

MULTIPLE MODES OF SPECIATION INVOLVED IN THE PARALLEL EVOLUTION OF SYMPATRIC MORPHOTYPES OF LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS*, SALMONIDAE)

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Abstract.—We performed a phylogenetic analysis of mtDNA variation among seven sympatric pairs of dwarf and normal morphotypes of whitefish from northern Québec and the St. John River drainage to address three questions relevant to understanding their radiation. Are all sympatric pairs reproductively isolated? Do phylogenetic analyses confirm that sympatric whitefish morphotypes found in eastern North America represent the outcome of polyphyletic evolutionary events? If so, did all sympatric pairs from the St. John River drainage originate from the same scenario of allopatric divergence and secondary contact? The hypothesis of genetic differentiation was supported for all sympatric pairs from the St. John River drainage, whereas lack of mtDNA diversity precluded any test of reproductive isolation for northern Québec populations. Patterns of mtDNA variation confirmed that dwarf and normal morphotypes evolved in parallel among independent, yet closely related, lineages, thus providing indirect evidence for the role of natural selection in promoting phenotypic radiation in whitefish. Patterns of mtDNA diversity among sympatric pairs of the St. John River indicated a complex picture of whitefish evolution that implied sympatric divergence and multiple allopatric divergence/secondary contact events on a small geographic scale. These results suggest that ecological opportunities, namely trophic niche availability, may promote population divergence in whitefish.

Key words.—*Coregonus*, mtDNA, parallel evolution, speciation, sympatric morphotypes.

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Ecological studies are the key to elucidating mechanisms driving the evolution of differences between populations (Schluter and McPhail 1992, 1993). However, gaining knowledge about the evolutionary history and genetic relationships remains essential because such processes are historical in nature. In many situations, the assessment of differentiation at neutral loci represents a powerful tool to test whether ecologically differentiated forms represent alternate life histories within a single gene pool (e.g., Sage and Selander 1975; Turner and Grosse 1980) or reproductively isolated populations (e.g., Foote et al. 1989; Ferguson and Taggart 1991). Patterns of origination such as allopatric versus sympatric divergence of populations cannot be understood without elucidating their evolutionary relationships (Behnke 1972). Accurate phylogenetic assessment is also necessary to elucidate whether similar ecological specializations observed in closely related populations are the result of unique or repeatable evolutionary events (e.g., Taylor and Bentzen 1993a). The understanding of such phenomena is of particular interest to the study of adaptive radiation. For instance, the demonstration of parallel evolution of closely related lineages that are phylogenetically distinct and experience similar environmental conditions represents the strongest nonexperimental evidence that evolution occurred by natural selection imposed by ecological forces (Bell and Foster 1994; Schluter and Nagel 1995).

Coregonid fishes have undergone extensive radiation in recent evolutionary history (Behnke 1972). Species flocks and sympatric forms are known from many taxa in the group (Steinman 1951; Lindsey 1970; McCart 1970; Clarke 1973; Svardson 1979; Vuorinen et al. 1981; Smith and Todd 1984). Among these, the lake whitefish (*Coregonus clupeaformis*

Mitchill) represents one of the best studied groups, from both ecological and evolutionary perspectives. The species is found throughout most of the northern half of North America (Scott and Crossman 1974). Population differentiation of life-history traits, morphology, and morphometry has been documented throughout the range as has nuclear and mitochondrial gene variation (Lindsey 1963; Lindsey et al. 1970; Bodaly 1979; Bodaly et al. 1992; Bernatchez and Dodson 1991, 1994; Foote et al. 1992; Bernatchez et al. 1996). Altogether, these studies have shown that the lake whitefish complex is composed of five geographic races that diverged as a result of isolation in different glacial refugia.

In eastern North America, sympatric pairs of whitefish morphotypes have been reported in several disjunct locations: the St. John River drainage of northern Maine and southern Québec (Fenderson 1964; Chouinard et al. 1996), northern Québec and Labrador (Fortin and Gendron 1990; Bodaly et al. 1992), and Ontario (Kennedy 1943; Bodaly et al. 1991). These morphotypes, referred to as dwarf and normal, display differences in life-history parameters, and also differ in behavioral and morphological traits associated with the use of distinct trophic niches (Fenderson 1964).

Genetic analyses of isozyme and mtDNA performed on three sympatric pairs of dwarf and normal morphotypes indicated that these are genetically differentiated (Kirkpatrick and Selander 1979; Bernatchez and Dodson 1990a; Vuorinen et al. 1993). Origins of different pairs of whitefish morphotypes is uncertain: evidence suggests that allopatric divergence followed by secondary contact, as well as sympatric radiation, may be involved. For example, it has been demonstrated that the existence of sympatric morphotypes in Cliff Lake Maine (St. John River drainage) resulted from the secondary contact of two monophyletic groups of whitefish that evolved allopatrically in the Atlantic and Acadian refugia

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TABLE 1. Locations, sample sizes, haplotype, and nucleotide diversity indices of lake whitefish dwarf and normal morphotypes. Letters in parentheses refer to localization in Table 2.

Lakes/reservoirs	Location	Morphotypes	N	Haplotype diversity	Nucleotide diversity
Cliff	46°23'51" N	Dwarf (a)	30	0.1310	0.000090
	69°15'05" W	Normal (b)	30	0.0667	0.000091
Webster	46°09'19" N	Dwarf (c)	12	0.3030	0.001449
	69°04'48" W	Normal (d)	10	0.6000	0.002689
East	47°11'00" N	Dwarf (e)	48	0.4787	0.001307
	69°33'00" W	Normal (f)	41	0.3402	0.000975
Témiscouata	47°36'00" N	Dwarf (g)	32	0.3871	0.000329
	68°45'00" W	Normal (h)	27	0.3846	0.000873
Outardes II	49°32'58" N	Dwarf (i)	7	0.0000	0.000000
	68°47'05" W	Normal (j)	19	0.1053	0.000072
Manicouagan V	51°30'00" N	Dwarf (k)	13	0.4231	0.000317
	68°30'00" W	Normal (l)	3	0.0000	0.000000
Caniapiscau	54°22'00" N	Dwarf (m)	21	0.0000	0.000000
	70°00'00" W	Normal (n)	18	0.2157	0.000153
Average/Total			22/311	0.2454 ± 0.0028	0.000596 ± 0.000000

during the last glaciation events (Bernatchez and Dodson 1990a). This scenario could explain the occurrence of all sympatric pairs found in the upper St. John River drainage. However, several sympatric pairs, such as those from northern Québec and Ontario, are apparently located away from potential zones of secondary contact between distinct races, suggesting sympatric divergence as a more likely explanation for their origin.

In this paper, we address three evolutionary questions relevant to the understanding of adaptive radiation in whitefish. First, are all sympatric pairs reproductively isolated? This would be demonstrated by a significant heterogeneity of mtDNA haplotype frequencies between dwarf and normal members of all given pairs of morphotypes (e.g., Bernatchez and Dodson 1990a; Taylor and Bentzen 1993a). Second, can phylogenetic analyses confirm that sympatric whitefish morphotypes found in eastern North America represent the outcome of polyphyletic evolutionary events? This would be supported by the demonstration that several populations of a given morphotype belonged to phylogenetically independent whitefish races. Third, did all sympatric pairs from the St. John River drainage originate from the same scenario of allopatric divergence and secondary contact, and did all sympatric pairs of northern Québec evolve from sympatric divergence events? The hypothesis of a common scenario of allopatric divergence and secondary contact would be supported by the grouping of all populations of a given ecotype from different lakes into phylogenetically distinct Acadian and Atlantic races. Alternatively, the finding that distinct morphotypes within lakes share uniquely derived genetic characters would support the hypothesis of incipient sympatric radiation.

MATERIALS AND METHODS

Samples

A total of 283 individuals representing dwarf and normal sympatric pairs of whitefish from seven lakes and reservoirs were sampled between 1992 and 1994 (Table 1, Fig. 1). In addition, 28 specimens (12 normal- and 16 dwarf-sized fish)

from Cliff Lake previously analysed by Bernatchez and Dodson (1990a) were also included in the study. Morphological and ecological differences between forms of all sympatric pairs have been documented elsewhere (Fenderson 1964; Fortin and Gendron 1990; Chouinard et al. 1996).

Mitochondrial DNA Extraction and Analysis

Tissue samples used for DNA purification consisted of fresh or ultra frozen (-80°C) liver and eggs or white muscle preserved at -20°C . In the case of fresh and ultra frozen tissues, mtDNA was purified according to Chapman and Powers (1984) as modified by Bernatchez et al. (1988), whereas total DNA was extracted from white muscle as described in Bernatchez et al. (1992). Mitochondrial DNA variation was analysed by restriction fragment length polymorphisms (RFLP) performed on two PCR amplified products for all 311 specimens and the whole mitochondrial genome for a subset of samples as described below. The PCR-RFLP procedure was adopted to circumvent problems related to the poor quality of many tissue samples. One PCR amplified segment encompassed the complete ND-5/6 region (approximately 2.4 kb) and the other (approximately 2.1 kb) comprised the cytochrome *b* gene, and the control region (D-loop). Procedures for PCR amplification, restriction analysis and electrophoresis are detailed in Bernatchez and Osinov (1995). Procedures used for the RFLP analysis of the whole mitochondrial genome were those described in Bernatchez and Dodson (1990b).

Ten restriction enzymes were used for the PCR-RFLP analysis (Table 2). The correspondence of mtDNA haplotypes identified by PCR-RFLP with those defined in earlier whitefish mtDNA studies was insured by performing a restriction analysis over the whole mtDNA genome on new PCR-RFLP variants with the same enzymes used in Bernatchez and Dodson (1991, 1994). Distinct single endonuclease patterns generated by both PCR-RFLP and total mtDNA-RFLP analyses were identified by a specific letter in order of appearance, and used in combination to define composite mtDNA haplotypes.

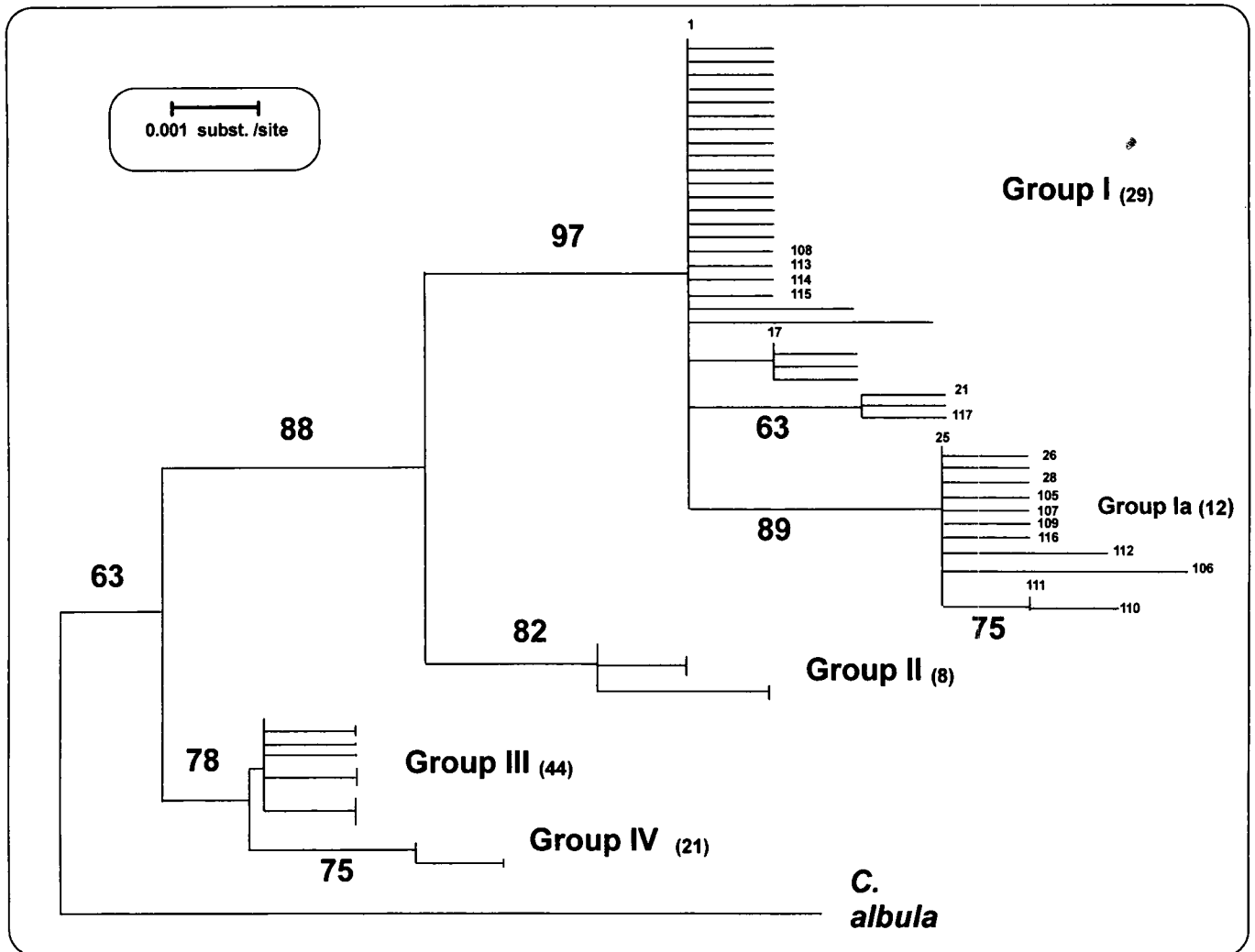


FIG. 1. Condensed majority-rule tree clustering 19 mtDNA haplotypes observed in the present study with all other haplotypes as detailed in Bernatchez and Dodson (1994). Numbers at the tip of branches refer to haplotypes observed in the present study. Numbers in parentheses refer to the total number of haplotypes observed thus far in each group. Bootstrap estimates are given along branches. The scales of 0.001 substitution/site corresponds to one restriction site difference.

Data Analysis

The restriction site matrix was used to estimate phylogenetic relationships among mtDNA haplotypes defined in this study according to a maximal parsimony criteria, using the program MIX of the PHYLIP 3.5c computer package (Felsenstein 1993). A majority-rule consensus tree and confidence statements on branches were obtained using the CONSENSE program performed on 10,000 different trees generated by MIX from bootstrap replicates produced by the SEQBOOT program.

Three enzymes (*Ava* II, *Ban* I, *Hinc* II) were used for RFLP analysis of both PCR amplified segments and total mtDNA (Table 2). Thus, identical polymorphic sites could potentially have been detected twice. This was the case for one *Hinc* II site, which was consequently considered only once in phylogenetic analyses. As a comprehensive rooted phylogenetic tree was previously produced by a parsimony analysis of mtDNA haplotypes distributed throughout the species range

(Bernatchez and Dodson 1994), new haplotypes identified in this study were incorporated in this overall tree.

The genetic differentiation between dwarf and normal forms of whitefish within each water body was evaluated by the analysis of frequency distribution of haplotypes using chi-square randomization tests (Roff and Bentzen 1989) with 1000 randomizations performed by the MONTE program of the REAP software package (McElroy et al. 1992). We also quantified the amount of genetic diversity between forms of each sympatric pair by computing fixation indices (F_{st} ; Wright 1978). Statistical significance of F_{st} estimates were tested using a random procedure available in the AMOVA computer package (Excoffier et al. 1992).

Inter- and intrapopulation genetic diversity were quantified by haplotype diversity (h) and by the maximum-likelihood estimation of the average number of nucleotide substitutions per site within (nucleotide diversity) and between populations (nucleotide divergence) using the program DA (REAP). Re-

TABLE 2. Composite definitions and location of mtDNA haplotypes observed among lake whitefish dwarf and normal morphotypes from seven lakes and reservoirs of eastern North America. The composite definitions are given for the total mtDNA molecule and for the ND5-ND6-Cyt *b*-D loop fragment. Capital letters correspond to fragment patterns observed for each enzyme and are independent for the analysis of total and PCR mtDNA segments. Small letters refer to populations described in Table 1. Haplotype numbering is in continuation of those used previously (Bernatchez and Dodson 1991, 1994; Bernatchez et al. 1996).

#	Total mitochondrial DNA molecule											Nd5-ND6-cyt <i>b</i> -D loop segment											Location
	<i>Ava</i> I	<i>Ava</i> II	<i>Ban</i> I	<i>Bgl</i> I	<i>Hae</i> II	<i>Hinc</i> II	<i>Hind</i> III	<i>Pvu</i> II	<i>Sma</i> I	<i>Xmn</i> I	<i>Pst</i> I	<i>Alu</i> I	<i>Cfo</i> I	<i>Ava</i> II	<i>Dde</i> I	<i>Mbo</i> I	<i>Rsa</i> I	<i>Hae</i> III	<i>Hinc</i> II	<i>Ban</i> I	<i>Bsp</i> 12861		
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	h-n	
17	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	B	A	A	j, k	
21	A	D	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	b-d	
25	A	A	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	a, c-h	
26	A	A	D	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	g	
28	A	A	D	A	E	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	g	
105	A	A	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	A	A	a	
106	A	A	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	A	C	A	e, f	
107	A	A	D	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	f	
108	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	B	A	A	A	n	
109	A	A	D	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	h	
110	A	A	D	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	C	h	
111	A	A	D	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	d, g	
112	A	A	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	g	
113	A	A	A	A	A	A	A	A	A	A	A	A	A	C	A	A	A	B	A	A	A	n	
114	A	A	A	A	A	A	A	A	A	A	A	A	C	A	A	A	A	B	A	A	A	k	
115	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	B	A	A	A	k	
116	A	A	D	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	a	
117	A	D	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	b	

relationships among all populations were assessed by using the resulting distance matrix of net nucleotide divergence to construct a neighbor-joining phenogram clustering all dwarf and normal morphotypes using the NEIGHBOR program of PHYLIP.

RESULTS

Mitochondrial DNA Diversity and Phylogenetic Differentiation

The PCR-RFLP analysis generated a total of 81 restriction sites. These resolved 19 mtDNA haplotypes that were distinguished by one to nine restriction sites (Table 2, Fig. 1). Combined with the 89 restriction sites surveyed by the RFLP analysis over the entire molecule, a total of 170 sites representing 858 base pairs (approximately 5.2% of the mitochondrial genome) was assayed for representatives of all mtDNA variants. Haplotypes 105 to 117 were not observed before, whereas haplotypes 1, 17, 21, 25, 26, 28 were previously reported (Bernatchez and Dodson 1991, 1994). All of these belonged to mtDNA phylogenetic group I of Bernatchez and Dodson (1994), which included all mtDNA haplotypes found thus far in North America outside Beringia (Alaska and Yukon). This major group is further subdivided into smaller assemblages that distinguish three glacial races of whitefish, based on their phylogenetic and/or geographic distinctiveness (Fig. 1; Bernatchez and Dodson 1991, 1994). Representative haplotypes of all three races were observed in this study (Fig. 1). Haplotypes 25, 26, 28, 105–107, 109–112, and 116 belonged to phylogenetic group Ia of Bernatchez and Dodson (1994), which characterizes whitefish issued from the Acadian race. This group was characterised by three diagnostic restriction sites, one *Hae* III and two *Ban* I sites, and was supported by 89% bootstrap level.

Haplotypes 21 and 117 belonged to a smaller subcluster characteristic of the Atlantic race, which was diagnosed by one *Ava* II and one *Mbo* I site, and supported by a moderate bootstrap level. Haplotype 17 belonged to a second small subcluster also characteristic of the Atlantic race and diagnosed by a *Hinc* II site. The association of these haplotypes to the Atlantic race does not rely on their phylogenetic distinctiveness, but was confirmed by their geographic pattern of distribution (Bernatchez and Dodson 1991).

Genetic Differentiation among Sympatric Ecotypes

Evidence for reproductive isolation between dwarf and normal morphotypes was provided by significant differences in the frequency distribution of haplotypes in all four sympatric pairs from the St. John River drainage (Table 3, 4, Fig. 2). In Cliff Lake, dwarf and normal morphotypes were alternatively fixed for haplotypes of Acadian (haplotype 25, 105, 116) and Atlantic (haplotype 21, 117) origins. These results were identical to those of Bernatchez and Dodson (1990a) based on fewer individuals, except that three additional rare haplotypes ($n = 1$) were detected. Evidence for highly restricted gene flow between forms was also indicated by an F_{st} estimate of 0.901. In Webster Lake, dwarf- and normal-sized fish were also alternatively dominated by, although not fixed for, mtDNA haplotypes of Acadian and Atlantic origins. Thus, haplotype 25 characterized 83% of the dwarf fish, whereas normals were dominated (60%) by haplotype 21. This translated into a lower but significant F_{st} estimate of 0.315. In East Lake, the frequency distribution of haplotypes 25, 106, and 107, all of Acadian origin, differed significantly between forms. However, a low fixation index ($F_{st} = 0.091$) indicated more extensive mitochondrial gene flow in this lake than in Cliff and Webster Lakes. In Tém-

TABLE 3. Absolute (N) and relative frequency distribution of 19 mtDNA haplotypes resolved among sympatric lake whitefish morphotypes in eastern North America. Haplotypes are grouped according to phylogenetic assemblages representative of whitefish races. Haplotypes 1–115: Mississippian race (Mis.); haplotypes 17–117: Atlantic race (Atl.); and haplotypes 25–116: Acadian race (Aca.).

Haplotype #	N	Webster		Cliff		East		Témiscouata		Outardes		Manicouagan		Caniapiscau	
		Dwarf	Norm.	Dwarf	Norm.	Dwarf	Norm.	Dwarf	Norm.	Dwarf	Norm.	Dwarf	Norm.	Dwarf	Norm.
1	79							0.15		1.00	0.95	0.76	1.00	1.00	0.88
108	1														0.06
113	1														0.06
114	1											0.08			
115	1											0.08			
Mis. total	83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	1.00	0.95	0.92	1.00	1.00	1.00
17	2										0.05	0.08			
21	37	0.17	0.60		0.97										
117	1				0.03										
Atl. total	40	0.17	0.60	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.05	0.08	0.00	0.00	0.00
25	110	0.83	0.30	0.94		0.38	0.12	0.79	0.77						
26	3							0.09							
28	1							0.03							
105	1			0.03											
106	63					0.62	0.80								
107	3						0.08								
109	1								0.04						
110	1								0.04						
111	3		0.10					0.06							
112	1							0.03							
116	1			0.03											
Aca. total	188	0.83	0.40	1.00	0.00	1.00	1.00	1.00	0.85	0.00	0.00	0.00	0.00	0.00	0.00

iscouata Lake, both morphotypes were also dominated by haplotypes of Acadian origin. Unlike East Lake, however, 15% of normal fish in this lake possessed haplotype 1, typical of the Mississippian race, which was not observed in the dwarf form. Extensive gene flow between morphotypes was also suggested by a low F_{st} value of 0.012.

The lack of mtDNA variation precluded any test of reproductive isolation in the three reservoirs from northern Québec. Thus, haplotype 1, largely dominated (76% to 100%) in dwarf and normal morphotypes of the three reservoirs. Consequently, F_{st} estimates were null or not significantly different from zero, except for Caniapiscau.

Polyphyletic Origins of Whitefish Ecotypes

The pattern of mtDNA variation observed among all seven sympatric pairs confirmed the hypothesis of a polyphyletic origin for dwarf and normal morphotypes of lake whitefish. No diagnostic haplotypes were found to characterize each morphotype, nor was there any evidence of more similar haplotype frequencies among all members of a given morphotype (Table 3). Consequently, dwarf and normal morphotypes did not compose distinct genetic clusters, as evi-

denced by the mtDNA population phenogram (Fig. 3). Multiple radiations of the two forms were best evidenced by the sharp pattern of mtDNA variation among sympatric pairs from northern Québec and the St. John River drainage (Table 3, Fig. 2, Fig. 3). All populations from northern Québec clustered together, being almost fixed for the Mississippian mtDNA group (94% of haplotype 1).

Further evidence for multiple origins of dwarf and normal morphotypes was indicated by the pattern of mtDNA variation and population clustering among the four sympatric pairs from the St. John River drainage (Table 3, Fig. 2, Fig. 3). Normal populations from Cliff and Webster Lakes, clustered very distinctively from either East and Témiscouata Lakes, normal populations. The normal population from Témiscouata Lake clustered with the dwarf populations from Webster, Cliff, and Témiscouata Lakes. Finally the dwarf and normal populations from East Lake clustered together.

DISCUSSION

Reproductive Isolation between Sympatric Morphotypes

The lack of mitochondrial DNA diversity precluded any test of reproductive isolation between morphotypes in the

TABLE 4. Within-lake genetic differentiation between sympatric dwarf and normal whitefish morphotypes based on chi-square analysis and fixation indices (F_{st}) estimates performed on mtDNA RFLP data.

	Webster	Cliff	East	Témiscouata	Outardes	Manicouagan	Caniapiscau
Chi-square probability	6.64 0.0190	60.00 < 0.0001	10.00 0.0040	13.02 0.0100	0.38 0.2920	0.85 0.4900	4.92 0.1200
F_{st} probability	0.315 0.015	0.901 < 0.001	0.091 0.0120	0.017 0.1868	0.000 0.2907	0.000 0.4685	0.040 < 0.0010

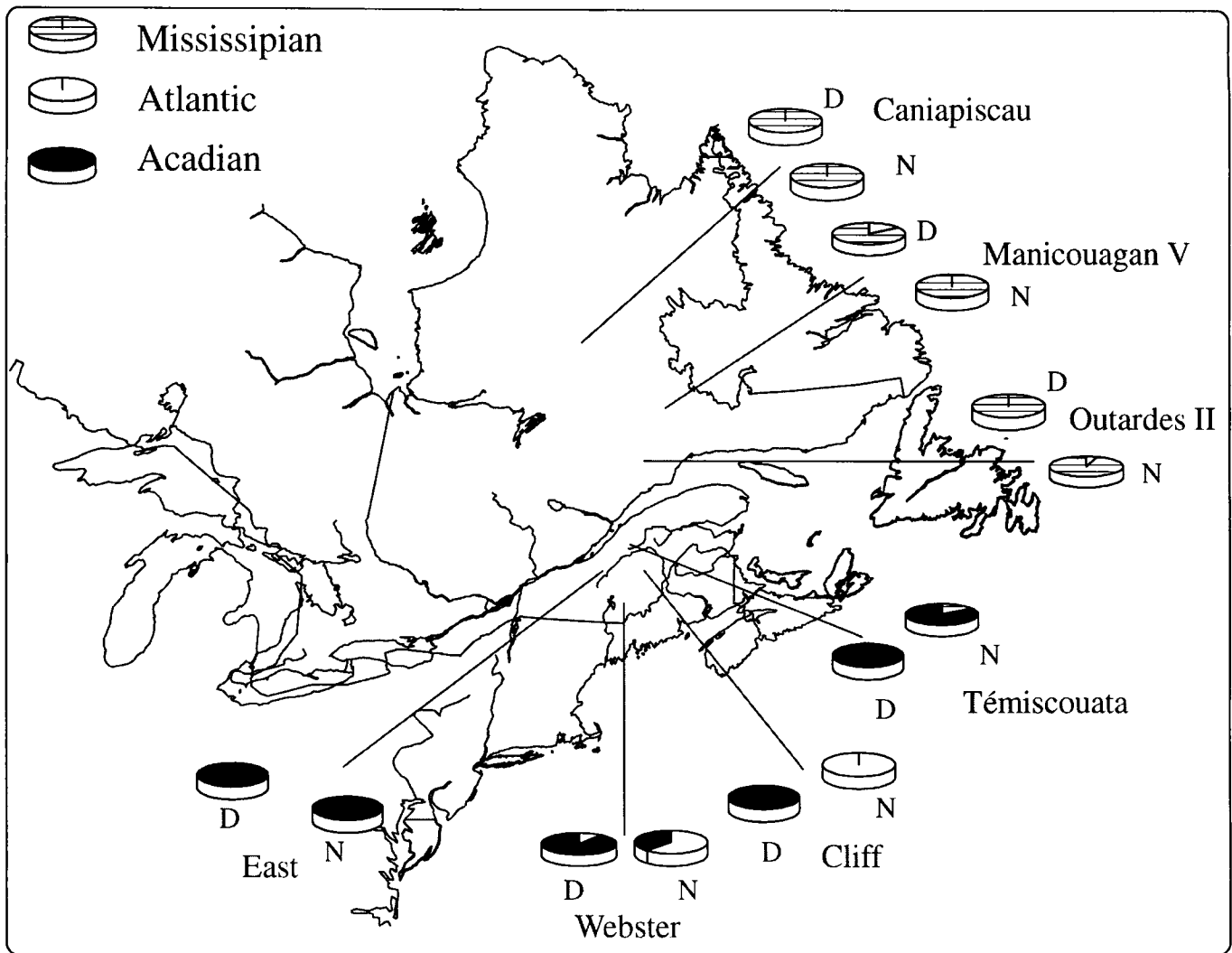


FIG. 2. Geographic distribution of mtDNA lineages representing three glacial races among dwarf (D) and normal (N) whitefish morphotypes.

three reservoirs from northern Québec. Consequently, these will not be discussed further. In contrast, the hypothesis of genetic differentiation between whitefish morphotypes was supported for all pairs from the St. John River drainage where significant differences in haplotype frequency distribution were observed in the different lakes. In some cases, the hypothesis of reproductive isolation between forms has also been supported by evidence for premating isolating mechanisms. For instance, Fenderson (1964) reported differential segregation of spawning time in Cliff Lake, although both morphotypes apparently use the same spawning grounds (Chouinard, unpubl. data). There is also strong evidence for spatial segregation of spawning in Témiscouata Lake, where the dwarf morphotype spawns in streams and normal fish spawn in the lake (Lamoureux and Sylvain 1986). The sympatric occurrence of genetically differentiated forms is not unique to whitefish. Indeed, there is growing evidence that reproductive isolation among sympatric morphotypes may be a general phenomenon among most subarctic and arctic freshwater fishes (Ryman et al. 1979; McPhail 1984; Smith and

Todd 1984; Foote et al. 1989; Verspoor and Cole 1989; Ferguson and Taggart 1991; Hindar et al. 1991; Taylor and Bentzen 1993a,b; Hindar 1994; Robinson and Wilson 1994; Skúlason and Smith 1995; Taylor et al. 1996).

Lake differences in mtDNA gene flow between sympatric dwarf and normal morphotypes is analogous to the situation observed in the Yukon Territory for sympatric benthic and limnetic ecotypes of whitefish (Bernatchez et al. 1996). Variation in the extent of genetic differentiation between coexisting morphotypes has been reported in the rainbow smelt *Osmerus mordax* (Taylor and Bentzen 1993a) and several salmonid fishes, including Arctic charr (*Salvelinus alpinus*), brown trout (*Salmo trutta*), Atlantic salmon (*S. salar*), and sockeye salmon (*Oncorhynchus nerka*) (reviewed in Hindar 1994). Bernatchez et al. (1996) hypothesized that sympatric morphotypes found in lakes with greater opportunity for trophic niche partitioning should have evolved more specialized traits for occupying these niches and selective pressures should favor the reinforcement of isolating mechanisms to maintain these differences. Alternatively, in lakes with lesser

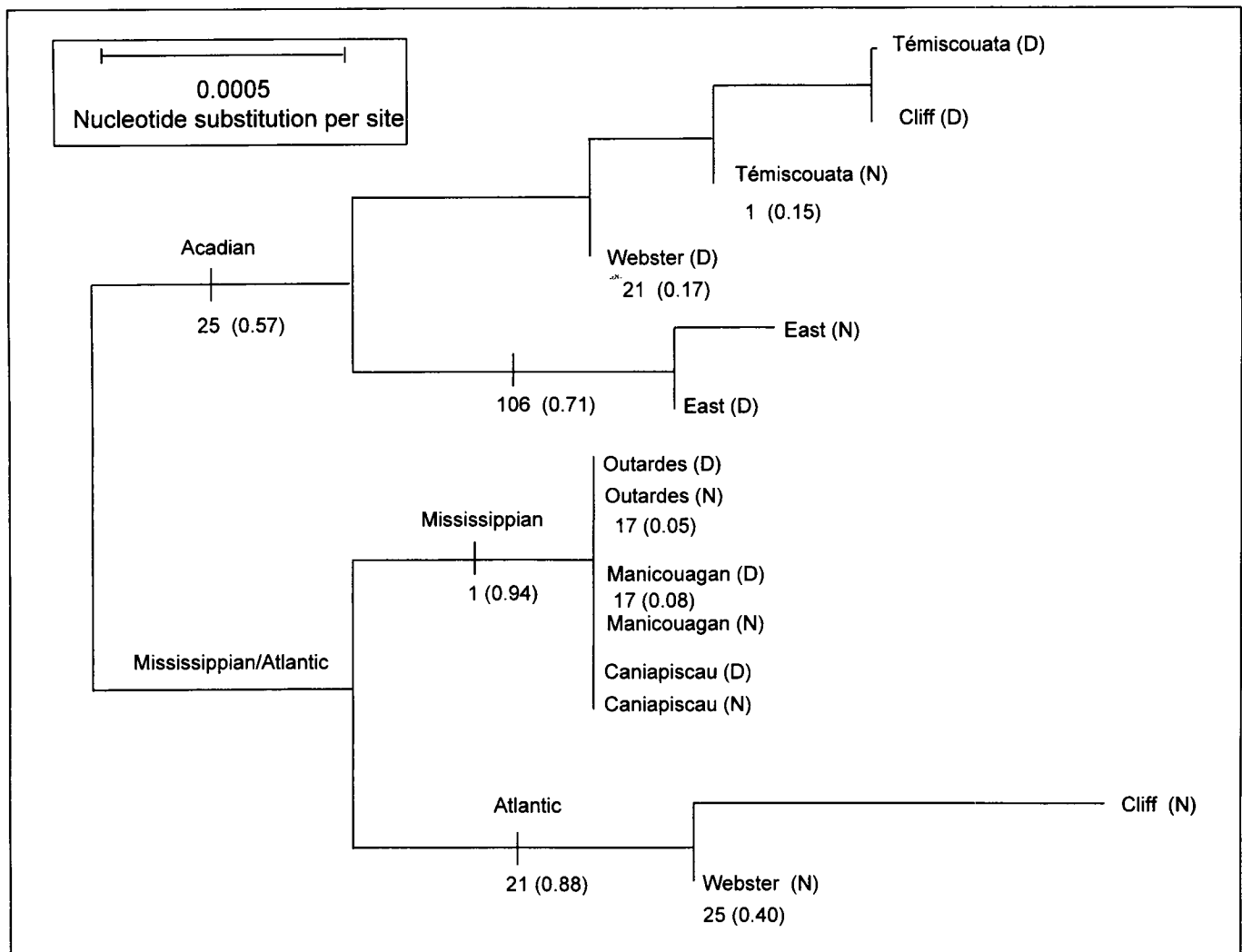


FIG. 3. Neighbor-joining phenogram clustering dwarf and normal sympatric morphotypes of lakes whitefish from seven lakes of north-eastern North America, according to the distance matrix resulting from the maximum-likelihood estimation of net average number of nucleotide substitution per site between populations or morphotypes. Numbers along branches designate synapomorphic haplotypes observed in more than one population and representative of Acadian, Atlantic, and Mississippian glacial races of whitefish. Numbers in parentheses refer to their relative frequency among all populations combined belonging to the same branch.

opportunities for differential trophic niche occupation, selective pressures promoting trophic specializations and reproductive isolation should be reduced, resulting in more extensive gene flow between forms.

Variation in the amount of gene flow between dwarf and normal morphotypes from different lakes in the St. John River drainage is partly congruent with this hypothesis. Morphological analysis suggested that dwarf and normal morphotypes from East Lake were less differentiated in traits potentially related to trophic ecology than morphotypes from Cliff Lake (Fenderson 1964; Chouinard et al. 1996). Spawning segregation is less pronounced in East Lake where both forms apparently spawn at the same time (Chouinard, unpubl. data) whereas there is an important delay in the spawning peak of both forms in Cliff Lake, although they use the same spawning sites (Fenderson 1964). Consequently, gene flow between forms was more extensive in East Lake than Cliff Lake.

Modes of Radiation of Sympatric Forms

The pattern of mtDNA variation confirmed our second working hypothesis that dwarf and normal sympatric whitefish pairs evolved more than once in northeastern America, thus refuting the scenario of a monophyletic origin of both forms. Thus, the occurrence of both forms in phylogeographic assemblages distinguishable by uniquely derived genetic characters provided evidence that similar phenotypic patterns evolved in parallel in separate but closely related lineages. This adds to the evidence that parallel evolution of traits potentially adapted for occupying distinct ecological niches may be a generalized phenomenon, having been described in most fish genera occurring in lakes of northern latitudes, including *Gasterosteus*, *Osmerus*, *Salvelinus*, *Oncorhynchus*, and *Salmo* (Hindar et al. 1986; Foote et al. 1989; McPhail 1993; Taylor and Bentzen 1993a; Bernatchez and Osinov 1995).

Whether all sympatric pairs of northern Québec evolved from sympatric divergence events could not be strictly assessed. Given that only the Mississippian glacial whitefish race recolonized that area in postglacial times (Bernatchez and Dodson 1991; this study), secondary contact could not have involved distinct glacial races. Instead, it could have implied two recolonization events from the same race, as has been hypothesized for explaining the origins of sympatric pairs of benthic and limnetic sticklebacks (McPhail 1994).

The pattern of mtDNA diversity among sympatric pairs of the St. John River drainage did not support the hypothesis that they all originated from the secondary contact between two monophyletic whitefish groups that evolved allopatrically in the Acadian and Atlantic glacial refugia during the last glaciation events (Bernatchez and Dodson 1990a). Rather, our results indicated more than one mode of radiation. Sound evidence for the allopatric divergence/secondary contact hypothesis was found in Cliff and Webster Lakes. In contrast, the presence of sympatric morphotypes in Témiscouata Lake cannot be explained by this same scenario. Instead, the Acadian race haplotypes dominated both morphotypes, those of Mississippian origin were only found in the normal form, and the Atlantic haplotypes are missing. These observations suggest that sympatric whitefish morphotypes in Témiscouata Lake resulted from the secondary contact between the Acadian and Mississippian (instead of Atlantic) races followed by important introgressive hybridization.

In contrast, the pattern of mtDNA diversity observed in East Lake better supports the hypothesis of intralacustrine origin of the sympatric morphotypes. Thus, all fish possessed mtDNA haplotypes typical of the Acadian race, and both populations shared a uniquely derived mtDNA haplotype observed nowhere else. The weak differentiation (two restriction sites) of this haplotype from others representative of the Acadian race is also plausible with its postglacial mutational genesis. The two morphotypes formed a distinct population cluster, being more closely related to each other than to any other group in the population phenogram. These observations can be explained by the postglacial colonization of East Lake by a single founding population of Acadian origin characterized by the original haplotype 25 still found in the lake, followed by the local genesis of haplotype 106, the radiation of the two morphotypes through disruptive selection, and the development of their partial reproductive isolation.

An alternative scenario to that of sympatric radiation is that, as in the other lakes of the St. John River drainage, the existence of two morphotypes in East Lake resulted from the secondary contact of distinct glacial races. This would imply that mtDNA introgression between the two colonizing populations as been such that the original mtDNA genome of one of them (either of Atlantic or Mississippian origin) has been completely replaced. Clearly, this scenario cannot be entirely ruled out, as complete replacement of mtDNA through introgressive hybridization has previously been reported in other fishes (e.g., Bernatchez et al. 1995). This, however, would imply either that one of the founding population was characterized by the occurrence of the unique haplotype 106, and that this population was not involved in the recolonization of any of the other nearby lakes connecting to the same river drainage, or that this haplotype vanished

everywhere else. Clearly, this represents a much less parsimonious explanation than the scenario of sympatric radiation, which we consequently favor for explaining the origin of sympatric morphotypes in East Lake.

Ecological Opportunities and Adaptive Radiation in Whitefish

It is generally assumed that natural selection plays a dominant role in population diversification (e.g., Mayr 1963; Endler 1977; Diehl and Bush 1989). Surprisingly, there is little direct support for its role in nature (Schluter and Nagel 1995). This is largely attributed to difficulties in obtaining evidence for selection through experimental studies in natural conditions (but see Schluter 1994). Alternatively, indirect evidence for selection may be obtained by the demonstration that similar evolutionary changes evolved in parallel among independent lineages experiencing similar environmental conditions (Schluter and Nagel 1995). In this study, similar phenotypic transitions from a normal to a dwarf whitefish morphotype occurred in at least three independent evolutionary lineages, thus arguing in favor of natural selection as a major evolutionary force driving phenotypic radiation in whitefish. Several observations also suggested that factors promoting population diversification in whitefish are ecological. All sympatric pairs of lake whitefish described thus far have been found in the absence of lake cisco (*C. artedii*), a broadly distributed coregonid specialized for limnetic mode of life (Scott and Crossman 1974). This suggests that the absence of this potential competitor is necessary for the persistence of dwarf morphotype. Dwarf and normal morphotypes differ in behavioral and/or morphological traits related to trophic ecology (Fenderson 1964). In addition, the whitefish dwarf morphotype is only found in sympatry with the normal morphotype, suggesting that it is derived from the ancestral normal form, likely reflecting character displacement that resulted from competition for trophic resources.

The role of ecological selection in promoting speciation in whitefish could be demonstrated by showing that traits differentiating dwarf and normal morphotypes also confer reproductive isolation (Schluter and Nagel 1995). Assortative mating through body size may act as strong premating isolating mechanisms in fishes (e.g., Foote 1988). Sympatric adults of dwarf and normal morphotypes differ in size by one order of magnitude and remain genetically differentiated even when using the same spawning grounds at the same time. This suggests that assortative mate choice may be one mechanism maintaining reproductive isolation in whitefish in the face of the potential to hybridize. The demonstration of assortative mating through body size would nevertheless only partly explain the selective processes involved in maintaining reproductive isolation in whitefish, since differential amounts of gene flow was observed in sympatric pairs from different lakes despite similar differences in body sizes between morphotypes. The elucidation of factors governing the extent of ecological specialization and reproductive isolation between sympatric morphotypes of whitefish may enable a better understanding of the role of environmental selection in promoting speciation events.

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