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GENETIC EVIDENCE FOR REPRODUCTIVE ISOLATION AND MULTIPLE ORIGINS OF SYMPATRIC TROPHIC ECOTYPES OF WHITEFISH (*COREGONUS*)

LOUIS BERNATCHEZ[1,5], JUKKA A. VUORINEN[2], R. A. BODALY[3], AND JULIAN J. DODSON[4]
[1]*Département de biologie, GIROQ Université Laval, Sainte-Foy, Québec G1K 7P4, Canada*
[2]*Department of Biology, University of Joensuu, P.O. Box 111, FIN 80101 Joensuu, Finland*
[3]*Canada Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6, Canada*
[4]*Département de biologie, Université Laval, Sainte-Foy, Québec G1K 7P4, Canada*

Abstract.—We assessed variation in mitochondrial DNA (mtDNA) by restriction fragment length polymorphism (RFLP) analysis and in nuclear genes by allozyme analysis among sympatric pairs of limnetic and benthic ecotypes of whitefish (*Coregonus*) coexisting in three lakes of southern Yukon to address three evolutionary questions regarding their origins. Are sympatric low and high gill-raker count ecotypes genetically differentiated? Are they issued from monophyletic or polyphyletic evolutionary events? If they are polyphyletic in origins, did they originate from multiple allopatric speciation events or intralacustrine radiation? Our results corroborated previous genetic and ecological studies of these ecotypes, indicating that they represent genetically distinct reproductive units, and therefore refuting the hypothesis of phenotypic polymorphism within a single population. However, the amount of gene flow between ecotypes varied among lakes, correlating with the extent of morphological differentiation and the potential for premating reproductive isolation. The results indicated a polyphyletic origin of ecotypes whereby each of them have been expressed independently more than once. In the two lakes of Squanga Creek drainage, the existence of sympatric pairs was best explained by the secondary contact of two monophyletic whitefish groups that evolved in allopatry during the last glaciation events. In Dezadeash L. of Alsek R. drainage, our results could not verify either sympatric or allopatric (or microallopatric) origin of ecotypes. Regardless of the mode of speciation involved in their origins, these sympatric whitefish populations provided further evidence that Pleistocene glaciation events created conditions favoring rapid divergence and phenotypic differentiation among northern freshwater fishes.

Key words.—Allozymes, *Coregonus,* evolutionary history, mtDNA, speciation.

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Sympatric ecotypic polymorphisms occur in many species of north temperate and subarctic freshwater fishes. This phenomenon has been most frequently reported in salmonid fishes (Ricker 1940; Lindsey 1963; Fenderson 1964; Ferguson and Mason 1981; Magnusson and Ferguson 1987; Verspoor and Cole 1989) but has also been observed in several other groups (Scott and Crossman 1973; Lanteigne and McAllister 1983; McPhail 1984; Ehlinger and Wilson 1988). Because phenotypic variation among these closely related forms has often been related to potentially adaptive differentiation in trophic ecology and/or reproductive behavior, they provide unique systems to address relevant questions concerning the forces promoting population evolutionary divergence and ultimately, speciation.

The use of molecular systematics has proven most useful in understanding the evolution and persistence of sympatric ecotypic polymorphisms in these fishes. For instance, the analysis of genetic variation among ecotypes has revealed that there is usually little relationship between genetic and phenotypic variation. It has been shown in several cases that morphologically and behaviorally differentiated sympatric ecotypes represent polymorphisms in a single gene pool (e.g., Ehlinger and Wilson 1988), whereas evidence of genetic differentiation between forms has been found in others (Foote et al. 1989; Ferguson and Taggart 1991; Hartley et al. 1992; Taylor and Bentzen 1993a). When similar ecotypes are found in disjunct locations, they may represent monophyletic entities (Bernatchez and Dodson 1990a).

Molecular phylogenetic analysis also revealed an unpreceeded occurrence of parallelism in phenotypic expression (Vuorinen et al. 1981; Foote et al. 1989; Hindar et al. 1986; McPhail 1993; Taylor and Bentzen 1993a). The most controversial outcome of these studies is that they most often argue in favor of sympatric speciation as the most likely explanation for the origin of ecotypic polymorphisms (Smith and Todd 1984; Foote et al. 1989; Taylor and Benzten 1993a,b). Typically though, most of these studies do not provide evidence for the critical test of incipient monophyly, whereby populations believed to have diverged sympatrically share uniquely derived characters observed nowhere else. Thus, sympatric speciation has most often been invoked from circumstantial evidence (but see Taylor and Bentzen 1993b). In such cases, the demonstration of allopatric or microallopatric speciation events may have been hampered by the limitations of the analytical approach and/or because of insufficient knowledge of population relationships.

One of the best studied groups from this point of view is the lake whitefish (*Coregonus clupeaformis*) population complex from North America. Patterns of population differentiation have been extensively documented over the entire range of distribution for life-history traits, morphology, and morphometry, as well as for nuclear and mitochondrial gene variation (Lindsey 1963; Lindsey et al. 1970; Bodaly 1979; Bodaly et al. 1992; Bernatchez and Dodson 1991, 1994). Sympatric ecotypes within this population complex have been reported from disjunct locations, including eastern Canada (Fortin and Gendron 1990; Bodaly et al. 1992), northern Maine (Fenderson 1964), and southern Yukon (Lindsey 1963; Lindsey et al. 1970; Bodaly et al. 1988). Where they are found, whitefish sympatric ecotypes usually differ in trophic

[5] Corresponding author. E-mail: Louis.Bernatchez@bio.ulaval.ca

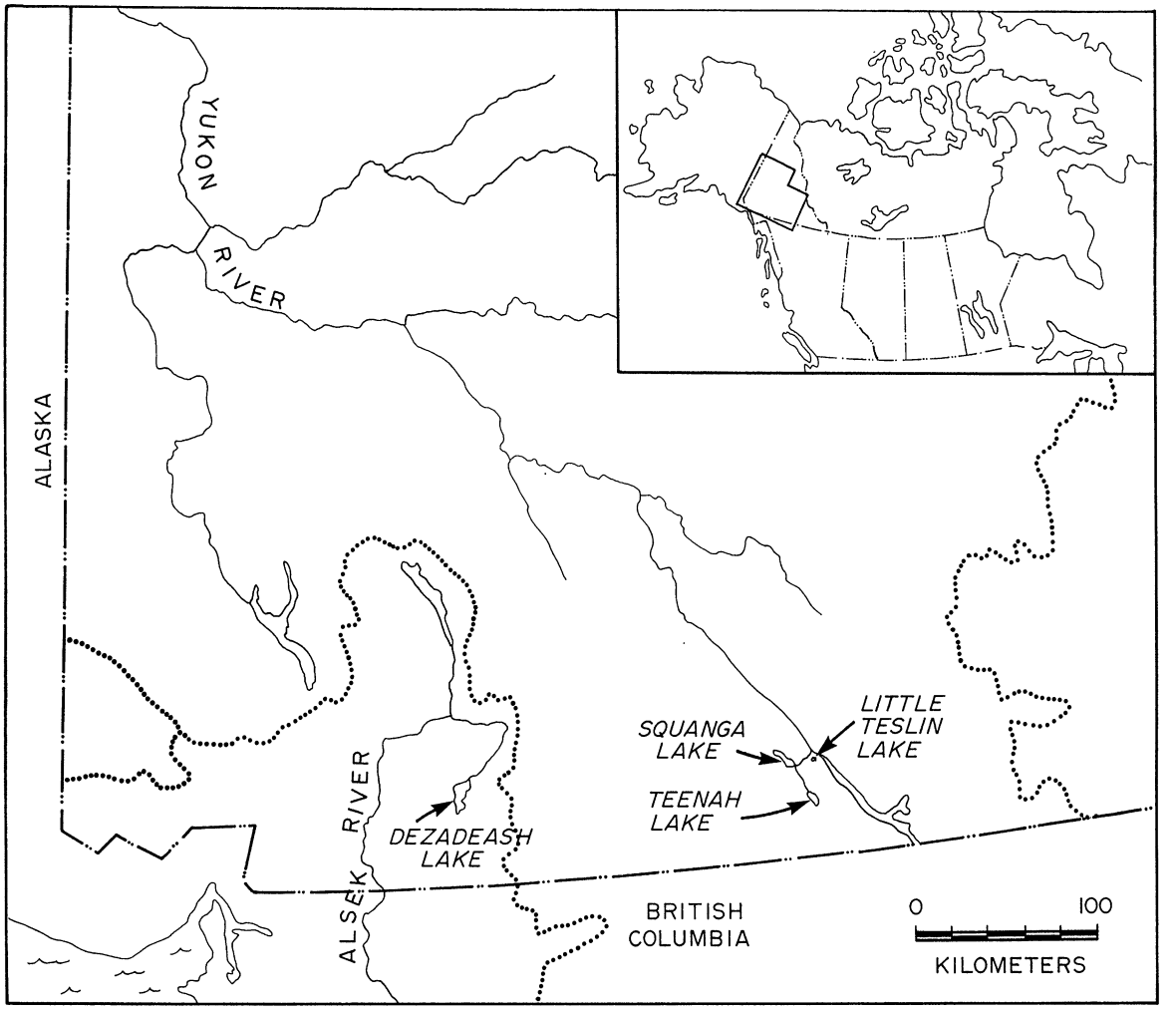

FIG. 1. Location map of Dezadeash, Squanga, and Little Teslin, and Teenah lakes, Yukon, where sympatric whitefish ecotypes coexist Teenah L. was not surveyed in the present study.

niches, place and/or time of spawning, and life-history traits, namely growth, fecundity, age at maturity, and generation time. Genetic analyses have often indicated that sympatric ecotypes are distinct reproductive units (Kirkpatrick and Selander 1979; Bodaly et al. 1988; Bernatchez and Dodson 1990a; Vuorinen et al. 1993).

Sympatric ecotypes from southern Yukon territory are generally reported as low and high gill-raker count forms, based on differences in gill-raker numbers, a heritable feature strongly related to trophic specialization in whitefishes (Svärdson 1979, Lindsey 1981). The name of Squanga whitefish has been used to describe the high gill-raker count form (Bodaly et al. 1988). For convenience, we will refer here to LGR ecotype and HGR ecotype to designate the forms. The LGR ecotype is phenotypically characteristic of most lake whitefish populations, its feeding apparatus and trophic behavior being adapted to a benthic mode of life. In contrast, the HGR ecotype is very rare, occurring in only four lakes of southern Yukon, and always in sympatry with the LGR ecotype (Fig. 1). The HGR ecotype also exhibits unique behavioral and morphological traits associated with planktivory. HGR ecotype is generally associated with the limnetic zone of the water column, whereas the LGR ecotype is typically associated with the bottom. These ecological differences translate into the differential use of trophic resources; the HGR ecotype consumes mostly pelagic, planktonic food, and the LGR ecotype feeds primarily on benthic prey (Bodaly 1979). Preliminary analyses of 15 enzymatic loci revealed significant differences in allele frequency at one locus between sympatric ecotypes in each of the four lakes where they are found, thus suggesting that they represent genetically distinct populations (Bodaly et al. 1992).

Previous studies have suggested that these ecotypes all belong to the single Beringian whitefish race and therefore represent a phenotypic radiation independent from more eastern populations (Lindsey et al. 1970; Bodaly et al. 1992). This conclusion excluded a scenario of secondary contact between populations from distinct evolutionary lineages as an explanation for the origins of sympatric ecotypes. Recent analyses of mitochondrial DNA (mtDNA) variation have also supported the view that whitefish of more eastern races were not present among populations from southern Yukon (Ber-

TABLE 1. Sample location, sample size, gill-raker counts, and proportion of catch of sympatric high and low gill-raker whitefish ecotypes in Yukon.

Population	Sample size		Gill-raker number		Proportion of catch	
	mtDNA	Allozyme	Mean	Range	Surface	Bottom
Dezadeash						
HGR	20	30	32.6	(30–36)	0.97	0.03
LGR	18	30	23.2	(21–25)	0.42	0.58
Little Teslin						
HGR	24	30	30.5	(28–33)	0.98	0.02
LGR	24	30	25.6	(24–27)	0.05	0.95
Squanga						
HGR	29	30	29.1	(26–32)	0.61	0.39
LGR	31	30	23.4	(22–26)	0.24	0.76

natchez and Dodson 1991). Nevertheless, they provided evidence for the existence of two distinct evolutionary lineages in southern Yukon, characterized by two monophyletic assemblages of mtDNA genotypes diverging by a mean percent sequence divergence of 1.29% and differentiated by six synapomorphies. One of these assemblages is endemic to Beringia (Alaska-Yukon), whereas the other arose in Eurasia and subsequently recolonized North America (Bernatchez and Dodson 1994). Therefore, these results raise the possibility that ecotypes from southern Yukon could represent two distinct monophyletic assemblages and that their occurrence in sympatry could result from secondary contact between two evolutionary lineages that evolved in allopatry. This hypothesis would be supported by the grouping of populations of a given ecotype from different lakes into phylogenetically distinct mtDNA lineages. Alternatively, the hypothesis of multiple origins of ecotypic variation would be favored by the demonstration that populations of each ecotype do not form monophyletic assemblages. Furthermore, the finding that distinct ecotypes within lakes share uniquely derived genetic characters would support the hypothesis of incipient sympatric radiation.

In this paper, we address three questions regarding the evolution of sympatric whitefish ecotypes of the Yukon territory. First, is the genetic differentiation between sympatric LGR and HGR ecotypes suggested previously by preliminary analyses of allozyme variation supported by a more thorough study involving the analysis of mtDNA variation and additional nuclear loci performed on temporally different samples? Second, do sympatric ecotypes represent the product of monophyletic or polyphyletic evolutionary events? Third, if they are polyphyletic in their origin, did they originate from multiple allopatric/secondary contact events or incipient sympatric radiation within each lake?

MATERIALS AND METHODS

Sample Collection

Whitefish were collected in August 1992 from Little Teslin and Squanga L., found in the Squanga Creek drainage (Yukon R. watershed), and Dezadeash L., located in the Alsek R. drainage (Fig. 1, Table 1). Experimental gill nets were set off shore in 7 m to 15 m of water depth. For each station, one surface and one bottom net were tended. For each lake, fish were assigned to LGR or HGR ecotypes based on gill-raker counts already known to discriminate these groups (Bodaly 1979). Tissues were kept frozen at −20°C from several weeks (allozymes) to several months (mtDNA) before analysis.

MtDNA Analysis

Isolation and Restriction Enzyme Analysis.—All mtDNA purification, enzyme digests, electrophoresis, hybridization procedures, and genotype designation followed protocols described previously for coregonines (Bernatchez et al. 1988; Bernatchez and Dodson al. 1990b). This methodology allowed complete compatibility with our earlier data set. Four hexameric (*Hin*dIII, *Pvu*II, *Sma*I. and *Xmn*I), three multihexameric (*Ava*I, *B*anI,and *Hin*cII), and one multipentameric (*Ava*II) restriction enzymes were used. These correspond to all polymorphic enzymes reported among whitefish populations from Alaska and Yukon (Bernatchez and Dodson 1991).

Data Analysis

A comprehensive rooted phylogenetic tree has previously been produced by a parsimony analysis of whitefish mtDNA genotypes throughout the species range (Bernatchez and Dodson 1994). The present study did not add new phylogenetic information to this tree as new genotypes differed only by autapomorphic characters from thoses previously found. Consequently, phylogenetic relationships among new genotypes were assessed by incorporating these to the overall tree already built. This allowed to assign them to one of the five significant mtDNA phylogenetic groups known in whitefish (Bernatchez and Dodson 1994).

The genetic differentiation of whitefish ecotypes within each lake was evaluated from the analysis of frequency distribution of each individual genotype and of mtDNA phylogenetic groups using χ^2 randomization tests (Roff and Bentzen 1989) with 1000 randomizations using the MONTE program of the REAP software package (McElroy et al. 1992). The extent of gene flow between pairs of ecotypic forms was evaluated from F_{st} estimates (Wright 1978) by considering the mitochondrial genome as a unique locus with mtDNA genotypes corresponding to distinct alleles (Chapman 1989).

Relationships among all populations were assessed by computing the maximum-likelihood estimation of the average number of nucleotide substitutions per site between populations (d, nucleotide divergence, Nei 1987) using the program DA (REAP). The resulting pairwise matrix of net nucleotide divergence among populations was used to construct a phenogram by clustering the populations analyzed in this study as well as others from Beringia surveyed previously for mtDNA variation (Bernatchez and Dodson 1991).

Allozyme Analysis

The isozyme products of 38 loci were analyzed by horizontal starch-gel electrophoresis according to the methods detailed in Bodaly et al. (1991b). Description of alleles and their mobilities was also provided by Bodaly et al. (1991b).

TABLE 2. Enzymes, International Union of Biochemistry enzyme classification numbers (EC), abbreviations, number of loci screened, and tissues of expression for. Tissues: M, muscle; L, liver; E, eye.

Enzyme	EC No.	Abbreviation	Number of loci screened	Tissue
Alcohol dehydrogenase	1.1.1.1	ADH	1	M
Aspartate aminotransferase	2.6.1.1	mAAT	1	M
		sAAT	2	M
Creatine kinase	2.7.3.2	CK-A	2	M
		CK-B	1	E
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	2	M
		G3PDH	1	L
Glucose-6-phosphate isomerase	5.3.1.9	GPI-A	2	L
		GPI-B	2	M
L-Iditol dehydrogenase	1.1.1.14	IDDH	2	L
Isocitrate dehydrogenase	1.1.1.42	mIDHP	2	M
Lactate dehydrogenase	1.1.1.27	LDH-A	2	M
		LDH-B	2	L
		LDH-C	1	E
Malate dehydrogenase	1.1.1.37	mMDH	1	M
		sMDH-A	2	L
		sMDH-B	2	M
NADP+-dependent malic enzyme	1.1.1.40	mMEP	2	M
		sMEP	2	L
Phosphogluconate dehydrogenase		PGDH	1	M
Phosphoglucomutase	5.4.2.2	PGM	2	M
Superoxide dismutase	1.15.1.1	sSOD	1	L

TABLE 3. Composite definition, absolute frequency (*N*), and relative frequency distribution of ten mtDNA genotypes resolved among sympatric whitefish ecotypes in the Yukon. Genotypes are grouped according to phylogenetic assemblages resolved in Figure 1; 41 to 104, group II; genotypes 29–103, group III. Genotype numbering, and designation of fragment patterns are in continuation with those used previously (Bernatchez and Dodson 1991, 1994). Restriction enzymes are in order: *Ava*I, *Ava*II, *Ban*I, *Hinc*II, *Hind*III, *Pvu*II, *Sma*I, and *Xmn*I. Frequencies of individual genotypes for Squanga L. ecotypes do not sum up to the total because some individuals could not be successfully characterized for all restriction enzymes but could be classified to either group II or III.

Composite definition	N	Distribution: Dezadeash LGR	Dezadeash HGR	Little Teslin LGR	Little Teslin HGR	Squanga LGR	Squanga HGR
41 DIAAGAAC	10	—	—	—	0.29	0.10	0.06
42 EIAAGAAC	1	—	—	—	—	0.03	—
100 DIAQGAAC	2	—	—	—	0.10	—	—
101 DUAQGAAC	2	—	—	—	0.10	—	—
104 DLAAGAAC	8	—	—	—	0.18	0.10	0.06
Group II total	**29**	**0.00**	**0.00**	**0.00**	**0.67**	**0.23**	**0.17**
29 AHGCGBAA	87	0.15	1.00	0.92	0.33	0.74	0.88
31 AHKCGBAA	17	0.85	—	—	—	—	—
39 ALGCGBAA	1	—	—	—	—	0.03	—
102 QHGCGBAA	1	—	—	0.04	—	—	—
103 AHNCGBAA	1	—	—	0.04	—	—	0.00
Group III total	**113**	**1.00**	**1.00**	**1.00**	**0.33**	**0.77**	**0.83**

Genetic models followed Vuorinen and Piironen (1984). The enzymes examined, their abbreviations, International Union of Biochemistry enzyme classification numbers (EC), the loci that code for them, and the tissues used are listed in Table 2. Designation of isoenzyme loci and allelic nomenclature followed Shaklee et al. (1990).

Data Analysis

Allele frequencies were estimated by direct allele counts. Levels of genetic variation and differentiation within and among populations were characterized from estimates of direct and Hardy-Weinberg expected mean heterozygosities per locus (*H*), proportion of polymorphic loci (*P*, 99% criterion), mean number of alleles per locus (n_a), and unbiased genetic distance (Nei 1978). Tests of the fit of observed genotype frequencies within population with expected Hardy-Weinberg equilibrium were estimated by χ^2 tests with adjusted probability levels following Lessios (1992). The matrix of pairwise unbiased genetic distance estimates was used to construct a phenogram relating all sympatric populations analyzed as well as all others from Beringia surveyed previously (Bodaly et al. 1991b) using the unweighed pair-group method of arithmetic averages (UPGMA; Sneath and Sokal 1973). Confidence on tree topology was estimated by bootstrap resampling of allele-frequency matrices as suggested by Felsenstein (1993). Briefly, we used the SEQBOOT program of the PHYLIP package (version 3.5, Felsenstein [1993]) to generate 100 bootstraped matrices of the allele frequency data. Nei's genetic distance was calculating for each matrix using the program GENDIST and 100 UPGMA trees were constructed from bootstraped distance matrices using the program NEIGHBOR (option UPGMA). A majority-rule consensus tree for the 100 UPGMA trees was built using the program CONSENSE. Significance of differences in allele frequencies among samples was estimated by χ^2 test of homogeneity. We also used randomizations χ^2 procedures with 1000 permutations to test whether significant allelic differences observed may be due to stochastic effect of multiple comparison of allele frequency differences.

RESULTS

Ecotypic Variation

Within each lake, whitefish captured exhibited bimodal gill-raker counts (Table 1). These were similar to those previously observed over the past 30 yr (Lindsey 1963, Bodaly 1979). As in previous studies, whitefish classified as HGR and LGR ecotypes also showed a differential pattern of distribution in the water column (Table 1). In each lake and for the same sampling station, the HGR ecotype was always more abundant in the surface net whereas the opposite pattern was observed for the LGR ecotype.

mtDNA Diversity and Phylogenetic Differentiation

The eight restriction enzymes used revealed a total of 10 mtDNA genotypes (Table 3). Five of these (29, 31, 39, 41, and 42) were found previously (Bernatchez and Dodson 1991), whereas genotypes 100 to 104 were new. All of these belonged to mtDNA phylogenetic groups already identified in whitefish (Fig. 2). Genotypes 41, 42, 100, 101, and 104 belonged to group II, endemic to Beringia; and genotypes 29, 31, 39, 102, and 103 belonged to group III, originating from Eurasia (Bernatchez and Dodson 1994). No genotypes representative of other whitefish races described in Bernatchez and Dodson (1991, 1994) were found.

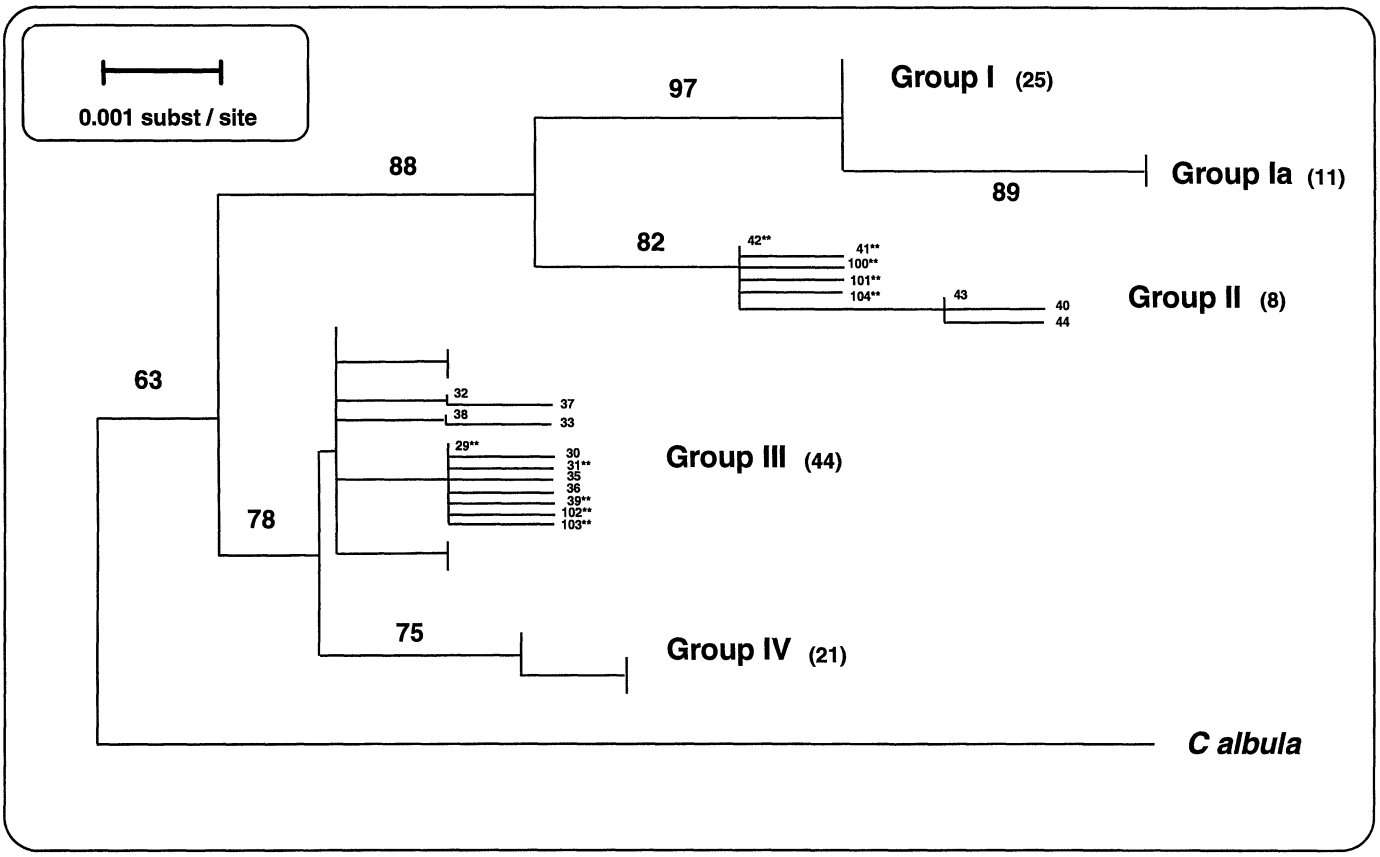

FIG. 2. Condensed majority-rule consensus tree (rooted with *C. albula*) clustering 104 mtDNA genotypes observed among whitefish populations throughout their circumpolar range of distribution. Numbers in brackets refer to the total number of genotypes observed within each phylogenetic group thus far. Branches are detailed in Bernatchez and Dodson (1994). Numbers at the tip of branches refer to all genotypes observed in Beringia (Alaska, Yukon), and those with asterisks were observed in the present study. Bootstrap estimates are given along branches. The scale of 0.001 substitution/site corresponds to one restriction site difference.

Nuclear Gene Diversity

A total of 38 loci, corresponding to 13 enzymatic systems were examined (Table 2). Variation was detected at 10 loci (Table 4). Five of these (*sAAT-2**, *G3PDH-3**, *GPI-B2**, *LDH-A2**, and *PGM-2**) exhibited polymorphisms not documented before among those populations. All samples were in Hardy-Weinberg equilibrium, with the exceptions of Squanga L. HGR that deviated significantly ($P < 0.05$) from equilibrium at *sMDH-B2**. The average observed heterozygosities ranged between 0.026 and 0.043 in Squanga and Little Teslin L. and between 0.060 and 0.066 in Dezadeash L. All samples were genetically characteristic of whitefish from Beringian origin as revealed by the presence of distinctive alleles of that race in all of them, and the complete absence of alleles characteristic of other races (Nahanni or Mississipian), as detailed in Bodaly et al. (1992).

Genetic Differentiation among Sympatric Ecotypes

mtDNA Differentiation.—Evidence for reproductive isolation between ecotypes was provided by differential frequency distribution of genotypes in two of the three lakes, refuting the hypothesis of phenotypic polymorphism within a single gene pool. (Tables 3, 5). In Dezadeash L., all whitefish belonged to phylogenetic group III. HGR ecotype was fixed for genotype 29, which was the most abundant in all lakes surveyed while 85% of LGR ecotype possessed genotype 31, derived from 29 by a single apomorphy and not observed elsewhere. Evidence for highly restricted mitochondrial gene flow was also indicated by an F_{st} estimate of 0.736.

Seven genotypes representing both phylogenetic groups II and III were present in Little Teslin L. Reproductive isolation between both ecotypes was indicated by a highly significant difference in the frequency distribution of these genotypes and a high F_{st} estimate. Ecotypes were strongly associated with different mtDNA phylogenetic groupings. Thus, all four genotypes belonging to group II were observed only in the HGR ecotype and characterized 67% of these fish (Table 3). In contrast, the LGR ecotype was fixed for phylogenetic group III. A randomization test of the null hypothesis that mtDNA genotypes representing phylogenetic groupings II and III should be evenly distributed between ecotypes indicated a very low probability that stochastic lineage extinction could explain the association between mtDNA of distinct phylogenetic groups and ecotypic forms ($\chi^2 = 4.18$, df = 1, $P < 0.001$).

Both phylogenetic groups II and III were also represented

TABLE 4. Allele-frequency distribution, average observed and expected heterozygosity (H), proportion of polymorphic loci ($P_{0.99}$), and mean number of alleles per locus (n_a) calculated for ten polymorphic loci among sympatric ecotypes of the Yukon.

		Squanga		Little Teslin		Dezadeash	
Locus	Allele	LGR	HGR	LGR	HGR	LGR	HGR
*sAAT-2**	100	1.00	0.98	1.00	1.00	1.00	1.00
	125	—	0.02	—	—	—	—
*G3PDH-1**	0	0.37	0.38	0.22	0.29	0.42	0.50
	210	0.63	0.62	0.78	0.71	0.58	0.50
*G3PDH-2**	100	0.90	0.85	0.87	0.77	0.95	0.98
	50	0.10	0.15	0.13	0.23	0.05	0.02
*G3PDH-3**	100	0.98	1.00	1.00	1.00	0.67	0.92
	65	0.02	—	—	—	0.33	0.08
*GPI-B2**	100	0.95	0.98	1.00	1.00	0.85	0.70
	50	0.05	0.02	—	—	0.15	0.30
*sIDHP-3**	100	0.98	0.97	0.88	0.81	0.97	0.97
	65	0.02	0.03	0.12	0.19	0.03	0.03
*LDH-A2**	100	1.00	0.97	1.00	0.98	1.00	1.00
	−50	—	0.03	—	0.02	—	—
*sMDH-A2**	100	0.75	0.77	1.00	0.97	0.32	0.38
	50	0.25	0.23	—	0.03	0.68	0.62
*mMDH**	100	1.00	1.00	1.00	1.00	0.95	0.98
	60	—	—	—	—	0.05	0.02
*PGM-2**	−100	0.93	0.80	0.87	0.68	0.73	0.87
	−155	0.07	0.20	0.13	0.32	0.27	0.13
	Hobs	0.033	0.040	0.026	0.043	0.066	0.060
	Hexp	0.035	0.043	0.027	0.043	0.066	0.058
	$P_{0.99}$	0.184	0.211	0.105	0.158	0.211	0.211
	n_a	1.2	1.2	1.1	1.2	1.2	1.2

in Squanga L. However, no significant difference in frequency distribution of genotypes was observed. Similarly, low F_{st} estimate indicated more extensive mitochondrial gene flow between ecotypes in Squanga L. than in the other two lakes. Although mtDNA variation did not provide evidence for genetic differentiation in Squanga L., significant differences in the frequency distribution of nuclear alleles was observed between forms.

Nuclear Gene Differentiation

Evidence for restricted gene flow between sympatric ecotypes was provided by differential allelic distribution for the three lakes. In Dezadeash L., the ecotypes differed significantly at *G3PDH-3** ($P = 0.007$) and *GPI-B2** ($P = 0.049$), whereas ecotypes from Squanga and Little Teslin L. differed at *PGM-2** ($P = 0.032$ and 0.013, respectively). Randomization χ^2 tests also supported the significance of these differences as randomized χ^2 values exceeded the observed values in 0/1000, 50/1000, 8/1000, and 4/1000 permutations, respectively. Allelic distribution between ecotypes was very similar at other loci, and consequently, overall F_{st} estimates and genetic distances between ecotypes were low within the three lakes (Table 5, Fig. 4).

Polyphyletic Origins of Whitefish Ecotypes

Mitochondrial and nuclear gene variation did not support the hypothesis of a monophyletic origin for HGR and LGR ecotypes. Thus, no diagnostic mtDNA or nuclear alleles were found to characterize each ecotype, and there was no evidence of more similar allele frequencies within them (Table 3–4). Consequently, ecotypes did not form distinct genetic clusters as evidenced by the mtDNA and allozyme UPGMA phenograms (Figs. 3–4).

In the mtDNA phenogram, populations representing HGR and LGR ecotypes clustered separately, reflecting their independent origins (Fig. 3). For instance, Little Teslin HGR ecotype, which was characterized by the dominance of phylogenetic group II genotypes, was very different from Dezadeash HGR, which was fixed for group III (genotype 29). In contrast, Dezadeash HGR and Little Teslin LGR could not be differentiated, both being dominated by genotype 29 (group III). Dezadeash LGR was very distinct from any other sympatric ecotypes, being almost fixed for genotype 31, observed nowhere else. Squanga L. ecotypes clustered more closely to each other than to any other populations.

In the allozyme phenogram, first population clustering occurred according to lakes of origin rather than to ecotypic differentiation (Fig. 4). Thus, in all cases, HGR and LGR ecotypes from a given lake clustered more closely to each other than to any other populations. This association was supported in 65% to 76% of the bootstrap replicates, depending on the lakes. Dezadeash L. populations clustered distinctively in 50% of the bootstrap replicates, namely as a result of differences at an *MDH* locus: allele *sMDH-A2** 50 dominated in Dezadeash L. but was almost absent in Little Teslin L. and found only at low frequency in Squanga L. (Table 4) and other Beringian populations that were surveyed in previous studies (Bodaly et al. 1992). Except for the sharp differentiation between Beringian and other North American populations that was supported in 100% of the bootstrap replicates, the remaining branching patterns of the UPGMA tree were not significant under the 50% criterion of the majority-rule consensus approach.

TABLE 5. Within-lake genetic differentiation between sympatric whitefish ecotypes based on χ^2 analysis and F_{st} estimates performed on mtDNA and allozyme data. χ^2 values for allozymes in Dezadeash L: (a) *G3PDH-3**, (b) *GPI-B2**; for Little Teslin L. and Squanga L.: *PGM-2**. F_{st} estimates represent the mean over all polymorphic loci observed for each lake and the range of values is given in brackets. Asterisks indicate probability of homogeneity: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Dezadeash		Little Teslin		Squanga	
Markers	χ^2	F_{st}	χ^2	F_{st}	χ^2	F_{st}
mtDNA	27.68***	0.736	23.66***	0.159	1.20	0.082
Allozymes	11.37**$_a$	0.020	6.18**	0.013	4.61*	0.010
	2.98*$_b$	(0.003–0.050)		(0.006–0.018)		(0.0005–0.015)

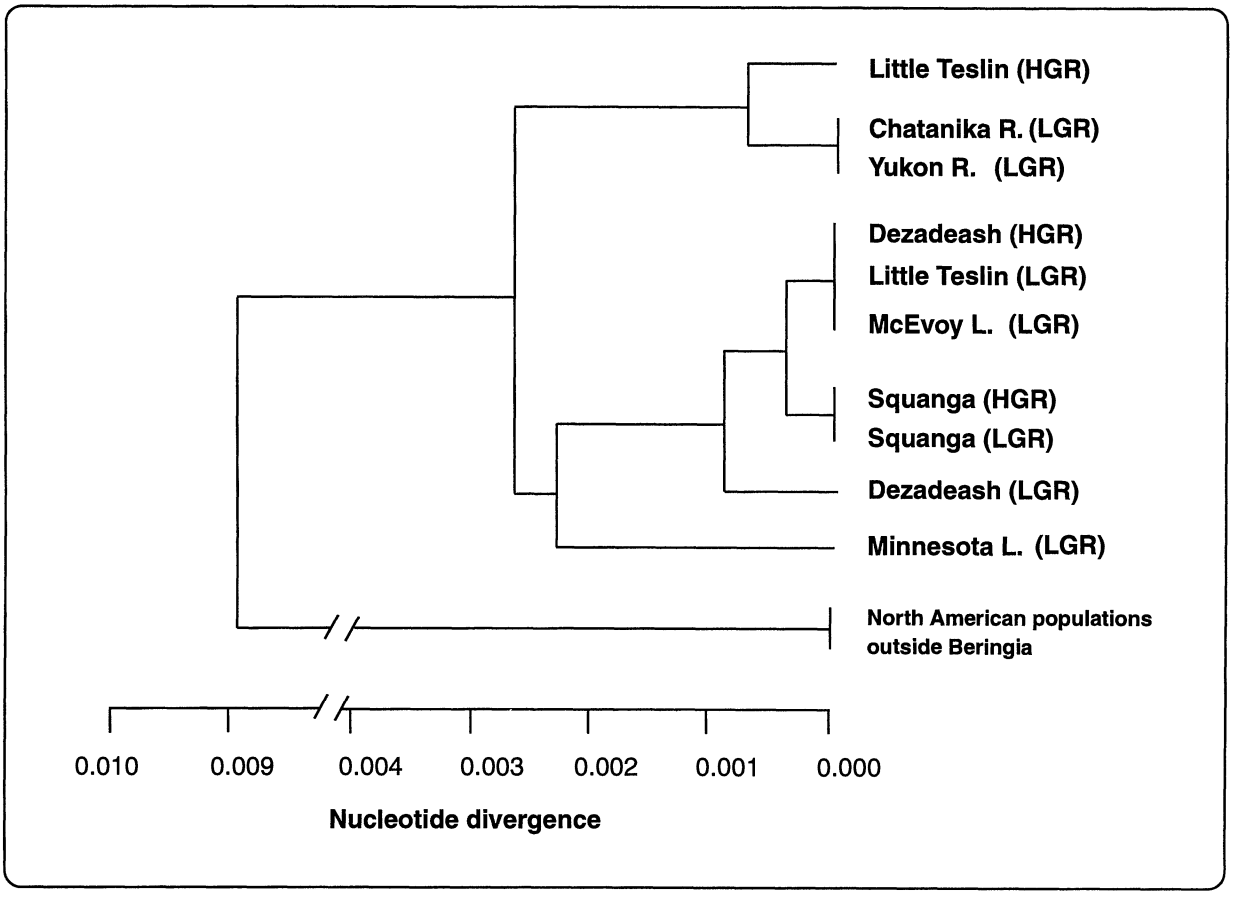

FIG. 3. UPGMA phenogram clustering sympatric whitefish ecotypes from the Yukon, with all other Beringian whitefish populations that were previously characterized (Bernatchez and Dodson 1991), according to the distance matrix resulting from the maximum-likelihood estimation of the net average number of nucleotide substitutions per site between populations. Details of the branches relating North American populations outside Beringia are given in Bernatchez and Dodson (1994).

DISCUSSION

Reproductive Isolation between Sympatric Ecotypes

This study reinforced earlier evidence based on morphological differentiation, spawning habits, trophic niche partitioning, and preliminary genetic analysis that whitefish sympatric ecotypes from Dezadeash, Little Teslin, and Squanga L. represent reproductively distinct populations (Bodaly 1979; Bodaly et al. 1988, 1992). Thus, our results revealed highly significant differences in mtDNA genotype distribution for Little Teslin and Dezadeash L. Significant differences in allele frequency between ecotypes of Dezadeash L. was also found at the *G3-PDH-3** and *GPI-B2** loci that had not been screened in Bodaly et al. (1992). Similarly, highly significant differences in allele frequency were found at the *PGM-2** locus in Little Teslin L. The weakest support for reproductive isolation between ecotypes was observed in Squanga L., which exhibited significant but mild genetic differences at *PGM-2** only. Nevertheless, these sympatric ecotypes strongly segregate for different spawning habitats that argues in favor of their partial reproductive isolation (Bodaly et al. 1988).

Differences were observed between the present study and previous allozyme studies of these populations. Namely, Bodaly et al. (1992) reported significant differences at the locus *G3PDH-1** between Squanga L. ecotypes whereas we observed an almost identical allele frequency. This may reflect temporal allelic fluctuations between samples that were collected 17 to 18 yr apart. Such variation could potentially be related to either drift or changes in the amount of gene flow between ecotypes. It is also possible that this difference is related to stochastic effects of sampling error. Another discrepancy was observed at the *sIDHP-3** locus for which three alleles showing significant frequency differences between all three sympatric pairs were observed in Bodaly et al. (1992), whereas only two alleles and no significant variation was found in the present study. This is most likely related to different electrophoretic methodologies used in both studies. Alleles "*a*" and "*d*" of Bodaly et al. (1992) could not be discriminated in the present study and collapsed into a single "100" allele. In such a case, reproductive isolation between sympatric ecotypes may be more important than we reported. However, it is not possible at present to refute the possibility that one of the alleles scored previously was not real.

Our results indicate that the amount of gene flow between ecotypes differed among lakes. The gene identity analysis of both mtDNA and nuclear genes revealed that gene flow was

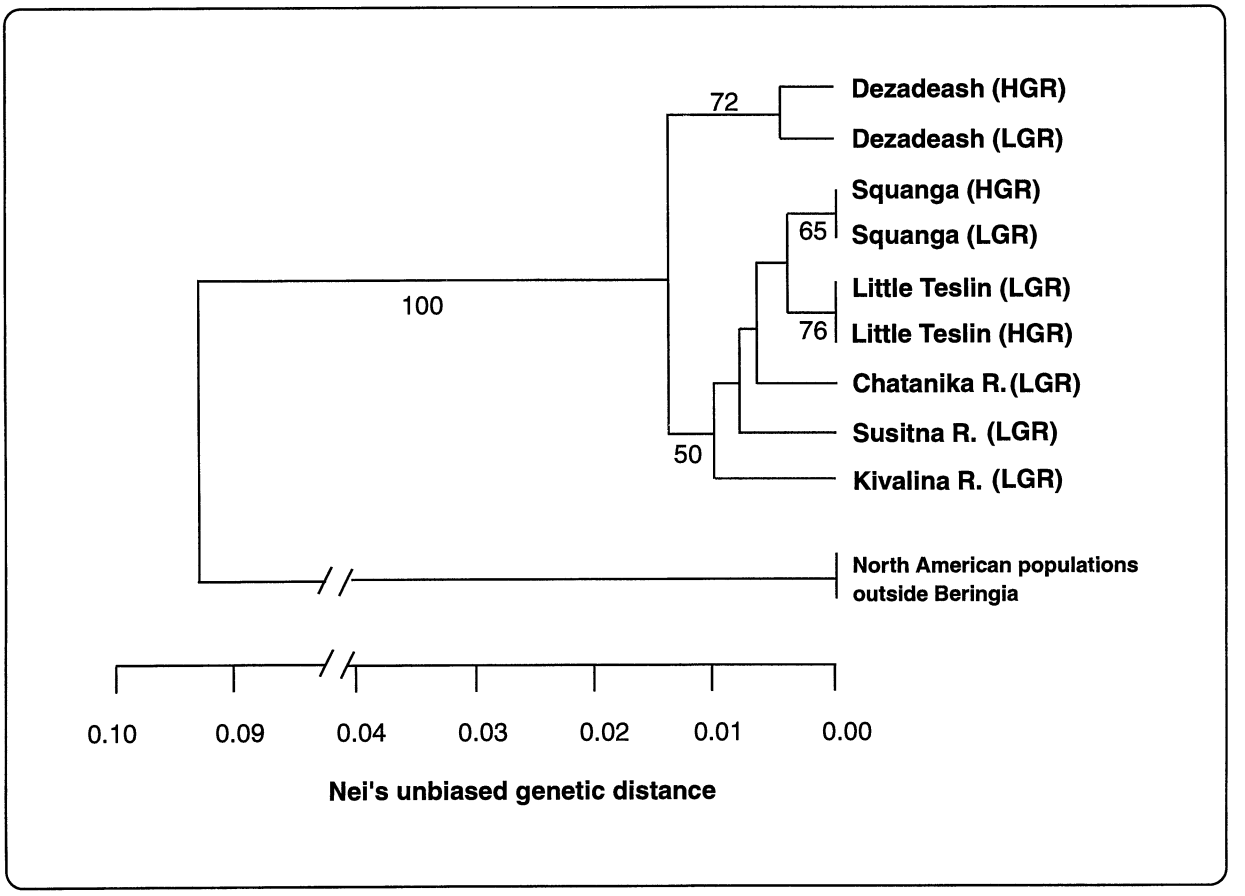

FIG. 4. Phenogram showing genetic relationships among sympatric ecotypes and other available whitefish populations from Beringia based on a UPGMA cluster analysis of Nei's pairwise unbiased genetic distance matrix. Bootstrap estimates exceeding the 50% majority-rule criterion are given below branches. Locations of allopatric Beringian populations and details of the branches relating North American populations outside Beringia are given in Bodaly et al. (1991).

most restricted in Dezadeash L., intermediate in Little Teslin, and more extensive in Squanga L. In general, mitochondrial gene flow was more restricted than nuclear gene flow. This corroborates theoretical predictions and empirical evidence that at equilibrium, mitochondrial gene flow should be more restricted because of its smaller effective population size resulting from haploidy and maternal transmission (Takahata and Slatkin 1984; Chapman 1989).

Morphological differentiation associated with trophic ecology between whitefish ecotypes may be partly governed by the extent of premating reproductive isolation. Patterns of mtDNA and isozyme differentiation correlate with morphological variation, best expressed by gill-raker counts. Bodaly (1979) found no overlap in gill-raker counts between ecotypes in Dezadeash L., almost none in Little Teslin L, whereas it was substantial in Squanga L. The same tendency was also observed in the 1992 sampling. Similarly, the patterns of genetic and morphological differentiation reflect the potential for premating reproductive isolation, as illustrated by behavioral preference in time and place of spawning. Spawning segregation is developed in all lakes, although to a variable extent (Bodaly 1977; Bodaly et al. 1988). In Squanga L., ecotypes spawn at different sites but at similar times. In Little Teslin L., spawning locations are not known, but differences in sexual maturity suggested only partial overlap in the spawning season, whereas the same criteria provided sound evidence for nonoverlapping spawning times for both ecotypes in Dezadeash L. Consequently, the number of morphological hybrids between ecotypes was the highest in Squanga L., intermediate in Little Teslin L., and lowest in Dezadeash L. (Bodaly 1977).

Differences in levels of premating reproductive isolation may be the consequence of the intensity of local natural selection, either directional or disruptive, that generated and is maintaining ecotypic adaptive divergence in each lake (Smith and Todd 1984). Directional selection would be favored in a traditional model of allopatric speciation whereby the extent of reproductive isolation reinforcement and character displacement would vary among lakes as a consequence of differential potential for competition among recolonizing populations. In contrast, disruptive selection would be invoked if variation in differentiation of ecotypes among lakes reflects the local potential for adaptive diversification from a single founding population. In the latter case, reproductive isolation may be achieved as a result of "niche-adapting" alleles that govern mate choice (Bush 1975; Rosenweig 1978; Rice 1984; Wilson 1989). This implies that mate preference must be genetically correlated with traits under disruptive selection,

for instance, place and times of reproduction (Bush 1994). A first step toward the understanding of the role of selection in promoting reproductive isolation and organismal diversification in these populations implies the elucidation of their origins.

Multiple Origins of Sympatric Whitefish Ecotypes

Both mtDNA and nuclear gene analyses refuted the hypothesis of monophyletic origin of LGR and HGR ecotypes. This would imply that different ecotypes arose in one place and subsequently immigrated into the lakes where they are found today. A corollary of this hypothesis is that populations of each ecotype should share unique apomorphies, or at least show distinct allele frequencies at some loci, which was not the case in this study (Tables 3–4). Rather, these results support the polyphyletic origins of ecotypes whereby each has been derived independently more than once.

The case of sympatric whitefish ecotypes from the Yukon adds to the growing evidence of the relative commonness of replicate expression of adaptive phenotypes among northern freshwater fishes. Thus, replicate ecotypic diversification has also been reported among European whitefish (*Coregonus lavaretus*) populations adapted to benthic and pelagic trophic niches (Bernatchez and Dodson 1994; Bernatchez 1995). Among eastern populations of lake whitefish (*C. clupeaformis*), a dwarf phenotype associated with a pelagic mode of life has been expressed independently in different races (Bernatchez and Dodson 1990a; Vuorinen et al. 1993). Similarly, Taylor and Benzten (1993a,b) provided sound evidence for multiple origins of trophic ecotypes in the rainbow smelt (*Osmerus mordax*). Parallel expression of life-history traits adapted to distinct habitats has also been reported in Arctic charr, *Salvelinus alpinus* (Hindar et al. 1986), threespine stickleback, *Gasterosteus aculeatus* (McPhail 1993), sockeye salmon, *Oncorhynchus nerka* (Foote et al. 1989), Atlantic salmon, *Salmo salar* (Ståhl 1987), and brown trout, *S. trutta* (Bernatchez et al. 1992; Bernatchez and Osinov (1995). Where it has been found, parallel expression of life-history traits in freshwater fishes has most often been interpreted as evidence for the potential of rapid, parallel evolution, which may reflect population response to local selection in the face of similar ecological opportunities.

Modes of Ecotypic Differentiation

Our working hypothesis was that the existence of sympatric whitefish ecotypes in the Yukon resulted from the secondary contact of two monophyletic groups of whitefish, best identified by mtDNA phylogenetic groupings II and III, that evolved in allopatry during the last glaciation events. Sound evidence for this scenario was found in Little Teslin L. of the Squanga Creek drainage. Thus, both mtDNA groups II and III were present in this lake, where they strongly segregated between ecotypes. Under the allopatric scenario, the two whitefish groups recolonized the Squanga Creek drainage following the Wisconsinian glacial retreat that occurred approximately 10,000 yr ago. Recolonization of the Squanga Creek drainage was possible from the Yukon R. basin until the development of waterfalls subsequently isolated the upper Squanga Creek drainage (Lindsey 1975). Because the HGR phenotype is very rare among lake whitefish, being reported in only four lakes, it is more likely that both recolonizing whitefish groups possessed the LGR phenotype that characterizes whitefish populations throughout North America. This assumption is also supported by the fact that other extant Beringian populations characterized by the same mtDNA genotypes are of LGR ecotype (Bernatchez and Dodson 1991). In Little Teslin L., reinforcement of premating reproductive isolation may have been such that gene flow remained restricted over time, maintaining sharper genetic and phenotypic differentiation. In Squanga L. populations, opportunities for character displacement and niche partitioning between founding populations originally characterized by mtDNA groups II and III may have been more limited, which reduced the potential for reinforcement of reproductive isolation and resulted in more important introgressive hybridization, as suggested by the complete admixture or mtDNA groups II and III, and by less phenotypic differentiation. This pattern of differences in mtDNA genetic flow among sympatric populations from different lakes is analogous to the situation observed in the contact zone between the Acadian and Atlantic races of lake whitefish in the Allegash basin (Bernatchez and Dodson 1990a). In this region, alternate fixation of distinct mtDNA groups was found in one lake, whereas variable levels of admixture were observed in others.

An alternative model to the allopatric hypothesis is that one founding population possessing both groups II and III mtDNA genotypes colonized the Squanga Creek drainage. Stochastic lineage sorting toward segregation of both mtDNA groups then occurred in Little Teslin L. following sympatric differentiation of the two ecotypes, whereas this has not been the case in Squanga L. Although plausible, this second scenario appears very unlikely, as indicated by the low probability that segregation of phylogenetically related mtDNA genotypes between ecotypes occurred as a result of stochastic lineage sorting. Also, the allopatric origins of mtDNA phylogenetic groups II and III have been clearly demonstrated by their phylogeographic structure, implying the existence of at least two founding populations in the Squanga Creek drainage (Bernatchez and Dodson 1994). Consequently, we conclude that an allopatric scenario implying secondary contact between two groups of whitefish, one endemic to Beringia, the other originating from Eurasia, best explains the existence of sympatric ecotypes in the Squanga Creek drainage. In such a case, lake differences in directional selection promoting character displacement and reinforcement of premating isolation would be favored as an explanation for genetic and morphological differences observed between sympatric ecotypes of Little Teslin and Squanga L.

Because this and previous studies have not provided diagnostic nuclear alleles discriminating whitefish belonging to mtDNA groups II and III, the test of allopatric origin could not be paralleled from allozyme data. Nevertheless, conclusions based on mtDNA analysis may appear to contradict the pattern of nuclear gene variation. Indeed, ecotypes within each lake showed close nuclear genetic similarity, clustering more closely to each other than to any other populations. This could be interpreted as evidence for intralacustrine radiation. However, genetic similarity between

ecotypes is based solely on patterns of frequency distribution of the same alleles found in other whitefish populations in Beringia. No diagnostic alleles were found to characterize ecotypes from each lake. Without such evidence of intralacustrine monophyly, sympatric speciation cannot be strictly claimed (Smith and Todd 1984). In contrast, more similarities in allele frequency between populations within lake is to be expected if historical and/or contemporary gene flow has been more important within than among lakes. Clearly, this is a strong possibility that is also reflected by low within-lake F_{st} estimates observed both within Little Teslin and Squanga L.

Our results indicate that the presence of sympatric ecotypes in Dezadeash L. of the Alsek R. drainage cannot be explained by the same scenario as that for Squanga Creek drainage. Thus, all specimens from Dezadeash L. belonged to the same mtDNA group III, which makes unlikely the possibility of secondary contact between groups II and III in this lake, although the possibility of complete stochastic lineage extinction for group II cannot be ruled out. However, not a single whitefish possessing mtDNA group II has been found so far outside the Yukon R. drainage (which includes the Squanga Creek drainage), suggesting that the group may be endemic to this watershed and has not invaded other drainages (Bernatchez and Dodson 1994). Additional support in favor of a distinct evolutionary history of origins for ecotypes from Dezadeash L. with those from Little Teslin and Squanga L. was provided by differences in allele frequencies at some loci in the present and previous studies (Bodaly et al. 1992), distant clustering in the population UPGMA phenogram, and the presence of a distinct protein band revealed by isoelectric focusing in an earlier study (Bodaly et al. 1988).

Although our data argue in favor of a different origin of Dezadeash L. sympatric populations from the Squanga Creek drainage, it is not sufficient to refute either sympatric or allopatric (or microallopatric) origin of whitefish ecotypes in Dezadeash L. Support of an allopatric origin/secondary contact hypothesis would be provided by the demonstration of an allopatric distribution outside Dezadeash L. of genotypes 29 and 31 that respectively characterized the HGR and LGR ecotypes. Genotype 29 has been observed among most whitefish populations in Beringia. This indicates that genotype 29 is associated with an ancestral whitefish group that dispersed extensively in postglacial times. Ample possibilities of dispersal for this group was provided at times of last glacial retreats by ancestral connections of the Alsek R. (which was ice covered during the Wisconsinian glaciation) with the Yukon R. basin, and the development of glacial lakes, such as Lake Champagne, that created a direct contact between the Alsek R. and Squanga Creek drainage (Lindsey 1975). In contrast, genotype 31 has only been observed in the Alsek R. drainage. It is plausible that this genotype is representative of a whitefish group that became geographically isolated in postglacial times until the double invasion of Dezadeash L. by this group and the one associated with genotype 29. The empirical test of this hypothesis must await additional sampling of lakes in the Alsek R. drainage to detect genotype 31 outside Dezadeash L.

Alternatively, it is possible that genotype 31 diverged from genotype 29 following postglacial sympatric differentiation of both ecotypes within this lake. This remains a plausible hypothesis as genotype 31 was derived from 29 by a single mutational event given the level of resolution of this study (which involved screening of 450 bp on average per individual). Empirical support for sympatric speciation event in Dezadeash L. is also provided by nuclear gene variation at locus *sMDH-A2** for which both ecotypes showed a very similar allele frequency that differed strikingly from all other whitefish populations. However, a clear case of sympatric divergence must await the findings of diagnostic alleles unique to Dezadeash whitefish populations which will require the use of finer resolution genetic techniques.

Regardless of the speciation process involved in their origins, sympatric whitefish populations from the Yukon add to the evidence that Pleistocene glaciations created conditions favoring rapid phenotypic differentiation among northern freshwater fishes, in the absence of large genetic divergence. The fact that they represent different stages of genetic and phenotypic differentiation makes them a unique system to better understand the role of evolutionary forces in the early stages of speciation processes.

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