) (Scott and Crossman, 1974; Bodaly, 1986). All of the actual distribution range of whitefish was repeatedly covered by glaciers during Pleistocene glaciation events except for central Alaska and Yukon which remained ice-free (Fig. 1) (Prest, 1970). Such vicariant events are assumed to have dramatically altered the genetic diversity of northern fishes in recent evolutionary times (Briggs, 1986). Glaciation may have affected whitefish population structure in opposite ways. First, while the overall genetic diversity may have decreased through population reduction as a result of habitat loss, several local races may have

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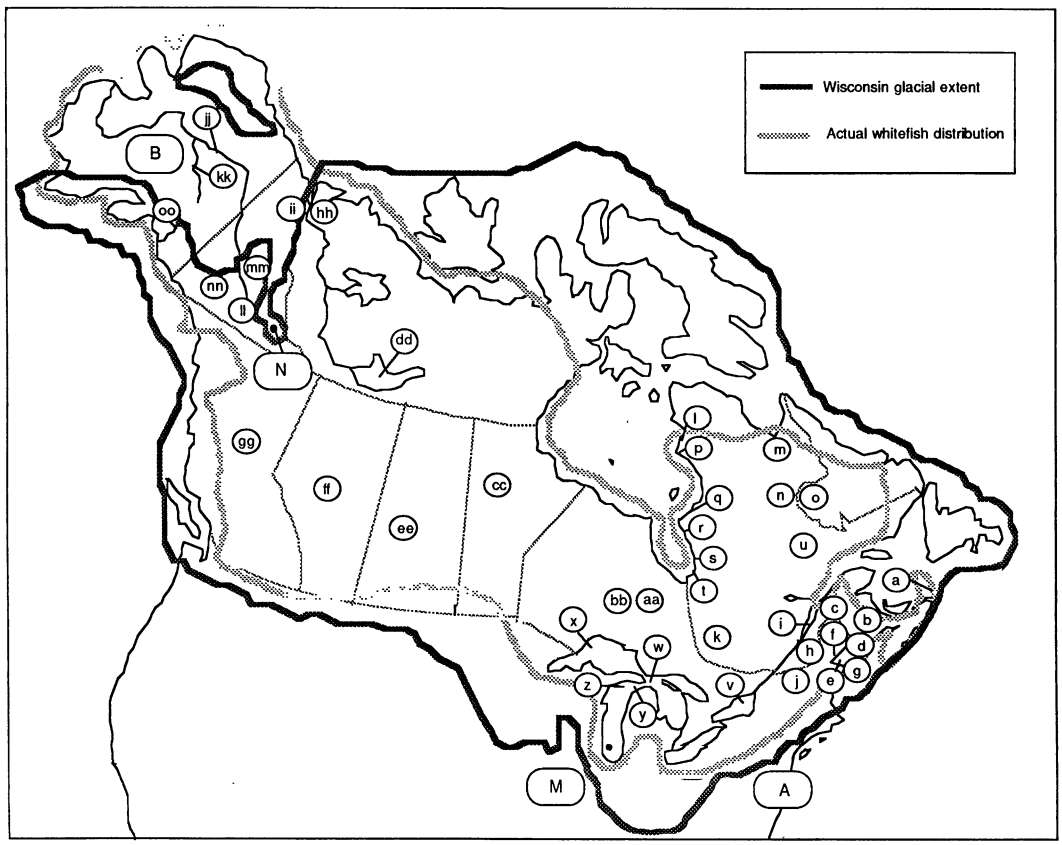

FIG. 1. Location map of lake whitefish sample sites, lake whitefish actual distribution, Wisconsinan glacial extent and location of Wisconsinan glacial refugia hypothesized for lake whitefish. B, Beringian; N, Nahanni; M, Mississippian and A, Atlantic. See Table 1 for corresponding names and sample sizes of populations identified by small letters.

originated from geographic and temporal isolation in separate glacial refugia (Crossman and McAllister, 1986). Second, huge proglacial lakes and river drainage connections provided formidable opportunities for dispersal and gene flow among newly created races. Therefore, it is likely that the broad scale pattern and magnitude of whitefish population genetic structure largely result from the historical interplay between the relative importance of these constraints and the potential of the species to cope with them.

Lake whitefish exhibit considerable phenotypic variation in morphological and life history characteristics. The interpretation of whitefish population differentiation has been based on such parameters for over a century (reviewed in Behnke, 1972) but conclusions remain conflicting (Lindsey, 1988). For instance, studies of geographic variation of gill-raker numbers, a key feature in coregonid systematics (Scott and Crossman, 1974), led to the recognition of geographic races and species (McPhail and Lindsey, 1970). On the other hand, comparable ranges of variation of gill-raker numbers may be generated within the same race by natural selection and environmental influence (Lindsey, 1981).

A better understanding of population differentiation has more recently been obtained by allozyme studies. Lindsey et al. (1970) showed that 3 sympatric pairs of whitefish populations found disjunctly in eastern, central and western North America evolved independently, which suggested that these populations survived in separate glacial refugia. More recently, Franzin and Clayton (1977) and Foote (1979) estab-

lished genetic discontinuities among populations of western Canada, suggesting that whitefish that recolonized that area survived in at least two and possibly three refugia. Taken together, allozyme studies hypothesized that whitefish survived the Wisconsinan glaciation in four refugia: the Atlantic, Mississippian, Beringian and the Nahanni, the latter corresponding to an ice-free corridor between the Laurentidan and the Cordillerian ice sheets (Fig. 1). However, these studies did not document historical evolutionary events such as the time of separation of the different races, the importance of population bottlenecks in reducing genetic diversity, and the phylogenetic relationships among differentiated populations.

Mitochondrial DNA analysis may prove more useful than allozymes in obtaining historical information on lake whitefish. For example, allozyme analysis established that sympatric pairs of whitefish populations in northeastern United States are reproductively isolated (Kirkpatrick and Selander, 1979). However, it failed to provide any clue about the evolutionary origin of these populations. MtDNA analysis of these populations clearly established that members of a sympatric pair of populations belonged to two phylogenetically and geographically distinct groups that evolved prior to the Wisconsinan glaciation and subsequently recolonized the same area but remained reproductively isolated (Bernatchez and Dodson, 1990*b*).

In this paper, we document the phylogenetic differentiation among mtDNA haplotypes, the geographic distribution and the diversity of mtDNA phylogenetic groupings in lake whitefish across its entire distribution range. We demonstrate that Pleistocene glaciation events represent the major factors responsible for the phylogeographic structure existing in lake whitefish mtDNA.

Material and Methods

Sample Collection

A total of 525 whitefish was collected from 1987 to 1989 among 41 populations (Table 1, Fig. 1). An average of 12 specimens was sampled at each locality. Most specimens were adult fish captured during their spawning run. Fresh or frozen egg and liver samples were shipped by collaborators or transported by ourselves to the laboratory.

Isolation and Restriction Enzyme Analysis

MtDNA was purified according to Bernatchez et al. (1988). MtDNA aliquots were digested separately as recommended by suppliers (Bethesda Research Laboratories, New England Biolabs, Pharmacia) with eight hexameric, four multihexameric and one multipentameric restriction enzymes (Table 2). MtDNA fragments were electrophoretically separated on 0.8% and 1.2% agarose gels run overnight at 25 V.

For most samples, ethidium bromide staining was sufficient to reveal digested fragments. For samples with low or poor quality yield of mtDNA, DNA was denatured, neutralized and transferred to nitrocellulose membranes (Maniatis et al., 1982). Membranes were hybridized with a highly purified radiolabelled total mtDNA probe as described in Bernatchez and Dodson (1990*a*). Fragments were sized by comparison with digests of phage lambda DNA with *Hind*III and *Eco*RI-*Hind*III double digest. No attempt was made to visualize fragments of fewer than 350 base pairs. Distinct single endonuclease patterns were identified by a specific letter in order of appearance. Each fish was assigned a multi-letter code that described its composite mtDNA genotype.

Data Analysis

Estimates of nucleotide sequence divergence (p) among mtDNA haplotypes were calculated by the fragment method of Upholt (1977). Sequence divergence was estimated independently for each type of enzyme and estimates were pooled following weighting for the number of base pairs sampled by each restriction enzyme. The resulting distance matrix was clustered by UPGMA (Sneath and Sokal, 1973) using the average linkage algorithm of the SAS statistical package. Changes in mtDNA fragment patterns could be accounted for by specific restriction site gains or losses. Therefore a data matrix consisting of site presence-absence information for each haplotype was used to

TABLE 1. Sample locations, sample sizes and nucleotide diversity indices of lake whitefish populations. Mentions of dwarf, normal and low gill-raker refer to known morphotypes of sympatric populations.

	Sample location	Sample size	Nucleotide diversity
a.	Mira River, Nova Scotia	12	0
b.	Gand Lake, New Brunswick	12	0.007
c.	Lake Témiscouata, Québec	12	0.020
d.	Cliff Lake, Maine (dwarf)	16	0
e.	Spider Lake, Maine (dwarf)	12	0.095
f.	Second Musquacook Lake, Maine (dwarf)	9	0.089
g.	Cliff Lake, Maine (normal)	12	0
h.	Lake Saint-François, Québec	12	0.057
i.	Saint Lawrence River, Québec	13	0.076
j.	Lake Champlain, Québec	12	0.081
k.	Réservoir Kipawa, Québec	12	0.010
l.	Povungnituk River, Québec	24	0.040
m.	Koksoak River, Québec	15	0.020
n.	Squaw Lake, Québec (Ungava drainage)	12	0
o.	Altikamagen Lake, Québec (Atlantic drainage)	9	0
p.	Inukjuak River, Québec	12	0.007
q.	Great Whale River, Québec	9	0
r.	La Grande River, Québec	20	0.029
s.	Eastmain River, Québec	31	0.006
t.	Rupert River, Québec	15	0.005
u.	Réservoir Manic V, Québec	10	0
v.	Lake Ontario, Ontario	12	0.010
w.	Lake Huron, Michigan	12	0.010
x.	Lake Superior, Ontario	10	0
y.	Lake Michigan, Michigan (grid 116)	11	0
z.	Lake Michigan, Michigan (grid 409)	11	0.011
aa.	Como Lake, Ontario (dwarf)	12	0.023
bb.	Como Lake, Ontario (normal)	12	0
cc.	South Indian Lake, Manitoba	12	0
dd.	Great Slave Lake, Northwest Territory	12	0.010
ee.	Jack Fish Lake, Saskatchewan	12	0.010
ff.	Wabamum Lake, Alberta	12	0.017
gg.	Crooked River, British Columbia	12	0.032
hh.	Fort McPherson, Northwest Territory	12	0
ii.	Arctic Red River, Northwest Territory	14	0.140
jj.	Yukon River, Alaska	11	0.306
kk.	Chatanika River, Alaska	12	0.351
ll.	Squanga Lake, Yukon	12	0.170
mm.	McEvoy Lake, Yukon	12	0.011
nn.	Dezadeash Lake, Yukon (low gill-raker)	9	0
oo.	Minnesota Lake, Alaska	12	0
Total		525	

generate a Wagner parsimony network by the MIX algorithm of the PHYLIP package provided by Joe Felsenstein (Department of Genetics, University of Washington, Seattle, WA 98195). The consensus tree and confidence statements on branches of the Wagner network were estimated by the bootstrapping method (BOOTM in PHYLIP package, Felsenstein, 1985). One hundred replicates of the bootstrapping procedure were performed.

We also estimated the intrapopulation diversity of mtDNA haplotypes using the nucleotide diversity index of Nei and Tajima (1981). This is defined by

$$\pi = \frac{n}{n-1} \sum_{ij} x_i x_j \pi_{ij}, \qquad (1)$$

where x_i is the population frequency of the ith mtDNA haplotype and π_{ij} is the percent sequence divergence estimate between the ith and jth haplotype. In a randomly mating population, π can be considered a measure of heterozygosity at the nucleotide level (Nei, 1987).

Estimates of divergence among major

phylogenetic groups were calculated using Nei's concept of genetic distance applied to mtDNA haplotype data as described in Wilson et al. (1985). This was estimated by

$$\delta = \delta xy - \delta A, \quad (2)$$

where δxy is the mean pair-wise divergence between randomly picked individuals of group x and those of group y, and δA is the mean pair-wise divergence between individuals within the common ancestry (A). δA was estimated by

$$\delta A = 0.5(\delta x + \delta y), \quad (3)$$

where δx and δy are the mean pair-wise divergence between randomly picked individuals in phylogenetic groups x and y respectively.

Results

Mitochondrial DNA Diversity and Phylogenetic Differentiation

The 13 enzymes used generated a total of 148 restriction fragments with a mean of 78 per individual (Table 3). All enzymes but two (*Bam*HI and *Dra*I) were polymorphic and discriminated 46 distinct mtDNA haplotypes (Table 2). Examples of gels are provided in Figure 2. Pairwise sequence divergence estimates among all lake whitefish haplotypes were generally low but highly variable [mean = 0.77% ± 0.44 (SD); range = 0.03 to 1.72%]. The relative abundance of these genotypes was also highly heterogeneous (Table 2). Haplotype 1 was the most common and composed 57% of all whitefish assayed. Only two other haplotypes (17 and 25) represented more than 5% of the total sample and 31 haplotypes were observed in single individuals. Omitting haplotypes 45 and 46, which represented two cases of introgressed mtDNA from *Coregonus nasus*, a close relative of *C. clupeaformis* (Bernatchez and Dodson, unpubl. data), UPGMA phenogram revealed three major phylogenetic groupings separated by average sequence divergence estimates of 1.15% (cluster C from cluster A-B) and 0.96% (cluster A from cluster B) (Fig. 3). *C. nasus* mtDNA haplotypes diverged from the *C. clupeaformis* cluster by 1.8%. Figure 4 illustrates parsimonious networks among restriction fragment patterns from which site changes were deduced. The overall Wagner parsimony analysis of site changes generated several equally parsimonious networks, each requiring a minimum of 58 site changes (Fig. 5). Major phylogenetic groups identified in UPGMA (A, B, C) were also discriminated in this analysis and were supported at more than 80% bootstrapping level. Furthermore, the Wagner analysis supported haplotypes 25, 26, 27 and 28 as a distinct assemblage 89% of the bootstrapping repeats. UPGMA also clustered these haplotypes distinctively among other group C clones (Fig. 3). Therefore, they represented a distinct phylogenetic entity which was named clonal group D.

Geographic Distribution of mtDNA Phylogenetic Assemblages

The major phylogenetic groups (A, B, C) exhibited a strong geographic pattern of distribution. All fish collected in Beringia (Yukon and Alaska) belonged to either phylogenetic group A or B whereas group C comprised all fish sampled outside this region (Fig. 6). Arctic Red R., N.W.T. (population ii), was the only location sampled where a Beringian group (B) overlapped with group C. Despite the considerable genetic difference between A and B, they did not exhibit a clear difference in their geographic distribution, overlapping at three different locations (jj, kk, ll).

Finer geographic structuring was observed within phylogenetic group C. Clonal group D showed a geographic distribution clearly distinct from all other group C haplotypes (Fig. 6). This assemblage was observed only among southeastern maritime populations (a–f). None of the other group C clones were observed in this region except in northern Maine where the distribution of both phylogenetic assemblages overlapped.

Mitochondrial DNA Haplotype Distribution and Population Differentiation Within Geographic Assemblages

The pattern of heterogeneity in the distribution of haplotypes within each geographic assemblage was variable. In Beringia, most populations surveyed were highly differentiated in their composition of mtDNA haplotypes. For instance, up to

TABLE 2. Composite definitions, absolute frequency (N) and distribution of mtDNA haplotypes resolved among lake whitefish sampled. Haplotypes are grouped according to major phylogenetic assemblages identified by UPGMA and Wagner parsimony analysis: 1–24, cluster C; 25–28, cluster D; 29–39, cluster A; 40–44, cluster B; 45, 46, *C. nasus* haplotypes. Restriction enzymes are in order: *Ava*I, *Ava*II, *Ban*I, *Bgl*I, *Hae*II, *Hin*cII, *Hin*dIII, *Pvu*II, *Sma*I, *Xmn*I and *Pst*I. Capital letters refer to fragment patterns described in Table 3. Small letters refer to sample location in Table 1.

	Composite genotypes											N	Distribution
1	A	A	A	A	A	A	A	A	A	A	A	300	h–ii
2	A	C	A	A	A	A	A	A	A	A	A	5	r, s
3	A	A	A	A	A	C	A	A	A	A	A	4	i, l
4	A	A	A	A	A	A	E	A	A	A	A	2	i, r
5	A	A	A	A	A	A	A	A	A	B	A	1	v
6	A	A	A	A	A	A	C	A	A	A	A	1	r
7	A	A	A	A	A	A	D	A	A	A	A	1	p
8	A	A	A	A	A	A	F	A	A	A	A	1	i
9	A	A	A	A	A	E	A	A	A	A	A	1	h
10	A	A	A	A	C	A	A	A	A	A	A	1	j
11	A	A	A	A	F	A	A	A	A	A	A	1	x
12	A	A	E	A	A	A	A	A	A	A	A	1	y
13	A	A	F	A	A	A	A	A	A	A	A	1	r
14	A	B	A	A	A	A	A	A	A	A	A	1	s
15	A	A	B	A	B	A	A	A	A	A	A	1	j
16	B	A	A	A	D	D	B	A	A	A	A	1	j
17	A	A	A	A	A	B	A	A	A	A	A	31	h–m, dd–gg
18	A	A	A	A	A	B	A	A	A	A	B	2	i
19	A	A	A	A	A	B	E	A	A	A	A	1	i
20	A	A	C	A	A	B	A	A	A	A	A	1	j
21	A	D	A	A	A	A	A	A	A	A	A	22	e–h
22	A	E	A	A	A	A	A	A	A	A	A	1	e
23	A	F	A	A	A	A	A	A	A	A	A	1	aa
24	C	A	A	A	A	A	A	A	A	A	A	1	aa
25	A	A	D	A	A	A	A	A	A	A	A	61	a–f
26	A	A	D	A	A	B	A	A	A	A	A	1	c
27	A	A	D	A	B	A	A	A	A	A	A	1	b
28	A	A	D	A	E	A	A	A	A	A	A	1	c
29	A	H	G	B	A	C	G	B	A	A	A	23	jj–mm
30	A	G	G	B	A	C	G	B	A	A	A	12	oo
31	A	H	K	B	A	C	G	B	A	A	A	9	nn
32	A	H	G	B	A	A	A	B	A	A	A	6	ii, jj
33	A	H	H	B	A	A	G	B	A	B	A	2	kk
34	A	H	A	B	A	A	G	B	A	B	A	1	kk
35	A	H	G	B	A	C	G	B	B	A	A	1	mm
36	A	H	G	B	A	F	G	B	A	A	A	1	kk
37	A	H	G	B	G	A	A	B	A	A	A	1	jj
38	A	H	H	B	A	A	G	B	A	A	A	1	kk
39	A	L	G	B	I	C	G	B	A	A	A	1	ll
40	E	I	A	B	A	A	G	A	C	D	A	6	jj, kk
41	D	J	A	B	A	A	G	A	A	C	A	3	jj, ll
42	E	I	A	B	A	A	G	A	A	C	A	2	jj, ll
43	E	I	A	B	A	A	G	A	C	C	A	2	jj, kk
44	E	I	I	B	A	A	G	A	C	C	A	1	kk
45	F	K	J	B	E	C	G	B	A	A	A	1	ii
46	F	K	J	B	H	C	G	B	A	A	A	1	jj

eight clones were observed within both the Yukon and Chatanika R., while populations such as Minnesota L. and Dezadeash L. were composed of a single haplotype observed nowhere else (Table 2). Most populations were much less differentiated outside Beringia. Among populations identified by clonal group D, all fish but three had the same mtDNA haplotype. Therefore, all populations were very homogeneous in mtDNA haplotype composition. Outside this region, the commonest clonal line (haplotype 1) was

TABLE 3. Fragment size estimates (in base pairs) of all fragment patterns observed among lake whitefish analyzed. Fragments marked with asterisks were not observed but assumed under the criterion of minimum mutational steps involved in fragment changes.

*Ava*I	A	B	C	D	E	F
5,700						–
3,700	–	–	–			
2,800						–
2,500	–	–	–	–	–	–
2,350				–	–	
2,300	–		–	–	–	–
2,150					–	
2,050		–				
1,950	–	–	–	–		
1,500	–	–	–	–	–	
1,450	–	–	–	–	–	–
1,400				–	–	
1,250	–	–	–	–	–	
1,150	–	–		–	–	–
850	–	–	–	–	–	–
770			–			
380			–			
*250		–				
*200	–	–	–	–		–

*Pst*I	A	B
11,670	–	–
5,320		–
4,850	–	
470	–	

*Hae*II	A	B	C	D	E	F	G	H	I
7,950			–						
7,600							–		
5,150	–	–		–	–	–	–	–	–
4,800	–	–	–	–	–			–	–
2,950						–			
2,800	–	–		–	–	–		–	–
1,850						–			
1,400	–	–	–	–	–	–	–	–	–
1,140					–			–	
1,000							–		
940	–		–	–	–	–	–	–	–
940								–	–
825	–	–	–		–				
825		–							
725				–					
700	–	–	–	–		–	–		–
440	–	–	–	–		–	–		–
*175	–	–	–	–	–	–		–	–
*115		–							
*115	–	–	–	–	–	–	–		
*100				–					

*Ava*II	A	B	C	D	E	F	G	H	I
3,400								–	
3,050	–	–	–			–	–		–
2,750	–	–		–	–	–	–	–	–
2,300		–							
2,250			–						
2,100				–	–				
1,625	–		–	–	–	–			
1,500									
1,475							–	–	–
1,450	–	–	–	–		–	–	–	–
1,300	–	–	–	–	–	–	–	–	–
1,300					–				
1,080									
1,050						–			
970	–	–	–	–	–	–	–	–	–
950				–	–				
830							–		
710	–	–	–	–	–		–	–	–
680	–		–	–	–	–		–	–
550	–	–	–	–	–	–	–	–	–
550	–	–	–	–	–	–	–	–	–
500	–	–	–	–	–	–	–	–	–
500			–						
470	–	–	–	–	–	–	–	–	–
470	–	–	–	–	–	–	–	–	–
360	–	–	–	–	–		–		–
350	–	–	–	–	–	–	–	–	–
350	–	–	–	–	–	–	–	–	–
*150								–	–
*150					–				

*Hinc*II	A	B	C	D	E	F
4,250	–	–	–	–	–	–
3,900	–		–			
3,875				–		
3,375			–			–
3,100					–	
2,350		–				–
2,250	–	–		–	–	
1,575		–				–
1,300	–	–	–	–	–	–
1,125	–	–	–	–	–	–
1,125	–	–		–	–	
1,075	–	–	–	–	–	–
930	–	–	–	–	–	–
810	–	–	–	–	–	–
800					–	
*25				–		

TABLE 3. Extended.

*Ban*I													*Bgl*I		
J	K	L		A	B	C	D	E	F	G	H	I	J	K		A	B
	–		4,900	–	–			–	–	–				–	9,400		–
–		–	4,000								–				8,100	–	
–	–	–	3,700	–	–	–	–		–	–	–	–	–	–	7,600	–	–
			3,650											–	1,300	–	
			3,550										–				
			3,250			–									*Dra*I	A	
			2,700				–					–					
	–		2,200				–					–			6,000	–	
–	–	–	2,050					–							4,600	–	
–	–	–	2,000	–	–	–	–	–	–	–	–	–	–	–	3,300	–	
–		–	2,000						–	–	–		–		2,275	–	
			1,650	–		–	–	–	–	–	–	–	–		630	–	
–			1,650			–											
			1,650					–							*Pvu*II		
–	–	–	1,575	–	–	–	–	–				–				A	B
			1,350										–		9,500	–	–
			1,150	–	–	–	–	–	–	–	–	–	–	–	5,000	–	–
–	–	–	890		–										1,640		–
		–	800								–				1,150	–	
–	–	–	760		–										860	–	–
–	–	–	730				–								490	–	
–			670	–	–	–		–	–	–	–	–	–	–			
			490	–	–	–	–	–	–			–	–		*Bam*HI		
–	–	–	425	–	–	–	–	–				–				A	
–	–	–	*60	–	–	–		–	–	–	–	–	–	–	16,800	–	
		–															
–	–	–															
–		–															
–	–	–															

*Hin*dIII									*Sma*I				
	A	B	C	D	E	F	G			A	B	C	
5,600	–	–	–	–	–	–			9,400	–		–	
4,220			–						5,900		–		
3,750	–	–		–	–	–	–		5,550	–	–	–	
3,750					–				3,500		–		
3,500	–	–	–	–	–	–	–		2,200			–	
3,500	–		–				–		2,000	–	–		
3,400		–							*200	–	–		
3,200							–						
3,150				–					*Xmn*I				
2,600						–				A	B	C	D
2,400							–		14,000				–
950						–			8,950			–	
470	–	–		–	–	–	–		7,600	–	–		
*350				–					5,050	–	–	–	
*250	–	–	–	–		–	–		4,200		–		
*100		–							2,850	–		–	–
									1,350	–			

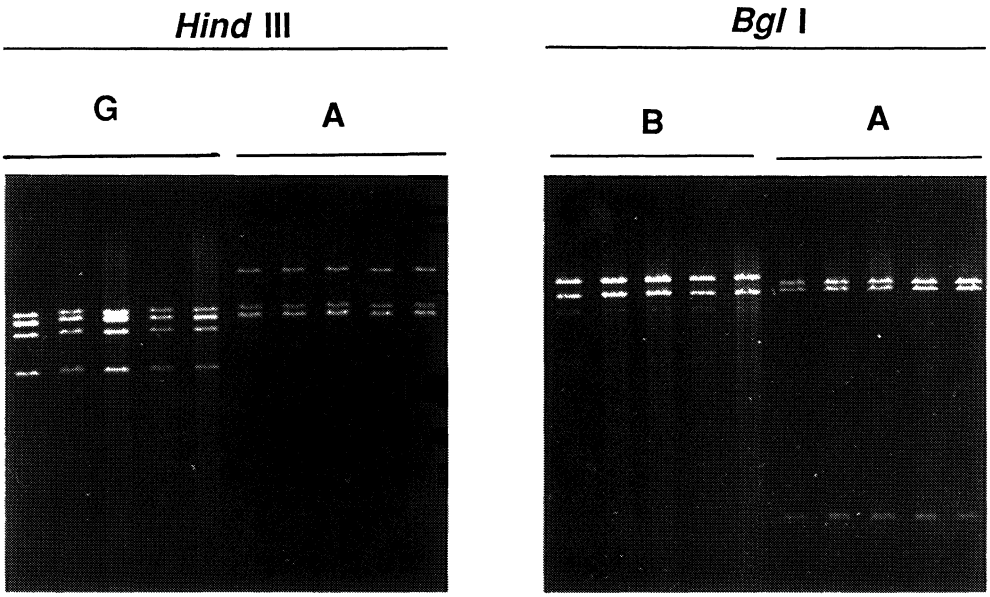

FIG. 2. Ethidium bromide stained agarose gels of mtDNA digestion patterns of lake whitefish. For *Hind*III digests, fragment pattern G was observed in 91% of whitefish from Beringia and fragment pattern A in 98% of whitefish outside Beringia. For *Bgl*I digests, fragment patterns B and A were fixed for whitefish observed within and outside Beringia respectively.

basically the only haplotype observed from Fort McPherson, N.W.T., through central Canada, including the Great Lakes and James-Hudson Bay watersheds, and east to Labrador (Fig. 7). All these populations were genetically identical on the basis of their mtDNA haplotype composition. Haplotype 1 was in lower abundance in British Columbia (gg), southwestern Québec (h, i, j) and in the Povungnituk R., northern Hudson Bay (l). It was not observed among southeastern populations. MtDNA haplotype 17 was the second most abundant haplotype belonging to phylogenetic group C and exhibited a very disjunct geographic distribution (Fig. 7). It was most abundant in southern Québec (populations h, i, j, k) but was also found in two northern Québec anadromous populations (l, m). It was not observed in any other northern, eastern or central populations but was found again in increasing abundance from Saskatchewan to central British Columbia (dd, ee, ff, gg). Finally, the third most abundant mtDNA haplotype in group C (clone 21) exhibited a very localized distribution (Fig. 7). It was observed only among four populations in southern Québec and northern Maine. The only other clones observed more than once (2, 3, 4) were distributed in more than one population (Table 1). Haplotype 3 exhibited a disjunct distribution being observed in the Saint-Lawrence R. and the Povungnituk R. in northern Hudson Bay (populations i and l) but nowhere else.

Geographic Pattern of mtDNA Haplotype Diversity

There was a clear difference in the overall mtDNA nucleotide diversity between the phylogeographic assemblages A-B and C. Figure 8 illustrates the relationship between the number of mtDNA haplotypes in each assemblage depicted as a function of sample size within each region. The number of haplotypes found for a given sample size was always at least twice as abundant in Beringia as in the rest of the whitefish distribution range. For example, 18 clones were observed among 74 fish sampled in the first region. However, 260 fish needed to be randomly sampled outside this region to detect the same number of clones.

The intrapopulation pattern of nucleotide diversity varied within each geographic assemblage (Table 1, Fig. 9). In Beringia, the highest diversity was observed in the Yukon and Chatanika R. populations (jj, kk) while

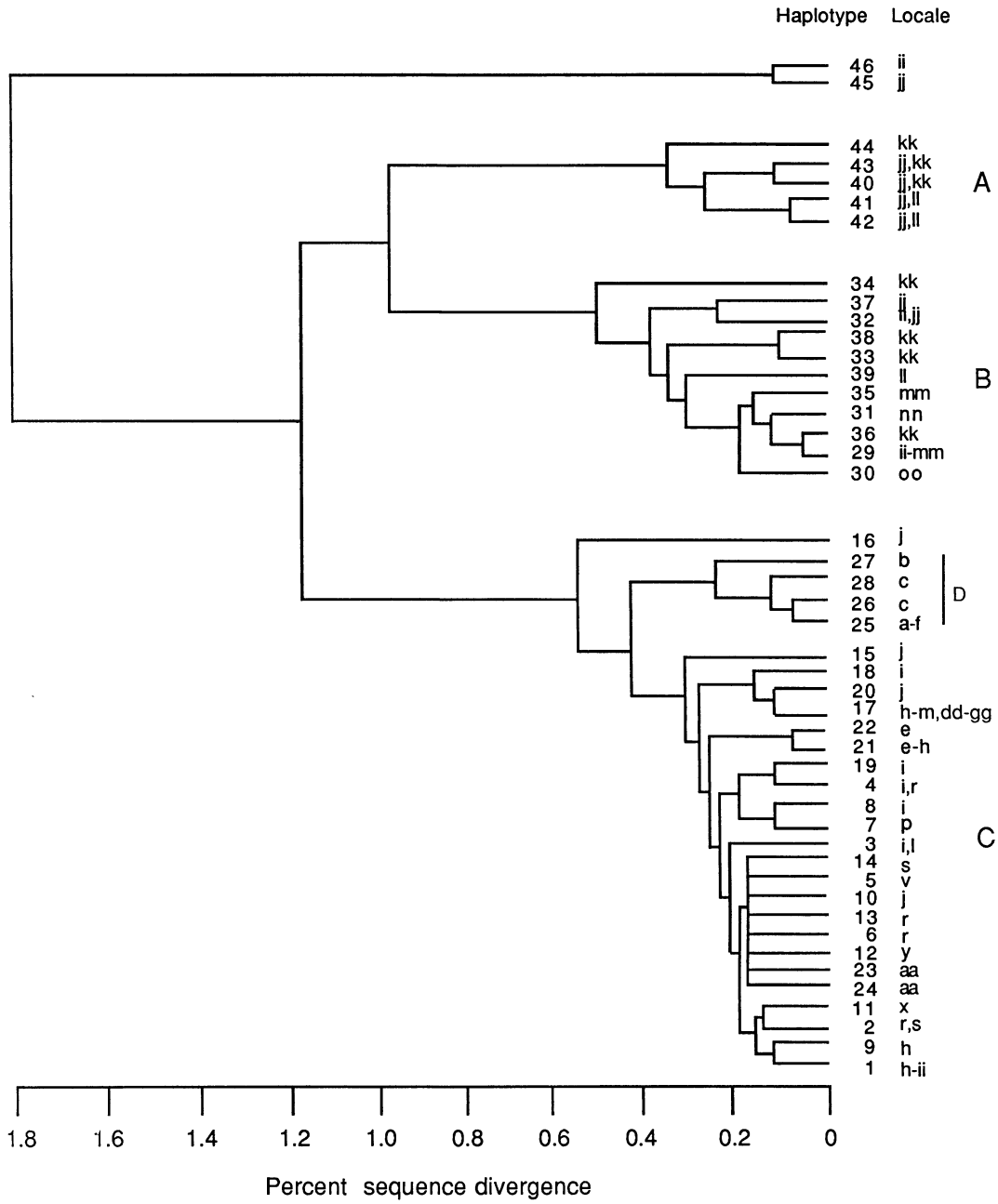

FIG. 3. UPGMA phenogram clustering the distance matrix of percent sequence divergence among lake whitefish mtDNA haplotypes. Numbers 1 to 46 refer to haplotypes defined in Table 2. Small letters refer to sample location in Table 1 and capital letters identify major phylogenetic groupings.

the Minnesota (oo) and Dezadeash L. (low gill raker, nn) populations exhibited no diversity. Outside Beringia, the nucleotide diversity index was generally null or extremely low except in southern Québec and northern Maine where some populations were highly polymorphic. For instance, the Saint-Lawrence R., Champlain L., and Saint-François L. populations, although representing only 8.2% of all fish belonging to phylogenetic group C, composed nearly half (46%) of all mtDNA haplotypes of this

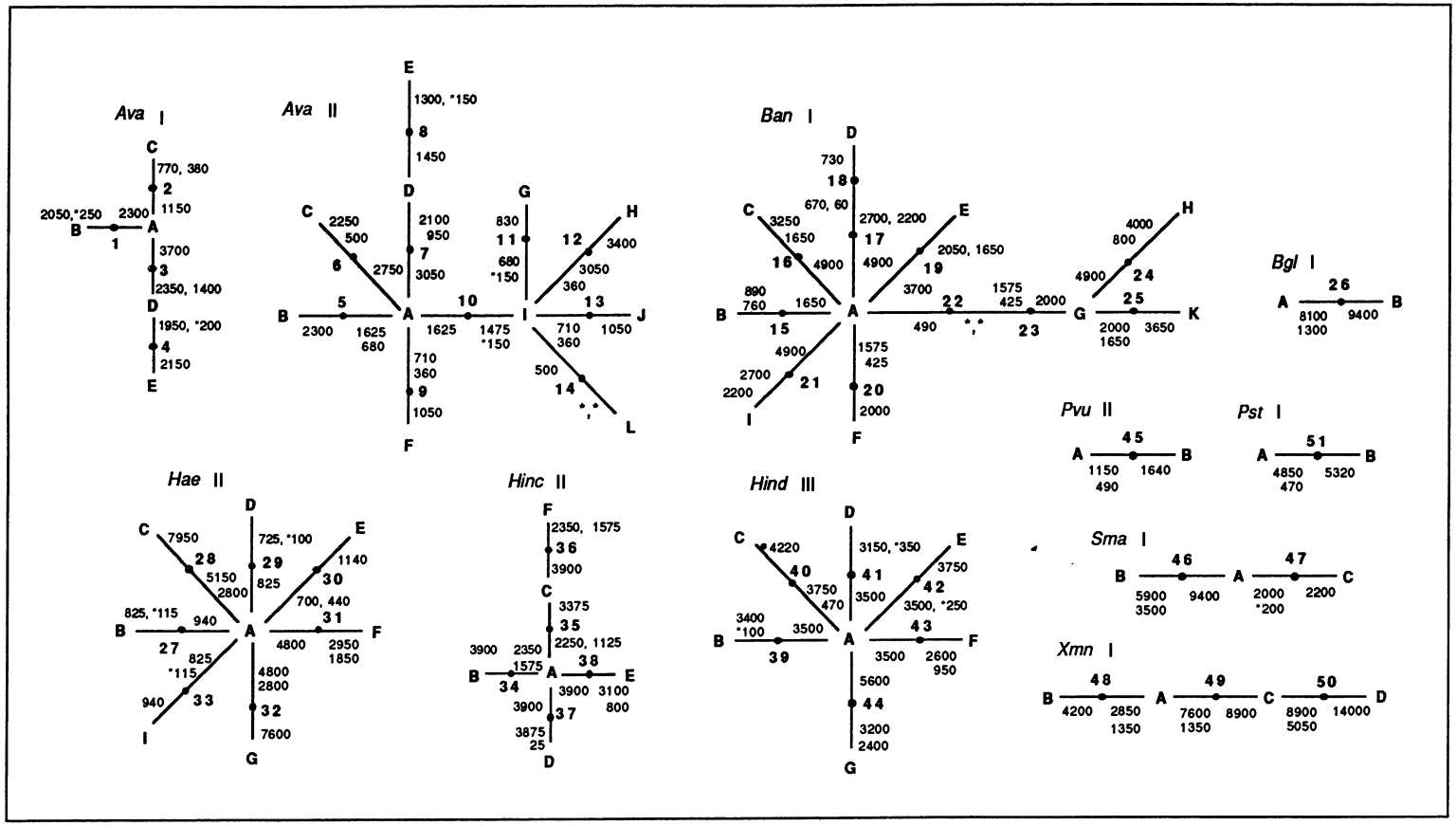

FIG. 4. Parsimonious, unrooted networks illustrating relationships among fragment patterns observed for all polymorphic restriction enzymes and described in Table 3. Fragment patterns specific to *C. nasus* (haplotypes 45 and 46 in Table 2) are omitted. Site changes involved in moving among patterns are numbered along branches and were parsimoniously deduced from observed fragment changes. Those fragments involved in differences among patterns are given along branches. Several fragments, identified by asterisks, were not observed but assumed under the criterion of minimizing mutational steps between restriction morphs.

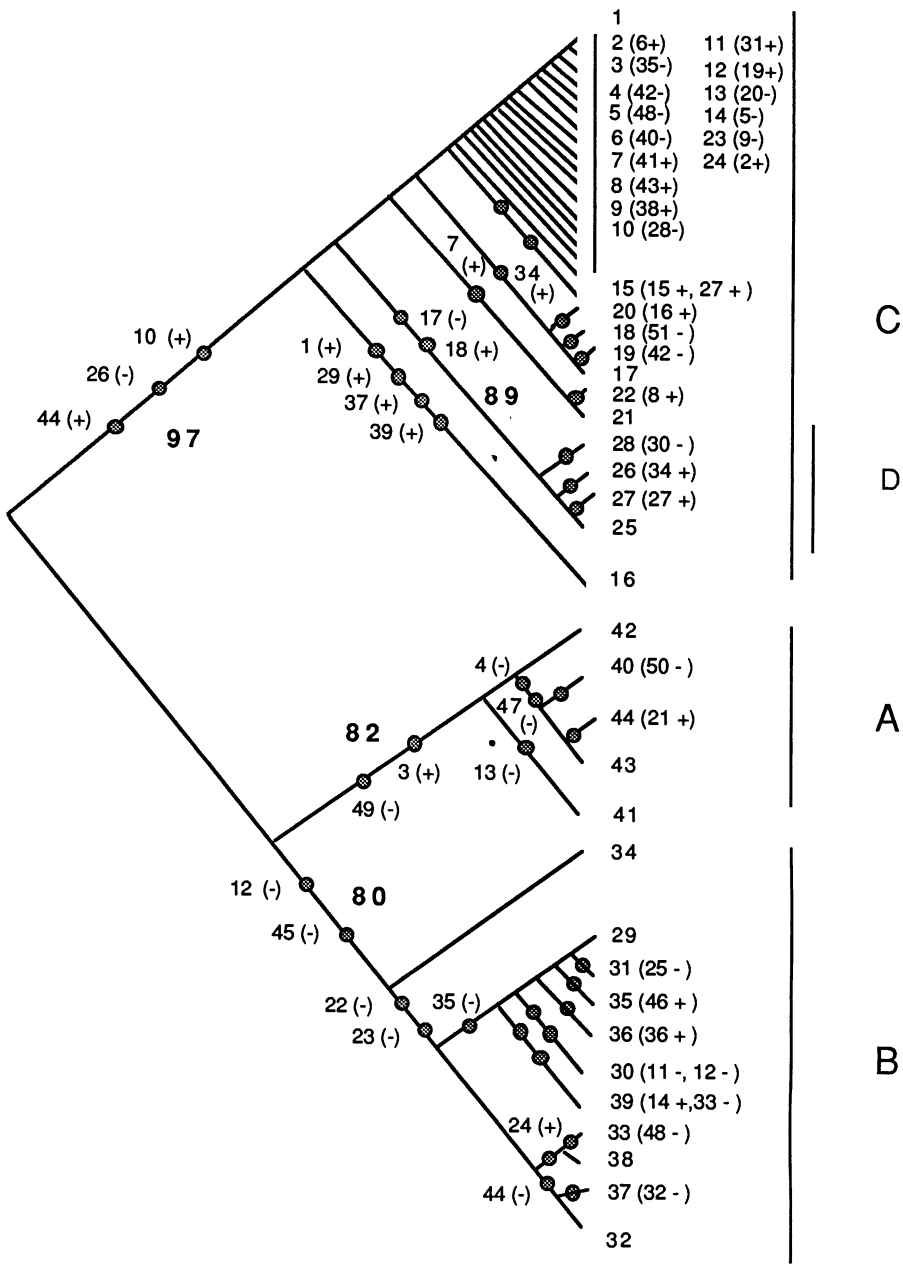

FIG. 5. Parsimony network linking 44 lake whitefish mtDNA haplotypes. Haplotypes 45 and 46 (*C. nasus* origin) were omitted. Dotted circles indicate mutational steps. Numbers given with circles or in parentheses next to haplotype number refer to restriction site resolved in Figure 4 and involved in moving along branches. Details on site gains (+) or losses (−) are also given. Confidence statements (in percentage) are given along branches dividing the major phylogenetic assemblages.

group. The majority of these haplotypes were endemic to these populations (Table 2). Nevertheless, the nucleotide diversity of these populations was much lower than the highest values observed in Beringia.

DISCUSSION

The distribution of mtDNA assemblages provides clues as to the evolutionary relationships of lake whitefish and to the existence of glacial refugia and subsequent re-

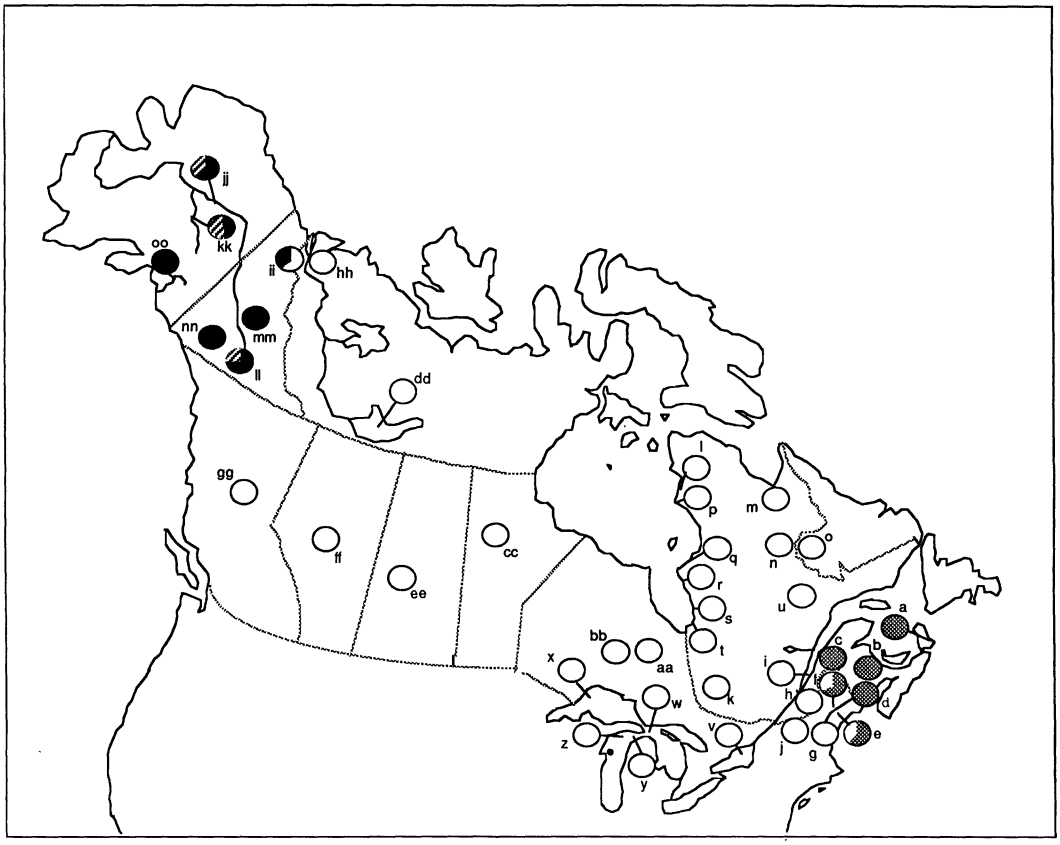

FIG. 6. Geographic distribution of major phylogenetic groups A (hatched), B (black), C (white) and D (dotted) identified by UPGMA and parsimony analysis.

colonization events. We identified three major phylogenetic groups but their degree of genetic divergence was not correlated with geographic distance. Two of the major phylogenetic groups overlapped in distribution in Beringia whereas lower phylogenetic groupings, such as clonal group D within group C, were distinctively distributed. This suggests that different groups of whitefish became isolated at different times. Thus, the following discussion describes the phylogeographic assemblages and treats the evolutionary history of lake whitefish in hierarchical order from the major groupings to individual clonal lines.

Phylogeographic Assemblages and Glacial Refugia

Phylogenetic groups A and B, confined to Beringia, were as divergent from each other as they were from group C but did not exhibit distinct geographic distributions. These two groups most likely identify whitefish that survived glaciation events in the Beringian refugium. The phylogenetic break observed between Beringia and the rest of North America corroborates previously documented discontinuities in gill-raker means and in isozyme frequencies that coincided with watershed boundaries between the two regions (McPhail and Lindsey, 1970; Lindsey et al., 1970; Franzin and Clayton, 1977).

Group C represents the second major phylogeographic assemblage and is composed of several subassemblages distinguishable by specific mtDNA haplotypes as well as by variation in mtDNA haplotype diversity. Clonal group D was phylogenetically distinct from other group C haplotypes and exhibited a very localized distribution among southeastern populations. It was found only east of the presumed Atlantic refugium that was located south of the

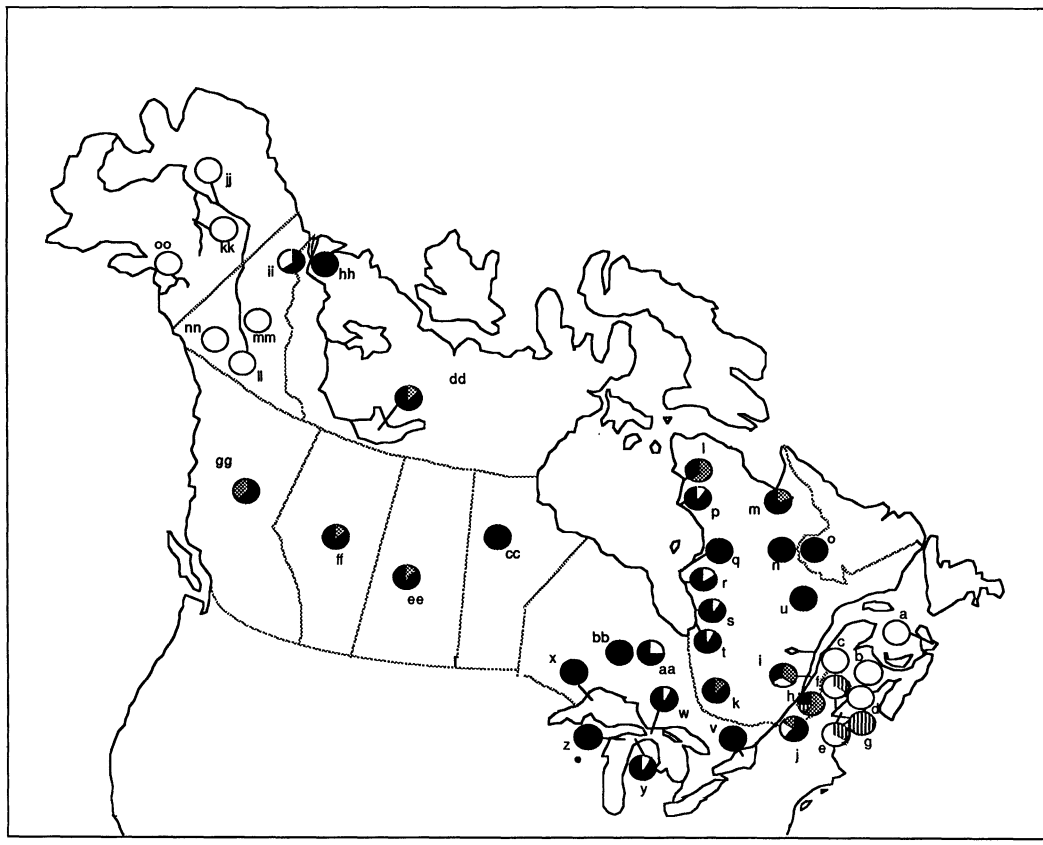

FIG. 7. Geographic distribution of the three major haplotypes belonging to phylogenetic group C observed among all sampled populations: haplotypes 1 (black), 17 (dotted) and 21 (hatched). The distribution of haplotype 17 also includes haplotypes 18, 19, and 20, which represented rare variants (four fish) that clustered with haplotype 17 in UPGMA and parsimony analysis.

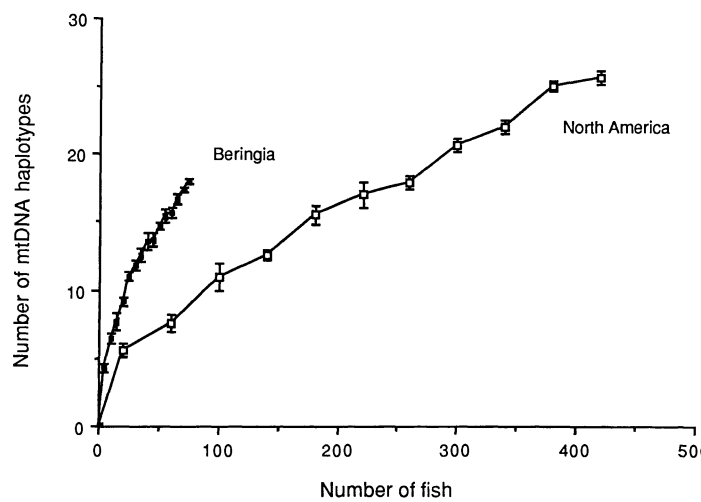

FIG. 8. Number of mtDNA haplotypes depicted as a function of random sample size within Beringia and the rest of North America. The relationship was estimated by an incremental random choice of 5 and 20 individuals in Beringia and the rest of North America respectively. The procedure was repeated 10 times for each sampling level. Standard errors are given by vertical bars.

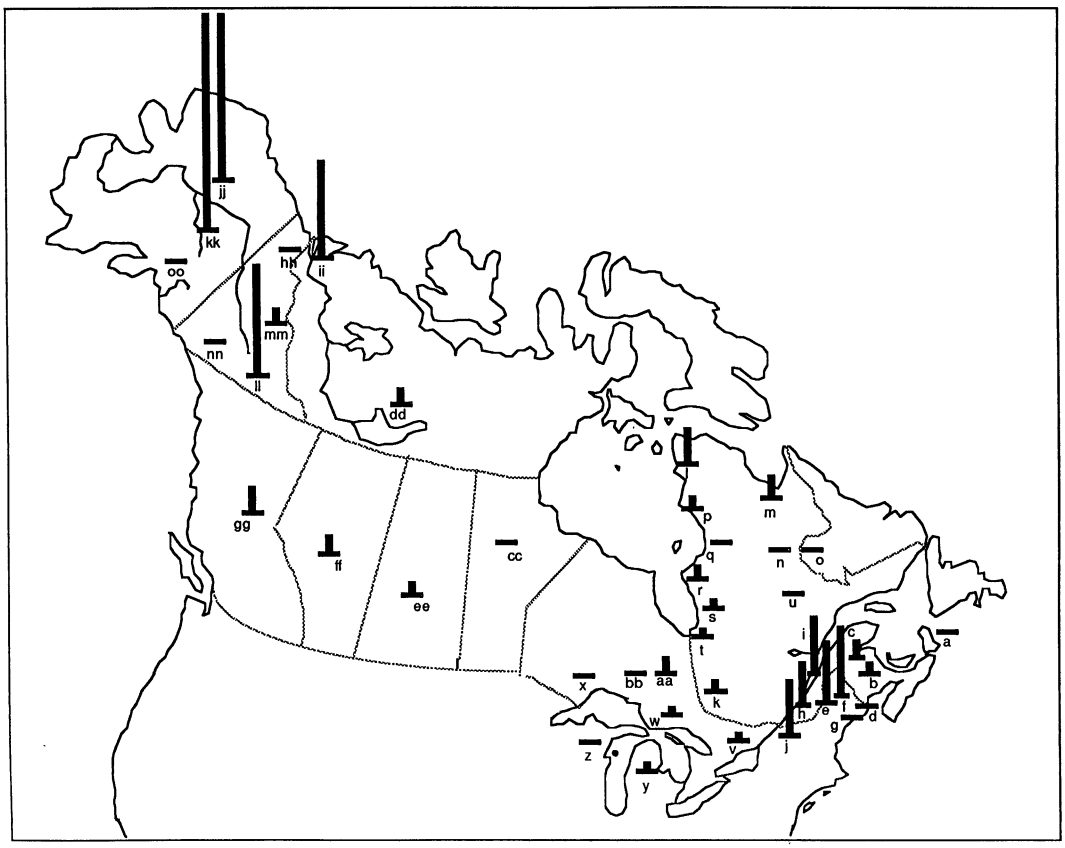

FIG. 9. Geographic variation of intrapopulation nucleotide diversity observed among lake whitefish populations. Corresponding values of nucleotide diversity for each population are given in Table 1.

Wisconsinan ice-sheet and comprised the unglaciated Atlantic Coastal Plain, especially the Hudson R. and the Susquehanna R. drainages (Schmidt, 1986). It was never observed in more western populations that had closer connections with the Atlantic refugium such as Champlain L. and the Saint-Lawrence R. (Underhill, 1986). In addition, genotypes characterizing the Champlain L. and Saint-Lawrence R. populations were absent in these eastern populations. It is well established that parts of Nova Scotia and adjacent continental shelf areas above sea level in glacial times remained unglaciated during recent glaciation events (Fulton and Andrews, 1987). Therefore, phylogenetic group D most likely originated from a refugium that existed in this region, possibly corresponding to the Northeastern Banks refugium described by Schmidt (1986). We propose the name of Acadian race for this eastern assemblage of populations. The low genetic divergence of clonal group D from other group C haplotypes suggests that it became isolated more recently than group C diverged from the Beringian phylogenetic assemblages.

No other distinct clonal groups were identified within group C. Nevertheless, different geographic patterns of distribution exhibited by individual clonal lines in this group suggest the existence of other refugia. Clonal line 1 was basically the only genotype observed over most of the actual whitefish range outside the distribution range of the Beringian and Acadian races. From its central distribution, it may be associated with the Mississippian refugium, which is generally accepted as the major refugium from which whitefish and most freshwater fish species dispersed to recolonize the continent (Briggs, 1986; Crossman and McAllister, 1986).

Populations sampled in southern Québec

and northern Maine may have been derived from the Atlantic refugium (Schmidt, 1986). Populations from this region exhibited the highest level of nucleotide diversity observed among all populations of group C and comprised the majority of all haplotypes observed. Such diversity contrasts sharply with the extremely low level of mtDNA polymorphism observed elsewhere and cannot be explained by simple random events such as differences in stochastic lineage extinction among individual populations (Avise et al., 1984). Furthermore, one of the most abundant haplotypes in group C (21) was restricted to this area. Also, clonal line 17 which exhibited a disjunct distribution was abundant in this area but was absent in adjacent populations to the east and west. Altogether, these observations support the hypothesis that whitefish survived in an Atlantic refugium located south of the Wisconsinan ice-sheet. The fact that the discrimination between the Mississippian and the Atlantic races is not based on phylogenetically distinct assemblages suggests that these populations became isolated in even more recent times than the separation of the Acadian race.

Historical Demography and Dispersal of Whitefish Races

Altogether, we identified four major phylogeographic assemblages that may be associated with the survival of lake whitefish in four glacial refugia: the Beringian, the Mississippian, the Atlantic and the Acadian. Mitochondrial DNA diversity varied greatly among these assemblages suggesting different demographic histories. A major difference in diversity was observed between the Beringian race and those observed in the rest of North America. MtDNA lineages have been demonstrated to become stochastically extinct as a function of time. The rate of extinction is in turn a function of the level of population structuring, long-term effective population size and variability in progeny survival (Avise et al., 1984). The overall reproductive strategy of whitefish is similar over its distribution range so that the variability in progeny survival is comparable. Differences in long-term effective population size and population subdivisions are more likely to explain the difference observed between the 2 groups. Estimates of long-term effective population size based on equation 5 of Wilson et al. (1985) gave a value of 72,000 for the Beringian assemblage compared to 25,000 outside this range. The absolute values generated by this equation may be questionable because many of the underlying assumptions of its application are rarely met (Wilson et al., 1985). Nevertheless, the ratio of 3/1 suggests that whitefish maintained a higher abundance in Beringia than in the rest of North America over historical times despite the smaller surface area inhabited by whitefish. Many catastrophic events could be responsible for the relatively higher population reductions of whitefish outside Beringia. Bernatchez et al. (1989) demonstrated that difference in mtDNA diversity between anadromous whitefish populations from the Baltic Sea and Hudson Bay resulted primarily from the more important population bottlenecks through habitat loss in North America than in Eurasia during Pleistocene glaciations. Similarly, the difference in mtDNA diversity between Beringia and the rest of North America may be attributed to differential population bottlenecks through loss of habitat. Most of Beringia remained unglaciated during Pleistocene glaciations. Furthermore, potential whitefish habitat was probably expanded in glacial times by the land bridge that connected Alaska to Eurasia so that the Beringian refugium also included northeastern Siberia (Lindsey and McPhail, 1986). Therefore, we propose that the difference in mtDNA diversity between Beringia and the rest of North America is due to differences in the extent of population bottlenecks associated with glacier advance.

The degree of population subdivision is also known to act as a buffer against mtDNA lineage extinction (Avise et al., 1988) and may also be responsible for differences in mtDNA diversity between both regions. The presence of two highly divergent phylogenetic groups in Beringia could theoretically result from the sorting and subsequent coexistence of highly divergent lineages within a single population lacking geographic subdivision. An alternative hypothesis is that Beringia represents a zone of secondary contact between two whitefish groups that evolved independently. One may have

evolved in Beringia and the other in Eurasia, later colonizing North America via the Bering land bridge during a glacial period. Another possible scenario is that the two assemblages evolved within Alaska-Yukon during a glacier advance and subsequently mixed following deglaciation. We are currently extending our sampling to Eurasia to test these alternative hypotheses.

The different phylogeographic groups identified outside Beringia showed extreme variability in diversity and geographic distribution extent. Nearly all whitefish (92%) from populations of proposed Mississippian origin belonged to a single haplotype distributed throughout most of the actual whitefish range. This suggests that descendants of a single female that persisted in a Mississippian population were responsible for the recolonization of over 5×10^6 square km of territory, extending from the Mackenzie R. to Labrador. The existence of huge proglacial lakes such as Agassiz and Ojibway-Barlow extending from central Canada to central Québec with connections to the Mackenzie R. system as well as ancestral connections between now separated river drainages on both sides of the Rockies all provided formidable dispersal routes for the Mississippian whitefish during Wisconsinan deglaciation (Briggs, 1986; Lindsey and McPhail, 1986).

In contrast to the extreme loss of mtDNA variability and the extensive postglacial dispersal of the Mississippian whitefish, the proposed Atlantic group retained most of the mtDNA diversity existing in whitefish outside Beringia but has not dispersed extensively through continental routes. However, the existence of two mtDNA lineages (Table 2) among anadromous populations from the northern Québec peninsula and also in the Saint-Lawrence R. drainage in southern Québec, and their absence elsewhere in the east, suggests that whitefish from the Atlantic refugium have been able to recolonize the north through coastal dispersal. During Wisconsinan deglaciation, glacier melting created low-salinity conditions on the Atlantic coast possibly favorable to whitefish survival (Vernal and Hilaire-Marcel, 1987). This observation contradicts the view that lake whitefish could not use euryhaline dispersal routes to recolonize northeastern America (Legendre and Legendre, 1984; Black et al., 1986).

Biological Barriers to Gene Flow among Races

Given the dispersal possibilities existing in glacial times and the apparent capability of whitefish to use these routes, gene flow and mixing among different phylogeographic assemblages have apparently remained very limited. There is ample evidence in the literature that other fish species used these routes to move from one refugium to another (reviewed in Hocutt and Wiley, 1986). Thus, some species dispersed out of Beringia (e.g., *Prosopium cylindraceum, Thymallus arcticus*) while some that survived in the Mississippian refugium have recolonized Beringia (e.g., *Percopsis omiscomaycus, Couesius plumbeus*). Many fishes that survived in the Mississippian and the Atlantic refugia reciprocally recolonized regions dominated by the influence of the alternate refugium (Underhill, 1986). In contrast, whitefish originating from different refugia are restricted to narrow zones. Contrary to what was previously deduced from allozyme studies (Franzin and Clayton, 1977), Beringian whitefish did not disperse toward central Canada but reached their eastern distribution in the lower Mackenzie R. system. This area is also the western limit of the Mississippian whitefish and represents the only location where both groups overlap. Mississippian whitefish overlap with whitefish of Atlantic origin only in the lower Saint-Lawrence R. drainage and northeastern Hudson Bay, and do not overlap at all with the Acadian race. The Acadian and the Atlantic races overlapped in northern Maine only. In this region, both phylogenetic assemblages coexist in sympatry by remaining reproductively isolated and adopting distinct ecological niches and life history strategies (Fenderson, 1964; Kirkpatrick and Selander, 1979; Bernatchez and Dodson, 1990*b*). Although isolated since relatively recent times, both groups apparently behave as competitive species that could potentially limit the range expansion of each other. Whether or not whitefish of different origins in other contact regions remain reproductively isolated has not been documented.

Divergence Times of Whitefish Phylogeographic Assemblages

One of the major assets of molecular phylogenetic data is the potential application of a molecular clock for estimating branching times of divergent assemblages. It is assumed that genetic distance between taxa is proportional to time since lineage separation. In mammals and birds, mtDNA rate of evolution has been estimated to be about 2% nucleotide substitutions per million years (Brown et al., 1979; Shields and Wilson, 1987). This rate is currently applied to other animal groups, including fish (Kessler and Avise, 1985; Bermingham and Avise, 1986; Gyllensten and Wilson, 1986; Avise et al., 1987*a*, 1987*b*; Billington and Hebert, 1988; Grewe and Hebert, 1988; Bentzen et al., 1989).

The divergence times among phylogenetic groups of lake whitefish estimated by the application of the 2% mutation rate correlate with the chronology of Pleistocene glacier advances. Converting the genetic divergence estimated from equations 2 and 3 to time estimates, the major genetic break observed between whitefish in and outside Beringia occurred some 375,000 years ago. This period corresponds to the Kansan ice advance. The second major genetic break observed resulting in the separation of groups A and B occurred at about the same time, some 360,000 years ago. Clonal group D (Acadian race) diverged from phylogenetic group C about 150,000 years ago. This time coincides with the Illinoian ice advance. Finally, the time of divergence between the Mississippian and the Atlantic assemblages was apparently too short to allow the evolution of new mtDNA lineages but was long enough for stochastic lineage sorting of ancestral lineages within each group. The most plausible event responsible for the isolation of these groups is the Wisconsinan glacier advance which started about 70,000 years ago and reached its peak some 18,000 years ago (Denton and Hugues, 1981). At best, the resolution of the mtDNA analysis used in the present study is at the limit for discriminating mtDNA genotypes differing solely by the accumulation of mutations over such a time scale, assuming a mutation rate of 2% (Wilson et al., 1985). The absence of alternate fixed genetic markers in these assemblages is consistent with this fact.

In conclusion, the correlations documented between the diversity, the geographic distribution, and the times of divergence of mtDNA phylogenetic assemblages and the Pleistocene glaciations emphasize the dominant influence of these catastrophic events on the population genetic structure and historical demography of lake whitefish. These results again demonstrate the usefulness of intraspecific mtDNA analysis in reconstructing historical zoogeography.

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