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CORRELATED TROPHIC SPECIALIZATION AND GENETIC DIVERGENCE IN SYMPATRIC LAKE WHITEFISH ECOTYPES (*COREGONUS CLUPEAFORMIS*): SUPPORT FOR THE ECOLOGICAL SPECIATION HYPOTHESIS

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Abstract.—There is ample empirical evidence that phenotypic diversification in an adaptive radiation is the outcome of divergent natural selection related to differential resource use. In contrast, the role of ecological forces in favoring and maintaining reproductive isolation in nature remains poorly understood. If the same forces driving phenotypic divergence are also responsible for speciation, one would predict a correlation between the extent of trophic specialization (reflecting variable intensity of divergent natural selection) and that of reproductive isolation being reached in a given environment. We tested this hypothesis by comparing the extent of morphological and genetic differentiation between sympatric dwarf and normal whitefish ecotypes (*Coregonus* sp.) from six lakes of the St. John River basin (eastern Canada and northern Maine). Eight meristic variables, 19 morphometric variables, and six microsatellite loci were used to quantify morphological and genetic differentiation, respectively. Dwarf and normal ecotypes in each lake differed primarily by traits related to trophic specialization, but the extent of differentiation varied among lakes. Significant but variable genetic divergence between ecotypes within lakes was also observed. A negative correlation was observed between the extent of gene flow between ecotypes within a lake and that of their morphological differentiation in trophic-related traits. The extent of reproductive isolation reached between dwarf and normal whitefish ecotypes appears to be driven by the potential for occupying distinct trophic niches and, thus, by the same selective forces driving tropic specialization in each lake. These results therefore support the hypothesis of ecological speciation.

Key words.—*Coregonus*, ecotypes, *Gasterosteus*, microsatellite, morphology, reproductive isolation, speciation, sympatry.

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How environments cause new species to form remains a fundamental problem in the search for the origin of diversity (Schluter 1998). Under the ecological theory of adaptive radiation, resource-based divergent natural selection is the ultimate cause of diversification (Dobzhansky 1937; Huxley 1942; Mayr 1942). Two major processes are inferred under this theory. The first postulates that phenotypic divergence of populations and species is driven by differences in the resource and competitive environment they experience. The second, coined "ecological speciation" by Schluter (1996a), infers that reproductive isolation evolves as a consequence of the same forces causing phenotypic and ecological divergence. A major argument for the role of ecologically divergent selection in speciation is the evidence for a fitness cost of producing hybrids of intermediate phenotype with reduced efficiency for resource exploitation relative to parental species, thus acting as a postmating isolation mechanism (Rice and Hostert 1993; Schluter 1993). In such a case, the theory of adaptive radiation predicts that selection will favor the development of mechanisms, either favoring mating among members of a given population or limiting reproduction between populations to reduce the probability of producing hybrids (Dobzhansky 1951). There is now ample empirical evidence that phenotypic diversification in an adaptive radiation is the outcome of divergent natural selection related to differential resource use (e.g., Grant and Grant 1989, 1996; Smith 1990; Schluter 1996b; Smith and Skúlason 1996). Despite recent theoretical and empirical evidence in favor of ecological speciation (e.g. Rice and Hostert 1993; Bush 1994; Liou and Price 1994; Noor 1995; Hostert 1997), however, the role of ecological forces in favoring and maintaining reproductive isolation in nature remains poorly understood (Schluter 1998).

Members of the northern temperate freshwater fish fauna share several attributes that make them of particular interest for the study of ecological speciation. Those comprise young populations arising via recolonization following the last glacier retreats, about 15,000 years ago. Polymorphism of sympatric ecotypes occurs frequently in salmonids, but exists as well in phylogenetically remote families, including Gasterosteidae, Osmeridae, Centrarchidae, and Catostomidae (reviewed in Robinson and Wilson 1994). These fishes reveal similarities in patterns of morphological, behavioral and life-history variation associated with specialization of different forms for exploiting distinct trophic niches (Schluter and McPhail 1993). This suggests an important role of divergent natural selection in driving diversification of these forms, possibly facilitated by ecological opportunities and relaxation of interspecific competition originally found in newly colonized habitats (Skúlason and Smith 1995). Genetic analyses generally provide evidence for restricted, yet variable, extent of genetic exchange among sympatric forms of a given group (Taylor and Bentzen 1993; Hindar 1994; McVeigh et al. 1995; Volpe and Ferguson 1996; Taylor et al. 1997; Thompson et al. 1997). This also suggests that evolutionary forces are promoting reproductive isolation in the face of gene flow, but that the process of speciation has not been completely achieved in many cases.

Three lines of evidence, largely derived from studies on stickleback *Gasterosteus* sp., support the hypothesis that the process of ecological speciation operates in sympatric fish species-pairs. First, experimental studies in laboratory and

natural conditions show that natural selection against hybrids of benthic and limnetic species of sticklebacks has an ecological basis (Schluter 1993, 1995; Hatfield and Schluter 1996; Foster et al. 1998). Second, premating isolation between these species partly depends on traits under divergent natural selection favoring distinct resource use (Nagel and Schluter 1998). Third, such premating isolation mechanisms evolved in parallel in independently evolved lineages (Taylor et al. 1997).

Beside these recent advances, no tests of ecological speciation have been performed in other fish species-pairs, partly because of the logistical constraints of performing experimental studies in natural conditions (see Schluter 1998; Bernatchez et al. 1999). A possible alternative is to compare the strength of reproductive isolation among species-pairs that evolved within the same time frame but that differ in the extent of trophic specialization, under the hypothesis that differentiation in traits involved in niche occupation reflects the intensity of divergent natural selection in different environments. The amount of gene flow occurring between sympatric populations should decrease as the strength of their reproductive isolation increases. Thus, the extent of genetic divergence at neutral loci can be used as a surrogate for their reproductive isolation. If ecological speciation is important in driving the reproductive isolation of species-pairs, one would then predict that gene flow should be more restricted between sympatric populations that are more specialized for distinct trophic niches. This prediction has never been systematically assessed in nature.

Sympatric dwarf and normal ecotypes of lake whitefish (*Coregonus clupeaformis* Mitchill) are found in several small lakes of the St. John River drainage in northern Maine, and southeastern Québec, Canada (Fenderson 1964; Chouinard et al. 1996). Dwarf individuals mature by the age of one or two years and seldom live beyond their fourth year, whereas the normal form does not generally mature until four years of age and may reach 12 years. In fact, a strong bimodal distribution in sizes of sexually maturing fish is the first indication for the existence of sympatric whitefish forms (Chouinard et al. 1996). Dwarf fish seldom exceed 20 cm in length and 100 g in weight while the normal fish commonly exceed 40 cm and 1000 g.

A phylogeographic study of this whitefish species complex based on mitochondrial DNA (mtDNA) variation and more recent microsatellite data revealed that the existence of sympatric dwarf and normal ecotypes in the St. John River drainage resulted from separate postglacial invasions by previously allopatric ancestors approximately 12,000 years ago (Bernatchez and Dodson 1990, 1991; Pigeon et al. 1997; Bernatchez et al. 1999). Because all known allopatric populations of whitefish outside the St. John River drainage possess the normal ecotype, it is most likely that populations of different races that colonized each lake were more adapted to a benthic mode of life and that the dwarf phenotype evolved in each lake by character displacement following secondary contacts (Pigeon et al. 1997; Bernatchez et al. 1999). Thus, the origin of sympatric populations in each lake is allopatric, but their morphological divergence is sympatric.

A recent study of ecological divergence between dwarf and normal ecotypes of two lakes (East Lake and Cliff Lake) from this system revealed that the extent of morphological differentiation (other than adult size) between ecotypes varied between lakes, and that this mainly involved traits related to trophic use (Bernatchez et al. 1999). More pronounced morphological specialization of both ecotypes in Cliff Lake translated into stronger trophic niche partitioning during periods of food depletion than in East Lake, with dwarf and normal fish mainly feeding on planktonic and epibenthic prey, respectively. These results provided evidence of a functional link between morphology and trophic use, and led Bernatchez et al. (1999) to hypothesize that the persistence of ecological opportunity for differential resource use throughout their ontogeny may be the selective force promoting the extent of specialization reached by whitefish ecotypes in a given environment.

In this study, we extend the analysis of morphological differentiation between dwarf and normal whitefish ecotypes to six lakes and compare it to the extent of their genetic differentiation assessed at microsatellite loci. Our main objective is to test the hypothesis that ecological processes driving phenotypic divergence are also responsible for determining the extent of their reproductive isolation, as would be expected if the process of ecological speciation operates. We report a negative correlation between the extent of morphological differentiation based on trophic-related traits and that of gene flow between ecotypes in different lakes, which provides support for this hypothesis.

Materials and Methods

Samples

Specimens of dwarf and normal ecotypes were sampled by gill nets from 1992 to 1996 in six lakes of the St. John River drainage (Fig. 1, Table 1). These lakes are geographically isolated from each other by either geographic barriers or unsuitable riverine habitat for lake whitefish such that contemporary gene flow among them is not possible. Whole fish used for morphological analyses were kept on ice in the field and then frozen until subsequent analyses. Additional samples for genetic analyses consisted of muscle tissues preserved in 95% ethanol. Fish were classified as dwarf or normal as detailed in Chouinard et al. (1996).

Morphological Analysis

Eight meristic and 19 morphometric variables were used to quantify morphological differentiation between dwarf and normal whitefish ecotypes. Details on variables and methodology are presented in Chouinard et al. (1996). The data were initially examined for differences between sexes. Because no differences were found, further analyses were based on datasets with sexes pooled. All datasets were first log-transformed and normalized to a mean of zero and a standard deviation of one. Morphological measurements were then adjusted to those expected for the mean body length by the allometric formula (Thorpe 1976; Reist 1985; Claytor and MacCrimmon 1987). Meristic and size-adjusted morphometric datasets were analyzed separately by discriminant function analysis (DFA) because meristic and morphometric variables did not meet the assumption of homogeneity in

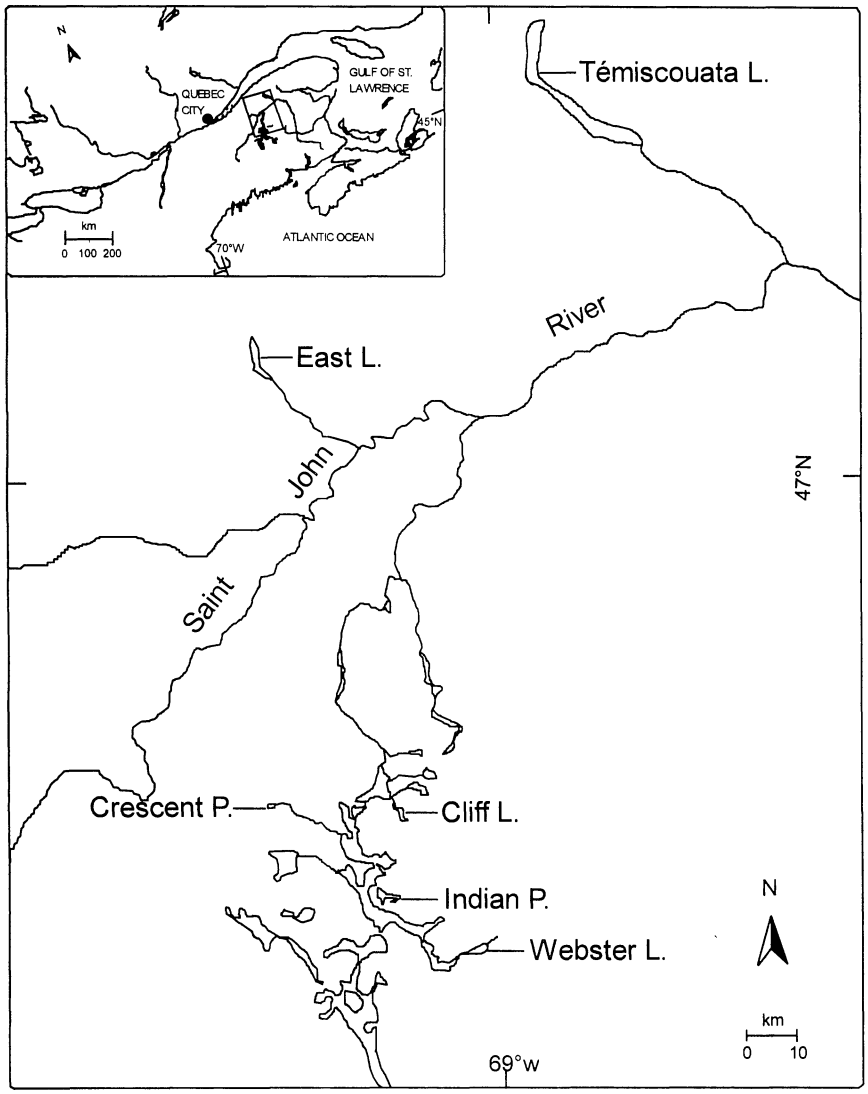

FIG. 1. Map of the study area, with locations of populations sampled.

TABLE 1. Sample location, sample size, fork length, and mean gill raker counts of dwarf and normal lake whitefish ecotypes. Letters m and g indicate the number of samples available for morphological and genetic analyses, respectively.

Lake	Latitude, longitude	Sample size				Fork length (mm) (standard deviation)		Gill raker counts (standard deviation)	
		Dwarf		Normal					
		m	g	m	g	Dwarf	Normal	Dwarf	Normal
Cliff	46°23′51″N, 69°15′05″W	48	40	40	40	167.6 (32.3)	285.1 (67.3)	27.6 (1.2)	25.1 (1.5)
Indian	46°15′25″N, 69°18′05″W	55	37	18	44	186.2 (9.0)	415.7 (18.7)	29.7 (1.1)	26.4 (2.1)
Webster	46°09′19″N, 69°04′48″W	40	41	22	40	213.3 (21.3)	306.2 (45.8)	30.6 (1.9)	28.0 (2.3)
Crescent	46°25′36″N, 69°36′20″W	51	41	21	21	216.5 (11.5)	309.7 (40.5)	26.4 (1.7)	24.6 (0.8)
Témiscouata	47°36′00″N, 68°45′00″W	34	44	25	35	188.3 (5.8)	237.5 (24.3)	25.3 (1.3)	24.6 (1.4)
East	47°11′00″N, 69°33′00″W	50	41	50	41	152.6 (17.4)	285.1 (67.3)	25.5 (1.5)	26.0 (1.6)

variance. To insure that the results were not biased by the allometric adjustment, we also used residual values (the difference between the observed and predicted size of the body part from the within-groups regression line) to adjust measurements for body size (Reist 1986; Fleming et al. 1994). Both methods led to similar results, and only those obtained by the allometric method is presented.

The *F*-values associated with the respective partial Wilk's lambda were used to quantify the significance of variable contributions to the discriminant function (variables with $F > 1$ were considered as contributing to the discriminant function). Eigenvalues, interpreted as the proportion of variance accounted for by the correlation between the respective canonical variates for canonical functions, were used to quantify the extent of morphological differentiation between ecotypes in a given lake. Values for both meristic and morphometric variables were summed up to express the overall extent of morphological differentiation between ecotypes within each lake. All statistical analyses were performed using STATISTICA, version 4.5 (Statsoft).

Genetic Analysis

The extent of genetic differentiation and gene flow between ecotypes in each lake was evaluated using six microsatellite loci (Table 2) with dinucleotide repeats specifically developed for *Coregonus* (Bernatchez 1996; Patton et al. 1997; Turgeon et al. 1999). Primer sequences of locus C4-157 (5′ GCTTGTTGAGGGCGTTTACC 3′ and 3′ CGAACCACTTTGTAGACACAC 5′) were not reported previously. Polymerase chain reaction (PCR) protocols were as described in Brunner et al. (1998) with an increase of the amount of [α-^{35}S] ATP from 1 μci to 1.5 μci to amplify loci in duplex (*Bwf-1/C2-157, Bwf2/Cocl23*). Loci *Cocl22* and *C4-157* were individually amplified. Amplifications were performed in a Perkin Elmer thermal cycler (model 480) and consisted of an initial 3-min denaturation step at 95°C, 15 cycles of 60 s at 94°C, 45 s at the annealing temperature, and a 10 s extension at 72°C, following by 20 additional cycles of 30 s at 94°C, 30 s at the annealing temperature, and 10 s at 72°C. The annealing temperature was 60°C for duplex *Bwf1/C2-157*, 55°C for *Bwf2/Cocl23*, 54°C for *C4-157*, and 52°C for *Cocl22*. PCR products were separated on 6% acrylamide sequencing gels run between 2.5 h to 4 h at constant voltage (1920 V). Gel fixation, drying, and autoradiography followed standard procedures. Alleles were sized by comparison with the standard M13 sequence and with standard controls consisting of two samples run on all gels. Each gel was also scored independently three times to minimize errors in assigning allelic size, which was always determined from at least two congruent scores.

Genetic polymorphism within each sample was quantified in terms of number of alleles per locus (*A*), observed heterozygosity (H_O), and gene diversity (H_E) using GENEPOP version 3.1a (Raymond and Rousset 1995). Differences in *A*, H_O, H_E among samples were assessed by Wilcoxon's signed-rank test (Snedecor and Cochran 1978). This test was performed after the number of alleles had been adjusted for a common sample size of 40 per population using equation 11 in Ewens (1972). Samples were tested for departure from Hardy-Weinberg equilibrium with the specific alternative hypotheses of either heterozygote deficiency or excess using the score (*U*) test of Rousset and Raymond (1995) available in GENEPOP. Significance values were computed for each locus by unbiased estimates of Fisher's exact test using the Markov chain method through 1000 iterations (Guo and Thompson 1992). Global tests across loci and across populations were also made. GENEPOP was also used to perform homogeneity tests of allele frequency distribution at each locus to test the null hypothesis of no genetic differentiation between ecotypes within each lake. The extent of genetic differentiation between ecotypes in each lake was first quantified by pairwise fixation indices based on allelic frequencies (θ of Weir and Cockerham 1984) using Fstat version 1.2 (Goudet et al. 1996). Genetic differentiation was also estimated using mutational differences among alleles by computing pairwise standardized R_{ST} based on the assumption of a strict stepwise mutation model with the program R_{ST} Calc (Goodman 1997). The 95% confidence intervals on both θ and R_{ST} estimates were obtained by the bootstrapping procedure over loci (1000 replicates) available in both programs. For both θ and R_{ST} estimates, departure from the null hypothesis (θ and $R_{ST} = 0$) were statistically assessed by permutation procedures (1000 iterations). The amount of gene flow between ecotypes within each lake was estimated from the private allele method (Slatkin 1985). Probability values in all of the above tests were adjusted for multiple simultaneous tablewide tests using the sequential Bonferroni adjustments (Rice 1989) to minimize Type I errors.

Relationships between the Extent of Morphological and Genetic Divergence

The Pearson correlation coefficient (*r*) and corresponding *P*-values were used to quantify the relationship between the extent of morphological differentiation (overall eigenvalues) and that of genetic divergence in sympatric ecotypes from each lake. Probability values for multiple individual correlations were obtained using Fisher's method (Fisher 1954).

Results

Morphological Differentiation

The size-adjusted morphometry of dwarf and normal fish was very similar in all six lakes. No significant difference ($P > 0.05$) between ecotypes were found in 19 morphometric variables, based on discriminant function analysis of samples (Table 3). Only four variables were considered discriminant ($F > 1$; marginal for adipose fin length, $F = 1.09$) and this occurred in only two lakes (Table 3). It is noteworthy that three of those variables (pectoral length, caudal peduncle length, and maxillary width) represent traits that are functionally related to trophic use efficiency (Gatz 1979; Webb 1984; Malmquist et al. 1992). The almost complete absence of size-adjusted morphometric differentiation between ecotypes translated into low and statistically nonsignificant eigenvalues, ranging from 0.009 to 0.221 depending on lake.

In contrast, meristic variables did differ between ecotypes in all six lakes (Table 3). Four to eight variables were considered as contributing ($F > 1$) to the discriminant function

depending on lake. Among meristic variables, gill raker counts were found to contribute most importantly to the discriminant function and to be significantly different between ecotypes in five of the six lakes (Table 3). In those cases, the dwarf ecotype consistently had higher mean gill raker counts than the normal ecotype (Table 1). It is noteworthy that this most discriminant meristic trait is considered the most functionally related to prey selection in whitefish, with a higher number of gill rakers being more efficient in selecting for small prey (Svärdson 1979; Lindsey 1981).

Histograms of discriminant (canonical) scores for meristic variables illustrate two points (Fig. 2). First, dwarf and normal ecotypes within each lake had distinct distributions of discriminant scores and different centroid values, implying that ecotypes were generally differentiated based on meristic traits. Second, the extent of differentiation varied substantially from lake to lake. Ecotypes from Cliff Lake and East Lake were respectively the most and least morphologically distinct, with a continuum of intermediate levels of differentiation observed in the other lakes. Accordingly, eigenvalues of canonical functions in morphological variables varied from lake to lake and were much higher for meristic variables than for morphometric variables (Table 3).

Genetic Differentiation and Gene Flow

Moderate to high levels of genetic diversity were observed within each sample, with the number of alleles per locus varying from two to 12, and gene diversity estimates varying between 0.16 and 0.82 (Table 2). Globally, dwarf ecotype had significantly more alleles per locus than the normal ecotype (Wilcoxon sign-rank test, $P = 0.0001$), but this did not translate into significant differences in either H_E or H_O at individual loci (Wilcoxon sign-rank test, $P = 0.7068$ for H_E and 0.4222 for H_O) or in averaged values (H_M) across loci (Kruskal-Wallis ANOVA, $P = 0.7738$ for H_E and 0.3857 for H_O). The average within-sample heterozygosity (H_M) across loci was generally similar in all lakes (Table 2). The magnitude of fixation indices is strongly correlated with that of genetic polymorphism (Jin and Chakraborty 1995). Thus, these observations are important because they support the assumption that genetic differentiation between ecotypes should not be strongly biased by variance in genetic diversity from lake to lake. Details on allele frequency distribution are presented in the appendix.

Hardy-Weinberg expectations for genotype frequencies in samples from each lake were rejected at the 5% level in 10 of 72 cases following Bonferroni sequential adjustment for multiple tests, a number slightly higher than expected by chance (Table 2). These departures were mainly due to a clustered heterozygote deficit at *C4-157* (five out of eight heterozygote deficits detected; *P*-value of global test across populations < 0.00001), strongly suggesting the presence of a null allele at this locus. The presence of a null allele at this locus is also substantiated by the observation that the five populations with overall heterozygote deficits were also those where *C4-157* was in heterozygote deficit. Thus, prior to performing additional statistical analyses, we estimated the gene frequencies of a null allele at *C4-157* in those populations with GENEPOP, using a maximum-likelihood procedure, that is, the EM algorithm of Dempster et al. (1977).

Significant genetic differentiation between sympatric whitefish ecotypes was observed in five of the six lakes (Table 4). Homogeneity tests of allele frequency distribution showed that dwarf and normal whitefish ecotypes were genetically differentiated in all lakes except Crescent Pond. This translated into estimates of both θ and R_{ST} as being significantly greater than zero in all lakes but Crescent. The extent of genetic differentiation between dwarf and normal ecotypes varied among lakes (Table 4). The number of loci that showed significant ($P < 0.05$) differences in allele frequency distribution, θ and R_{ST} values were all greatest in Cliff, least in Crescent, and intermediate in other lakes. Depending on lake, R_{ST} estimates could be either higher (e.g. Cliff Lake), lower (e.g., Indian Pond), or similar (e.g., Crescent Pond) to θ.

Correlation of Morphological Differentiation and Genetic Divergence

A positive correlation value was generally observed between the extent of morphological differentiation of ecotypes in each lake and that of their genetic divergence (Fig. 3, Table 5). However, no single locus value could be considered significant (except at *Bwf1*) for comparisons involving θ. Nevertheless, a trend for low *P*-values was obvious such that the global *P*-value for multiple comparisons (Fisher 1954) was significant. The trend was not significant for comparisons involving R_{ST} when considering all loci, but it was so when excluding either locus *Bwf2* ($\chi^2 = 19.88$, df = 10, $P < 0.05$) or *C2-157* ($\chi^2 = 20.16$, df = 10, $P < 0.05$) from the comparison. A positive correlation was found between the extent of morphological differentiation and that of genetic divergence based on either multilocus θ or R_{ST} estimates (Fig. 4). Similarly, a negative correlation between morphological differentiation and the extent of gene flow derived from the private-allele method was observed. Although only the comparison involving θ was significant, *P*-values for those involving R_{ST} and *Nm* were near 0.05, thus indicating an overall correlation between indices of morphological and genetic differentiation (Table 5).

Discussion

Divergent Natural Selection and Reproductive Isolation

The main objective of this study was to test the hypothesis that ecological processes driving phenotypic divergence are also responsible for determining the extent of reproductive isolation in sympatric fish species-pairs. To achieve this, we compared the strength of reproductive isolation among whitefish species-pairs evolved within the same time frame, but differing in the extent of their potential for occupying distinct trophic niches. Under the hypothesis that ecological speciation operates in whitefish, we predicted that gene flow should be more restricted between sympatric populations that are more specialized for occupying distinct trophic niches.

We summarized evidence that dwarf and normal ecotypes differ mainly in phenotypic traits known to be related to trophic efficiency in whitefish. We demonstrated that the extent of differentiation in such traits varied among lakes from

TABLE 2. Allelic variability at six microsatellite loci from sympatric lake whitefish ecotypes in six lakes. Number of samples successfully used for genetic analysis (N), number of alleles at each locus (A), total number of alleles at six loci (A_T), most common alleles (A_C; in base pairs), frequencies of the most common alleles (F_C), range of allele size (A_R), observed heterozygosity (H_O) and gene diversity (H_E) at each locus, and mean within-ecotype heterozygosity at six loci (H_M). Superscripts e and d indicate significant heterozygote excess and deficit, respectively, following the sequential Bonferroni correction ($\alpha = 0.05$, k = 72), whereas superscript D indicates significant heterozygote deficit (global test, Fisher's method) prior to correcting for the presence of a null allele at *C4-157*.

Lake	Ecotype		*Bwf1*	*Bwf2*	*C2-157*	*Cocl22*	*Cocl23*	*C4-157*	A_T	H_M
Cliff	Dwarf	N	40	40	40	36	40	39		
		A	9	3	9	4	4	6	35	
		A_C	220	157	147	123	266	293		
		F_C	0.44	0.91	0.51	0.60	0.59	0.50		
		A_R	212–228	153–161	133–167	117–127	260–266	281–301		
		H_E	0.75	0.16	0.70	0.57	0.55	0.66		0.57
		H_O	0.93	0.18	0.68	0.75	0.75	0.49		0.63
	Normal	N	37	40	40	40	40	38		
		A	4	3	7	3	4	3	24	
		A_C	214	157	147	123	262	297		
		F_C	0.70	0.69	0.68	0.41	0.58	0.72		
		A_R	210–220	147–157	147–169	121–127	260–266	293–301		
		H_E	0.45	0.44	0.49	0.57	0.60	0.44		0.50
		H_O	0.54	0.48	0.60	0.70	0.65	0.39		0.56
Webster	Dwarf	N	39	41	40	40	39	34		
		A	8	5	12	8	7	11	51	
		A_C	220	157	147	123	266	293		
		F_C	0.49	0.82	0.54	0.54	0.71	0.40		
		A_R	210–224	147–161	143–171	117–131	252–270	283–303		
		H_E	0.70	0.32	0.69	0.65	0.49	0.75		0.60
		H_O	0.72	0.29	0.70	0.78	0.41	0.35^{d}		0.54^{D}
	Normal	N	40	40	40	40	40	40		
		A	6	2	6	5	5	5	29	
		A_C	214	147	147	123	262	297		
		F_C	0.38	0.74	0.84	0.46	0.38	0.38		
		A_R	212–226	147–157	145–171	119–131	262–272	277–303		
		H_E	0.73	0.39	0.29	0.68	0.60	0.72		0.59
		H_O	0.60	0.33	0.33	0.88^{e}	0.53	0.33^{d}		0.46^{D}
Indian	Dwarf	N	44	44	44	42	43	43		
		A	7	5	12	7	6	10	47	
		A_C	220	157	147	123	266	293		
		F_C	0.50	0.84	0.57	0.61	0.57	0.50		
		A_R	212–224	147–161	147–171	109–129	256–270	278–303		
		H_E	0.70	0.29	0.65	0.59	0.58	0.71		0.59
		H_O	0.57	0.16^{d}	0.73	0.67	0.70	0.58^{d}		0.57^{D}
	Normal	N	32	37	31	30	33	22		
		A	5	4	5	5	5	6	30	
		A_C	220	157	147	123	266	297		
		F_C	0.38	0.78	0.84	0.62	0.68	0.66		
		A_R	212–224	147–161	145–159	119–129	260–270	273–305		
		H_E	0.73	0.36	0.29	0.55	0.49	0.55		0.49
		H_O	0.53	0.35	0.06^{d}	0.73	0.42	0.41		0.42^{D}
East	Dwarf	N	39	40	40	39	40	39		
		A	8	6	9	6	5	7	40	
		A_C	220	157	147	125	266	293		
		F_C	0.61	0.55	0.39	0.55	0.63	0.68		
		A_R	212–216	147–159	121–167	105–127	260–270	285–301		
		H_E	0.60	0.63	0.76	0.60	0.56	0.52		0.61
		H_O	0.62	0.67	0.63	0.89^{e}	0.59	0.30^{d}		0.62^{D}
	Normal	N	40	40	40	37	40	35		
		A	6	4	10	6	6	5	38	
		A_C	220	157	145	123	266	293		
		F_C	0.61	0.91	0.29	0.46	0.55	0.36		
		A_R	212–224	147–159	121–167	105–127	260–270	285–301		
		H_E	0.57	0.16	0.82^{d}	0.64	0.58	0.76		0.59
		H_O	0.51	0.15	0.63	0.76	0.58	0.66		0.55

TABLE 2. Continued.

Lake	Ecotype		*Bwf1*	*Bwf2*	*C2-157*	*Cocl22*	*Cocl23*	*C4-157*	A_T	H_M
Témiscouata	Dwarf	N	44	44	42	34	43	21		
		A	8	4	8	3	5	4	32	
		A_C	220	157	147	123	266	297		
		F_C	0.58	0.64	0.83	0.62	0.51	0.64		
		A_R	212–226	147–159	143–163	121–127	258–268	293–301		
		H_E	0.63	0.56	0.30	0.54	0.63	0.51		0.53
		H_O	0.68	0.48	0.31	0.65	0.63	0.14[d]		0.48
	Normal	N	35	35	35	35	35	28		
		A	10	5	9	4	5	4	37	
		A_C	214	157	147	123	266	297		
		F_C	0.31	0.57	0.63	0.60	0.46	0.43		
		A_R	208–226	147–163	145–163	119–127	158–168	289–301		
		H_E	0.77	0.59	0.58	0.54	0.65	0.66		0.63
		H_O	0.69	0.54	0.54	0.71	0.69	0.36		0.59
Crescent	Dwarf	N	36	42	38	34	36	22		
		A	7	6	8	6	4	5	36	
		A_C	220	157	147	123	266	293		
		F_C	0.68	0.82	0.62	0.49	0.82	0.59		
		A_R	208–228	141–161	143–171	117–127	262–268	289–301		
		H_E	0.52	0.32	0.55	0.58	0.32	0.40		0.45
		H_O	0.53	0.29	0.58	0.65	0.36	0.27		0.45
	Normal	N	19	19	14	21	18	12		
		A	6	3	4	4	4	4	27	
		A_C	220	157	147	123	266	293		
		F_C	0.76	0.84	0.46	0.55	0.72	0.76		
		A_R	214–228	147–161	147–171	119–127	264–270	289–301		
		H_E	0.42	0.28	0.62	0.55	0.46	0.67		0.50
		H_O	0.37	0.21	0.43	0.81	0.56	0.42		0.46

almost complete overlap (e.g., East Lake) to almost complete separation (Cliff Lake). These observations, along with previous results that revealed a functional link between morphological specialization and the potential for trophic niche partitioning throughout the ontogeny (Bernatchez et al. 1999), lend support to the hypothesis that the extent of phenotypic divergence reached in sympatric whitefish ecotype-pairs is driven by the strength of resource-based divergent natural selection operating in a given environment (Bernatchez et al. 1999). The previous demonstration of parallel evolution of dwarf/normal whitefish ecotypes also provided strong indirect evidence for the role of natural selection in driving their divergence (Pigeon et al. 1997). Similarly, we showed that whitefish sympatric ecotypes were genetically differentiated, but that the extent of gene flow between them varied from lake to lake. Finally, we documented a negative correlation between the extent of gene flow occurring between sympatric ecotypes and that of their morphological differentiation. Based on the hypothesis that differentiation in traits involved in trophic resource use reflects the intensity of divergent natural selection (reviewed in Robinson and Wilson 1994), these results suggest that the extent of reproductive isolation reached between whitefish ecotypes is mainly driven by resource-based divergent selection in different environments.

Alternative Explanations

Before concluding that the pattern we observed does indeed provide evidence for ecological speciation, we must consider the possibility that factors other than divergent selection can also produce a correlation between niche shift and reproductive isolation. An obvious alternative explanation is the possibility that ecotypes found in a given lake were already morphologically differentiated prior to their postglacial dispersal, and consequently, their differentiation is not related to local divergent selection. However, this appears very unlikely because all known allopatric populations of whitefish outside the zone of secondary contact possess the normal ecotype (Edge et al. 1991), such that it is most likely that populations of different glacial races that colonized each lake were also both characterized by the normal ecotype. Second, no morphological trait consistently differentiates extant allopatric whitefish populations from these different glacial races (Edge et al. 1991). Another explanation may be that the extent of morphological differentiation in each lake reflects random drift processes and is not driven by selection. In such a case, however, one would predict that traits with no obvious functional link to trophic ecology (e.g., number of scales) would be as likely to generate the pattern of differentiation that we observed. Although such traits did contribute to the differentiation of whitefish ecotypes in several cases, the traits involved varied among lakes (as predicted if drift operates), and their contribution was always clearly less than trophic-related traits, namely the number of gill rakers. Finally, it is also possible that the extent of reproductive isolation reached in each lake has not evolved as a consequence of divergent selection related to the fitness cost of producing hybrids of intermediate phenotype, but instead by chance dispersal that created a reproductive habitat shift of sympatric ecotypes, instantly producing prezygotic

TABLE 3. Summary of discriminant function analysis for morphometric and meristic variables of dwarf and normal whitefish ecotypes. Description of morphological variables is detailed in Chouinard et al. (1996). *F*-values larger than one are considered as contributing to the discriminant function. Asterisk indicates significant *P*-values following the sequential Bonferroni correction ($\alpha = 0.05$, k = 19 for morphometric variables, k = 8 for meristic variables). C, count; D, depth; L, length; S, scale; W, width.

Lake	Cliff		Indian		Webster		Crescent		Témiscouata		East	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Morphometric												
Preorbital L	0.762	3.386	0.000	0.983	0.077	0.783	0.038	0.847	0.038	0.846	0.030	0.862
Orbital L	0.098	0.755	0.008	0.929	0.297	0.589	0.518	0.475	0.426	0.518	0.299	0.586
Postorbital L	0.099	0.754	0.005	0.944	0.090	0.766	0.688	0.411	0.002	0.962	0.007	0.932
Trunk L	0.026	0.872	0.000	0.984	0.007	0.934	0.059	0.808	0.204	0.654	0.483	0.489
Dorsal L	0.569	0.453	0.001	0.982	0.144	0.706	0.596	0.443	0.069	0.794	0.576	0.450
Lumbar L	0.051	0.821	0.001	0.977	0.640	0.428	0.072	0.790	0.006	0.939	0.085	0.771
Anal fin L	0.672	0.415	0.000	0.991	0.914	0.344	0.007	0.934	0.024	0.877	0.000	0.998
Caudal peduncle L	0.002	0.966	0.000	0.989	0.039	0.845	0.000	0.988	0.322	0.573	0.253	0.617
Maxillary L	0.458	0.501	0.001	0.973	0.019	0.890	0.049	0.826	0.008	0.927	0.000	0.991
Mandible L	0.117	0.733	0.016	0.901	0.002	0.963	0.029	0.866	0.709	0.405	0.437	0.511
Maxillary W	0.002	0.965	0.006	0.938	0.936	0.339	2.370	0.130	0.393	0.534	0.030	0.862
Pectoral L	3.158	0.080	0.000	0.983	0.000	0.996	0.417	0.521	0.128	0.723	0.781	0.379
Pelvic L	0.059	0.808	0.016	0.899	0.351	0.557	0.229	0.634	0.817	0.372	0.639	0.426
Body D	0.002	0.966	0.004	0.951	0.018	0.894	0.075	0.785	0.166	0.686	0.009	0.926
Head D	0.505	0.480	0.000	0.994	0.049	0.827	0.160	0.690	0.606	0.441	0.120	0.730
Caudal peduncle L	2.799	0.099	0.027	0.870	0.343	0.561	0.029	0.864	0.071	0.792	0.027	0.870
Adipose L	0.045	0.833	0.028	0.867	0.164	0.687	1.096	0.300	0.000	0.984	0.495	0.484
Interorbital W	0.332	0.566	0.015	0.902	0.176	0.677	0.101	0.751	0.006	0.940	0.129	0.721
Multivariable *P*-level		0.833		1.000		0.990		0.947		0.947		0.981
Eigenvalue		0.173		0.009		0.015		0.170		0.221		0.093
Meristic												
S above the lateral line	1.136	0.290	6.073	0.016	0.003	0.956	0.243	0.624	3.366	0.073	0.907	0.344
Suprapelvic S	19.332	3×10^{-5}*	0.744	0.392	0.016	0.901	0.734	0.395	6.364	0.015	2.467	0.120
Lateral line S	14.849	2×10^{-4}*	5.287	0.025	1.423	0.238	5.740	0.020	3.110	0.084	2.695	0.104
Dorsal ray C	1.062	0.306	2.670	0.107	4.150	0.047	5.888	0.018	0.090	0.765	0.757	0.387
Anal ray C	1.885	0.174	3.607	0.062	0.170	0.682	2.270	0.137	0.548	0.462	0.014	0.905
Pectoral ray C	7.289	0.008	1.068	0.305	0.475	0.494	0.139	0.711	0.004	0.952	4.428	0.038
Pelvic ray C	1.135	0.290	0.444	0.508	18.163	1×10^{-5}*	0.763	0.386	0.861	0.358	1.684	0.198
Gill raker C	64.452	1×10^{-5}*	51.314	1×10^{-5}*	18.921	1×10^{-5}*	13.412	0.001*	13.662	0.001*	1.519	0.221
Multivariable *P*-level		<0.001		<0.001		<0.001		<0.001		<0.001		<0.018
Eigenvalue		3.003		2.089		1.435		0.974		0.796		0.217

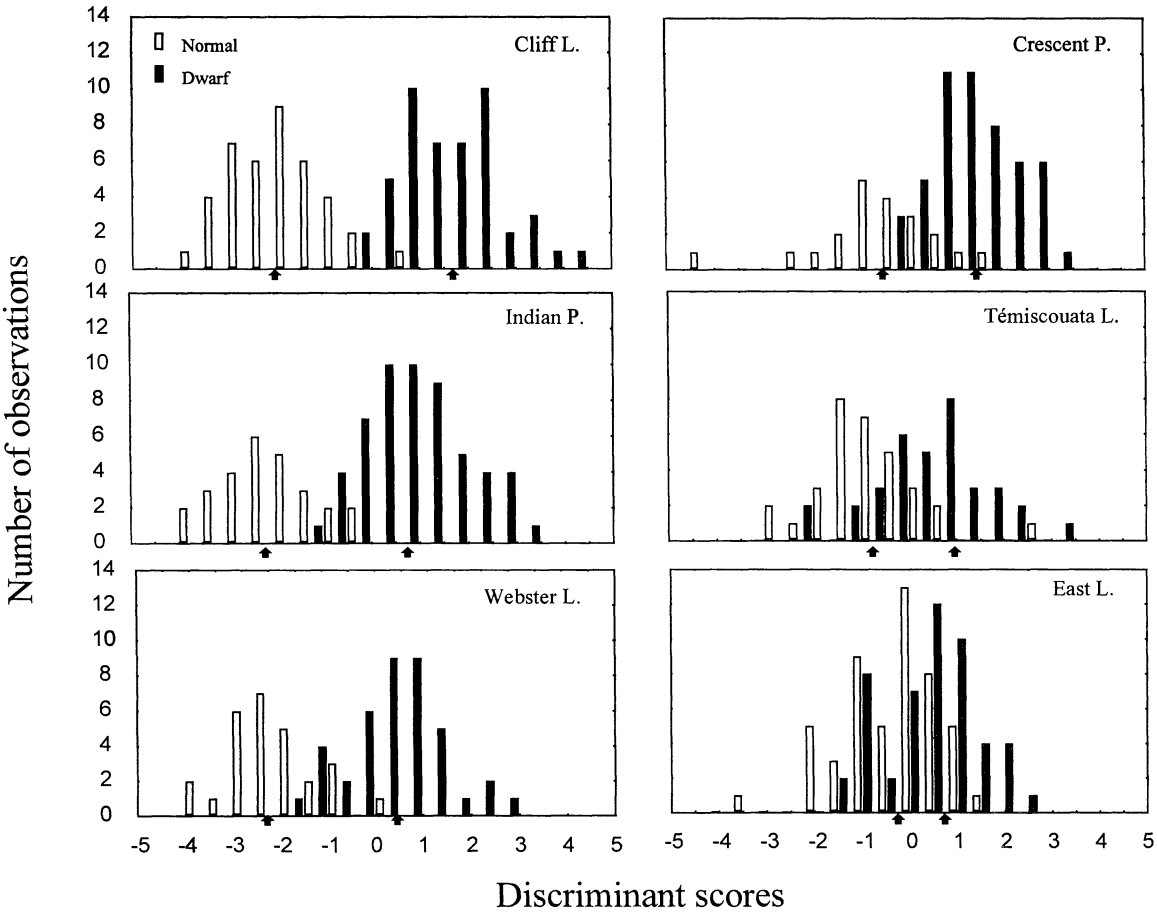

FIG. 2. Discriminant analysis of dwarf and normal whitefish ecotypes from six lakes based on eight meristic variables. Arrows indicate centroids of discriminant scores.

isolation through an alteration of breeding time and location. We cannot entirely refute this explanation until a comprehensive study of reproductive ecology has been completed in each lake. Nevertheless, information available to date does not support this possibility. For instance, both ecotypes use the same spawning grounds in Cliff Lake where reproductive isolation is maximal, whereas normal and dwarf ecotypes from Témiscouata Lake (much less genetically differentiated) spawn in the lake littoral zone and tributaries, respectively (Chouinard and Bernatchez 1998). Consequently, the most parsimonious explanation at this time for the correlation between the extent of morphological specialization and that of gene flow observed in this study is that the extent of reproductive isolation reached between whitefish ecotypes evolved as a consequence of the intensity of local divergent selection in each lake. Further investigation will be required to document the functional link between the extent of morphological specialization and that of trophic niche partitioning achieved between ecotypes throughout their ontogeny in additional lakes. Critical experiments designed to test the hypothesis of a trade-off in fitness versus trophic specialization must also be performed in whitefish.

TABLE 4. Estimation of genetic differentiation between dwarf and normal whitefish ecotypes based on allele frequency distribution, allelic variance (θ), and molecular variance (R_{ST}), respectively. The multilocus P-value was estimated by the Fisher's method using individual P-values calculated from each of the six loci.

	Cliff	Webster	Indian	East	Témiscouata	Crescent
Multilocus P	<0.001	<0.001	<0.001	<0.001	<0.001	0.126
No. of loci with $P < 0.05$	6/6	5/6	3/6	4/6	4/6	1/6
θ	0.256	0.140	0.084	0.058	0.041	0.020
95% interval	0.147–0.339	0.020–0.302	0.012–0.175	0.013–0.110	0.006–0.083	−0.006–0.052
P	<0.001	<0.001	<0.001	<0.001	<0.001	0.055
R_{ST}	0.392	0.188	0.039	0.079	0.085	0.019
95% interval	0.331–0.449	0.111–0.259	0.019–0.072	0.037–0.126	0.036–0.140	−0.015–0.073
P	<0.001	<0.001	<0.001	0.002	<0.001	0.690

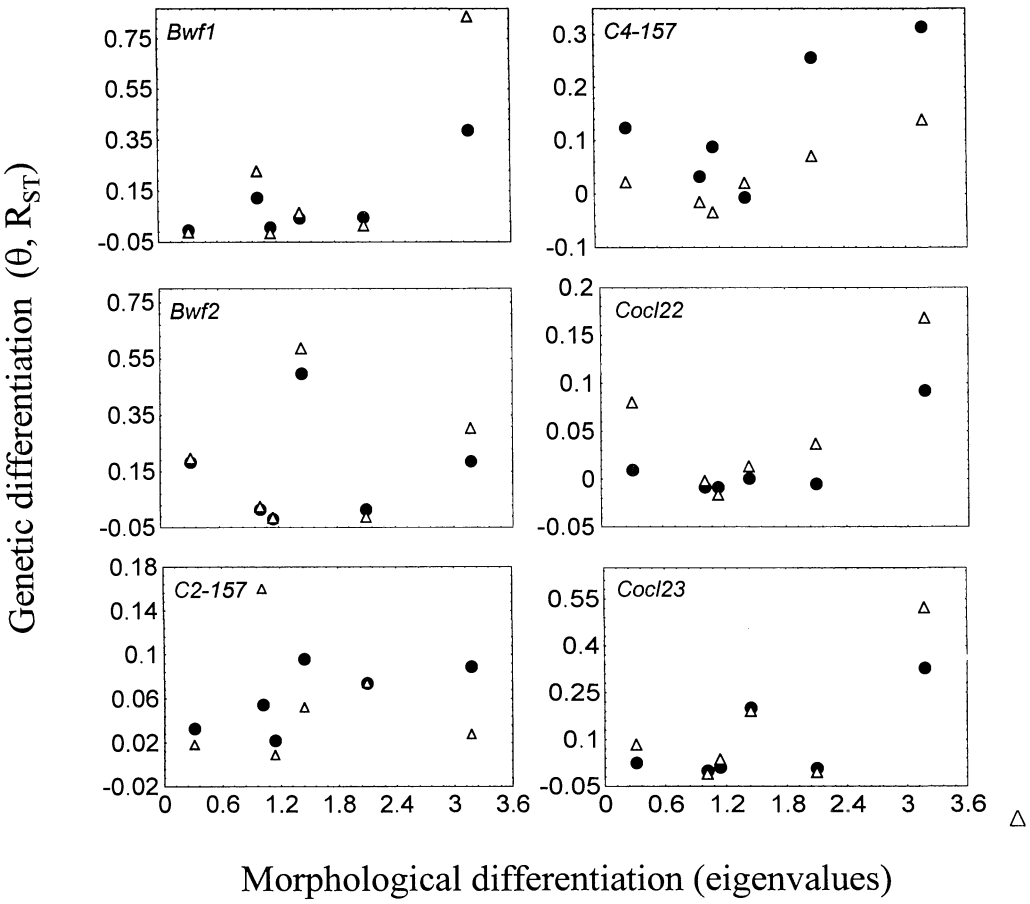

FIG. 3. Scatter diagrams of θ (solid circles) and R_{ST} (open triangles) of pairwise whitefish populations from six lakes illustrated for each of the six loci analyzed. The horizontal axis indicates morphological eigenvalues. Correlation coefficients and corresponding *P*-values of θ versus eigenvalues and R_{ST} versus eigenvalues are given in table 5. Symbols from left to right represent East Lake, Témiscouata Lake, Crescent Pond, Webster Lake, Indian Pond, and Cliff Lake, respectively.

A Scenario for the Evolution of Reproductive Isolation in Whitefish Ecotypes

The results of this study, along with earlier work on the evolutionary history and ecology of whitefish, allow us to generate a scenario of resource-based ecological selection in the diversification process resulting in whitefish ecotypes. Earlier work revealed that the existence of sympatric dwarf and normal ecotypes in the St. John River drainage resulted from separate postglacial invasions by previously allopatric ancestors (Bernatchez and Dodson 1990, 1991; Pigeon et al. 1997; Bernatchez et al. 1999). As mentioned above, it is most likely that populations of different races that colonized each lake were of normal ecotype. The absence of the most likely important competitor for the planktonic trophic niche, *Coregonus artedii* (Lindsey 1981), and competitive interactions between phenotypically similar forms would have led through character displacement to the development of a life cycle adapted for exploiting a planktonic trophic niche. As documented in other fish species-pairs, niche shift to planktivory is almost invariably accompanied by a reduction in growth, younger age at maturity, and smaller size at maturity (Sandlund et al. 1992a,b; Taylor and Bentzen 1993; McPhail 1994). In the case of dwarf/normal whitefish species-pairs, this translated into a 1:10 ratio in average size at maturity between forms. As hypothesized by Bernatchez et al. (1999), the persistence of ecological opportunity for differential resource use throughout ontogeny may have been important in promoting the development of additional trophic specialization, such as the number of gill rakers. Thus, the extent of specialization reached in a given lake would be determined by the persistence in ecological opportunity for differential resource use encountered. Bernatchez et al. (1999) hypothesized that lake-to-lake variation in the persistence of ecological opportunity would be a direct outcome of the level of secondary production throughout the growing season.

The one-order magnitude difference in adult size would have provided a major premating mechanism of reproductive isolation between sympatric ecotypes that evolved as a by-product (pleiotropy and/or genetic hitchhiking) of trophic niche specialization. Although yet to be experimentally demonstrated in whitefish, size assortative mating is a common feature of other fish species-pairs that differ much less in size than the dwarf/normal ecotypes of this study (e.g., Ridgway and McPhail 1984; Foote 1988; Foote and Larkin 1988; Nagel and Schluter 1998). The demonstration of size-assortative mating would nevertheless only partly explain the process of

TABLE 5. Correlation coefficient and probability of significance between indices of morphological differentiation (eigenvalue; E) versus either genetic divergence (θ, R_{ST}) or gene flow (*Nm*). Multicorrelation *P*-values estimated by the Fisher's method. *Nm* was calculated using the private-allele method (Slatkin 1985).

	θ vs. E		R_{ST} vs. E	
	r	*P*	*r*	*P*
Individual locus				
Bwf1	0.814	0.049	0.778	0.0684
Bwf2	0.051	0.923	0.169	0.749
C2-157	0.670	0.129	0.092	0.862
C4-157	0.735	0.096	0.835	0.039
Cocl22	0.745	0.089	0.605	0.204
Cocl23	0.722	0.105	0.724	0.104
Multicorrelation *P*	0.018 (χ^2 = 24.308, df = 12)		0.579 (χ^2 = 20.457, df = 12)	

	Multilocus θ vs. E		Multilocus R_{ST} vs. E		*Nm* vs. E	
	r	*P*	*r*	*P*	*r*	*P*
Combined loci	0.826	0.043	0.727	0.101	0.781	0.067

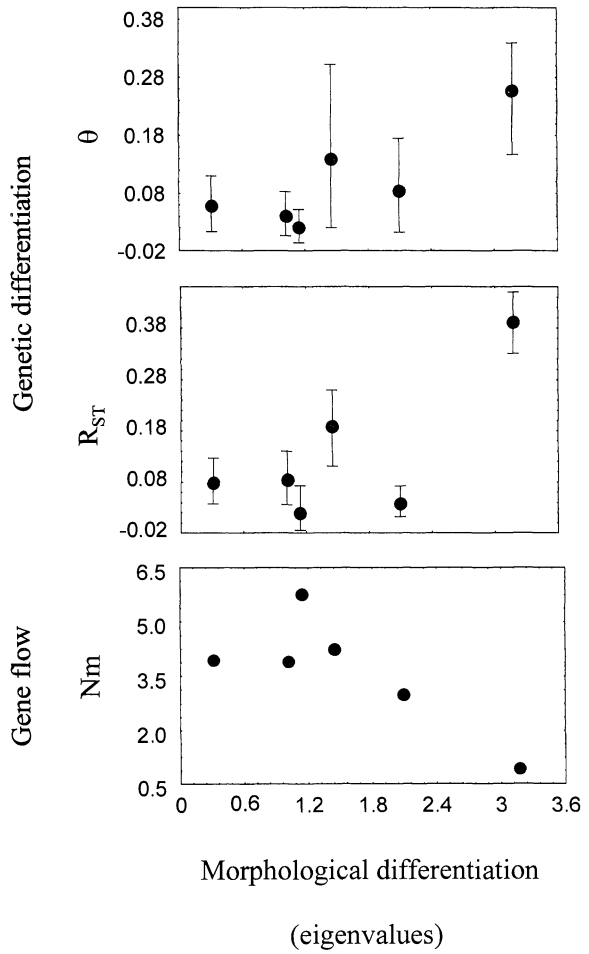

FIG. 4. Relationships of morphological differentiation index (eigenvalues) versus either genetic differentiation indices (θ, R_{ST}) or gene flow (*Nm*) estimated by the private-allele method. Confidence intervals (means ± 1 SE) are provided for θ and R_{ST}. Correlation coefficients and corresponding *P*-values are provided in Table 5. Spots from left to right represent East Lake, Témiscouata Lake, Crescent Pond, Webster Lake, Indian Pond, and Cliff Lake, respectively.

reproductive isolation in whitefish dwarf/normal ecotypes, because varying amounts of gene flow were observed between ecotype-pairs despite a similar degree of size differentiation at reproduction. This strongly suggests that additional reproductive isolation mechanisms evolved at a different pace in each lake. For example, the degree of temporal prezygotic isolation varies among lakes, even when both ecotypes use the same spawning grounds (Fenderson 1964; Chouinard et al. 1996; Chouinard and Bernatchez 1998). Under the hypothesis of ecological speciation, the evolution of differential levels of premating isolation would be associated with lake-to-lake variation in fitness costs of producing hybrids of intermediate phenotype (Schluter 1993). Such costs are expected to increase as trophic specialization increases. This hypothesis implies reproductive reinforcement, the process whereby natural selection strengthens prezygotic barriers to gene exchange between populations in response to hybrid fitness reduction (Dobzhansky 1940). Although long considered rare, there is now growing theoretical and empirical evidence favoring the possibility for reinforcement in nature under certain circumstances (Liou and Price 1994; Noor 1995; Hostert 1997; Saetre et al. 1997; Servedio and Kirkpatrick 1997; Rundle and Schluter 1998). These circumstances are likely met in whitefish ecotypes. First, heterospecific mating (interecotype) surely occurs in nature, as evident by the resulting gene flow quantified in this study. Second, reduced fitness of hybrids due to higher embryonic mortality has recently been shown experimentally from laboratory crosses between dwarf and normal ecotypes belonging to distinct glacial races (Lu and Bernatchez 1998). Embryonic mortality rates estimated by Lu and Bernatchez (1998) would result in an initial selection coefficient against hybrids ranging between 0.24 and 0.47 compared to pure progeny. Such high intensity of selection may be compatible with the view that postmating isolation arising as a product of genetic changes (either through mutations, drift, or selection) in once physically isolated populations may enhance speciation by reinforcement (Hostert 1997). Third, the morphology of dwarf and normal ecotypes mainly varies by gill raker counts, a heritable character capable of responding to selection (Svärdson 1979; Lindsey 1981).

To conclude, dwarf and normal whitefish ecotype-pairs from the St. John River drainage represent a continuum of both morphological and genetic differentiation. Each lake may be viewed as different temporal snapshot taken during the evolution of sympatric ecotypes. Consequently, lake-to-lake comparisons represent a powerful approach to further investigate the evolutionary processes driving population divergence and ultimately speciation. Knowledge of the evolutionary history, ecology and population genetics of whitefish species-pairs strongly supports the view that ecological opportunity and divergent natural selection are promoting both phenotypic divergence and reproductive isolation in fish species complexes. The most likely mechanism involved in the evolution of reproductive isolation in whitefish is the "divergence-with-gene-flow speciation via pleiotropy/hitchhiking" (sensu Rice and Hostert 1993), although the additional role of reproductive reinforcement is likely. Variation in the amount of gene flow and in the extent of morphological differentiation between sympatric ecotypes in different environments has also been reported in other fish species complexes (Hindar 1994; Smith and Skúlason 1996). However, little attention has been paid to this phenomenon, and to our knowledge no attempt has been made to elucidate the causes for such variation. The result of this study suggests that such patterns may also be indicative of ecological speciation, and, consequently, their investigation may allow generalization about the role of ecological processes in driving the evolution of northern temperate freshwater fishes.

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APPENDIX

Allelic frequency of sympatric dwarf and normal whitefish ecotypes from Crescent Pond, Indian Pond, Webster Lake, Cliff Lake, East Lake, and Témiscouata Lake, for the six loci *Bwf1, Bwf2, C2-157, Cocl22, Cocl23,* and *C4-157.*

		Population											
		Crescent		Indian		Webster		Cliff		East		Témiscouata	
Locus	Alleles	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal
Bwf1	208	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
	210	0.014	0.000	0.000	0.000	0.026	0.000	0.000	0.014	0.000	0.000	0.000	0.029
	212	0.000	0.000	0.080	0.156	0.051	0.188	0.013	0.257	0.054	0.024	0.148	0.314
	214	0.056	0.079	0.057	0.109	0.218	0.375	0.013	0.703	0.176	0.232	0.034	0.286
	216	0.000	0.053	0.023	0.000	0.077	0.000	0.025	0.000	0.054	0.049	0.091	0.014
	218	0.056	0.000	0.034	0.000	0.013	0.000	0.087	0.000	0.027	0.000	0.057	0.014
	220	0.681	0.763	0.500	0.375	0.487	0.313	0.438	0.027	0.608	0.610	0.580	0.243
	222	0.042	0.053	0.182	0.031	0.103	0.075	0.075	0.000	0.054	0.012	0.045	0.043
	224	0.139	0.026	0.125	0.328	0.026	0.038	0.175	0.000	0.014	0.073	0.034	0.029
	226	0.000	0.000	0.000	0.000	0.000	0.013	0.150	0.000	0.014	0.000	0.011	0.014
	228	0.000	0.026	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
Bwf2	141	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	147	0.095	0.105	0.057	0.176	0.098	0.738	0.000	0.300	0.218	0.012	0.148	0.286
	149	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
	151	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.064	0.012	0.000	0.014
	153	0.012	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.013	0.000	0.000	0.000
	155	0.012	0.000	0.011	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.102	0.000
	157	0.821	0.842	0.841	0.784	0.817	0.262	0.913	0.688	0.551	0.915	0.636	0.571
	159	0.000	0.000	0.068	0.027	0.061	0.000	0.000	0.000	0.141	0.061	0.114	0.114
	161	0.048	0.053	0.023	0.014	0.012	0.000	0.063	0.000	0.000	0.000	0.000	0.000
	163	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
C2-157	121	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000
	123	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
	133	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.013	0.025	0.000	0.000
	141	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	143	0.013	0.000	0.000	0.000	0.087	0.000	0.075	0.000	0.000	0.000	0.012	0.000
	145	0.013	0.000	0.011	0.016	0.000	0.013	0.000	0.000	0.211	0.287	0.048	0.043
	147	0.618	0.464	0.568	0.839	0.538	0.837	0.512	0.675	0.395	0.175	0.833	0.629
	149	0.000	0.000	0.034	0.016	0.063	0.013	0.050	0.025	0.066	0.188	0.000	0.000
	151	0.000	0.000	0.034	0.000	0.000	0.025	0.112	0.000	0.026	0.000	0.036	0.014
	153	0.000	0.000	0.057	0.000	0.013	0.000	0.000	0.250	0.000	0.000	0.012	0.014
	155	0.000	0.036	0.011	0.000	0.038	0.000	0.000	0.013	0.000	0.000	0.012	0.057
	157	0.039	0.071	0.000	0.065	0.025	0.000	0.000	0.000	0.000	0.013	0.000	0.014
	159	0.263	0.429	0.159	0.065	0.075	0.025	0.075	0.000	0.197	0.200	0.036	0.086
	161	0.013	0.000	0.034	0.000	0.075	0.000	0.013	0.013	0.066	0.013	0.000	0.014
	163	0.000	0.000	0.045	0.000	0.025	0.087	0.025	0.013	0.026	0.000	0.012	0.129
	165	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	167	0.013	0.000	0.000	0.000	0.025	0.000	0.125	0.000	0.000	0.038	0.000	0.000
	169	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
	171	0.026	0.000	0.011	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX
Continued.

		Population											
		Crescent		Indian		Webster		Cliff		East		Témiscouata	
Locus	Alleles	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal
Cocl22	105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000
	109	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	117	0.015	0.000	0.000	0.000	0.025	0.000	0.014	0.000	0.000	0.000	0.000	0.000
	119	0.015	0.024	0.048	0.017	0.025	0.063	0.000	0.000	0.014	0.014	0.000	0.014
	121	0.441	0.405	0.190	0.233	0.250	0.275	0.167	0.075	0.311	0.392	0.279	0.314
	123	0.485	0.548	0.607	0.617	0.525	0.463	0.597	0.412	0.554	0.459	0.618	0.600
	125	0.029	0.000	0.024	0.050	0.013	0.000	0.000	0.000	0.000	0.041	0.000	0.000
	127	0.015	0.024	0.060	0.083	0.112	0.188	0.222	0.512	0.081	0.041	0.103	0.071
	129	0.000	0.000	0.060	0.000	0.038	0.013	0.000	0.000	0.014	0.000	0.000	0.000
	131	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.027	0.000	0.000	0.000
Cocl23	252	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	256	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	258	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.140	0.071
	260	0.000	0.000	0.035	0.015	0.000	0.000	0.038	0.200	0.000	0.025	0.000	0.057
	262	0.042	0.000	0.302	0.212	0.103	0.512	0.038	0.575	0.179	0.350	0.314	0.371
	264	0.125	0.167	0.070	0.076	0.013	0.075	0.338	0.050	0.051	0.013	0.023	0.000
	266	0.819	0.722	0.570	0.682	0.705	0.375	0.587	0.175	0.628	0.550	0.512	0.457
	268	0.014	0.028	0.000	0.000	0.077	0.000	0.000	0.000	0.026	0.013	0.012	0.043
	270	0.000	0.083	0.012	0.015	0.064	0.025	0.000	0.000	0.115	0.050	0.000	0.000
	272	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
C4-157	273	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	277	0.000	0.000	0.000	0.023	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	278	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	279	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.014	0.000	0.000	0.000
	281	0.000	0.000	0.000	0.023	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	283	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
	285	0.000	0.000	0.012	0.000	0.000	0.013	0.000	0.000	0.000	0.071	0.000	0.000
	287	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.027	0.000	0.000	0.000
	289	0.068	0.208	0.023	0.000	0.015	0.013	0.000	0.000	0.014	0.186	0.000	0.018
	291	0.000	0.000	0.035	0.000	0.015	0.000	0.051	0.000	0.108	0.000	0.000	0.000
	293	0.773	0.500	0.500	0.091	0.368	0.338	0.500	0.079	0.676	0.357	0.286	0.375
	295	0.045	0.000	0.070	0.000	0.074	0.000	0.000	0.000	0.000	0.014	0.048	0.000
	297	0.045	0.250	0.140	0.659	0.338	0.375	0.141	0.724	0.122	0.100	0.643	0.429
	299	0.000	0.000	0.023	0.000	0.044	0.000	0.026	0.000	0.000	0.000	0.000	0.000
	301	0.068	0.042	0.151	0.045	0.074	0.175	0.269	0.197	0.041	0.271	0.024	0.179
	303	0.000	0.000	0.035	0.000	0.029	0.063	0.000	0.000	0.000	0.000	0.000	0.000
	305	0.000	0.000	0.000	0.136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000