Lack of specialization in trophic morphology between genetically differentiated dwarf and normal forms of lake whitefish (*Coregonus clupeaformis* Mitchill) in Lac de l'Est, Quebec

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Abstract: We recently observed a bimodal distribution in size of sexually maturing lake whitefish (*Coregonus clupeaformis* Mitchill) in Lac de L'Est, Quebec. The objective of this study was to test the hypothesis that these two spawning size classes represent genetically distinct ecotypes, potentially adapted in morphology for occupying different trophic niches. This was accomplished by quantifying the extent of genetic (mitochondrial DNA (mtDNA) and enzyme loci) and morphological differences. Significant differences in haplotype and allele frequency distributions confirmed the hypothesis that whitefish maturing at small (dwarf) and normal sizes are structured into two distinct gene pools. However, low F_{st} values at mtDNA and enzyme loci, coupled with the apparent lack of spatial and temporal spawning segregation, suggest that reproductive isolation is incomplete and that gene flow is still occurring between the two forms. Patterns of mtDNA diversity favoured the origin of genetically distinct dwarf and normal-size lake whitefish forms in Lac de l'Est through sympatric divergence. However, a lack of differences in morphological traits potentially related to trophic ecology did not support the hypothesis that the two forms represent ecotypes which are morphologically specialized in trophic niches. This study established that sympatric dwarf and normal-size spawning groups of lake whitefish in Lac de l'Est represent two genetically distinct populations despite the potential for gene flow between them. This, coupled with their low level of morphological diversification, suggests that they represent an early stage of population divergence, and therefore makes them of particular interest for understanding the gene–environment processes involved in the early steps of speciation events.

Résumé : Nous avons récemment observé une bimodalité dans la distribution de la taille des Grands corégones (*Coregonus clupeaformis* Mitchill) en cours de maturation sexuelle dans le Lac de l'Est, Québec. L'objectif de cette étude était de vérifier l'hypothèse voulant que ces deux classes de taille représentent des écotypes génétiquement différenciés qui possèdent une morphologie potentiellement adaptée à l'utilisation différentielle de niches trophiques. Pour ce faire, nous avons quantifié leur degré de différenciation génétique (ADN mitochondrial et loci enzymatiques) et morphologique. Des différences significatives ont été observées dans la distribution des fréquences alléliques aux deux types de marqueurs, confirmant ainsi que l'hypothèse que les corégones qui atteignent leur maturité à une petite et grande tailles sont structurés en deux pools génétiques distincts. Cependant, les faibles valeurs de F_{st} observées, jumelées à l'absence apparente de ségrégation spatiale et temporelle au moment de la reproduction, suggèrent que l'isolement reproducteur est incomplet et qu'un flux génique se maintient entre les deux formes. Le comportement de la diversité mitochondriale favorise l'hypothèse d'une origine sympatrique de ces deux formes. Cependant, l'absence de différences au niveau des traits morphologiques potentiellement reliés à l'écologie trophique ne supporte pas l'hypothèse que ces deux formes représentent des écotypes morphologiquement spécialisés pour l'occupation de niches distinctes. En conclusion, l'ensemble de ces résultats suggère que les formes sympatriques du corégone du Lac de l'Est représentent un stade primaire de divergence des populations, ce qui en fait un système d'intérêt particulier pour la compréhension des interactions génome–environnement impliquées dans les premiers stades du phénomène de la spéciation.

Introduction

Populations still capable of exchanging genes but showing the capacity to retain separate gene pools are of particular interest for studying the early stages of species formation.

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Indeed, they provide an opportunity to observe the effects of evolutionary forces promoting reproductive isolation (Mallet 1995). However, such situations are relatively rare, and consequently, speciation studies have more often relied on the comparison of closely related yet fully isolated species (Otte and Endler 1989). A major drawback of such an approach is that it is more likely to provide information about the characteristics of species than about the processes that gave rise to them (McPhail 1993). Therefore, it is still relevant to identify populations between which reproductive barriers may have begun to develop but are still incomplete.
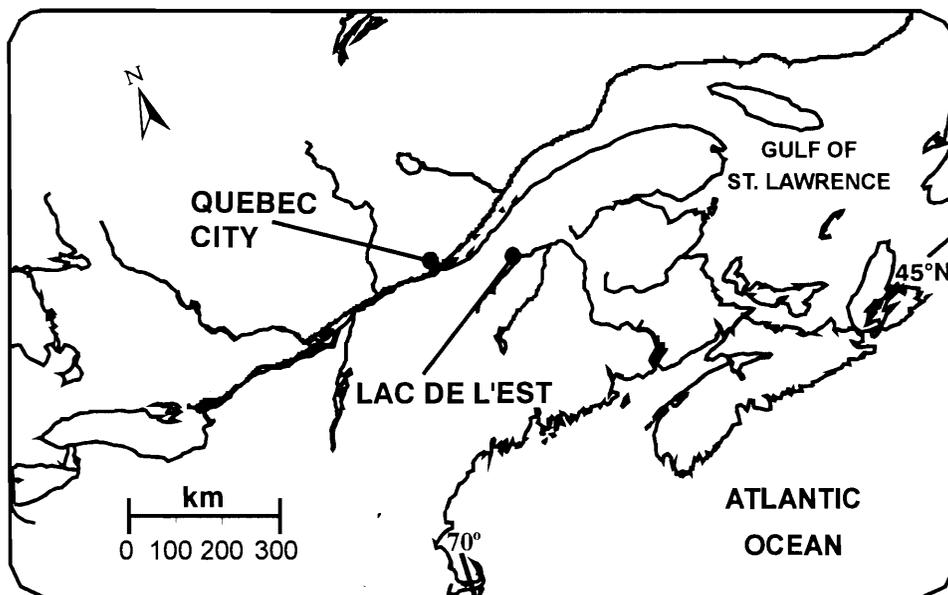
In fishes, the sympatric occurrence of closely related forms belonging to the same species complex probably pro-

Fig. 1. Location of Lac de l'Est.

vides the best available models to study genetic, ecological, and behavioural changes that may occur during species formation (Skúlason and Smith 1995). This phenomenon has been reported in several temperate-zone and northern freshwater species but remains unusual (e.g., Bodaly 1979; Ferguson and Mason 1981; Lindsey and Klatt 1982; Galat and Vucinich 1983; Verspoor and Cole 1989; Taylor and Bentzen 1993a, 1993b; Schluter and McPhail 1993; Snorrason et al. 1994).

The lake whitefish (*Coregonus clupeaformis* Mitchill) constitutes one of the species complex in which the occurrence of sympatric forms has been observed at several locations in North America, including southern Yukon (Lindsey 1963; Lindsey et al. 1970; Bodaly et al. 1988), northern Maine (Fenderson 1964), Ontario (Kennedy 1943; Bodaly et al. 1991a), Quebec (Fortin and Gendron 1990), and Labrador (Bodaly et al. 1992). As in most other groups of fish in which sympatric forms have been investigated, studies in lake whitefish have indicated that ecological, behavioural, and morphological differences between sympatric forms are functionally related and are apparently adaptations for occupying different trophic niches (e.g., Bodaly 1979); this suggests that opportunities for using distinct food resources may be an important mechanism driving ecotypic radiation in fish (Schluter and McPhail 1993). Genetic analyses of isozyme and mitochondrial DNA (mtDNA) variation have also indicated that sympatric whitefish ecotypes are genetically differentiated to variable degrees, and may constitute reproductive units having the capacity to retain separate gene pools despite the potential for hybridization (Kirkpatrick and Selander 1979; Bernatchez and Dodson 1990; Vuorinen et al. 1993; Bernatchez et al. 1996). Therefore, detailed ecological, morphological, and genetic studies of these populations could increase our knowledge of gene–environment interactions during the development of reproductive isolation. However, these have not been undertaken thus far, because of the difficulty of accessing (because of either remoteness or legislation) those lakes harbouring whitefish sympatric forms reported to date.

During a search for a more accessible system where such studies could be undertaken, we observed a strong bimodal distribution in sizes of sexually maturing lake whitefish in Lac de l'Est (Quebec), which suggested the existence of sympatric forms in this lake. The objective of this study was to test the hypothesis that these two spawning size classes of lake whitefish represent genetically distinct ecotypes that are potentially adapted in morphology for occupying different trophic niches. This was accomplished by quantifying the extent of genetic (mtDNA and isozymes) and morphological differences between fish belonging to the two spawning groups.

Material and methods

Study area and sampling

Lac de l'Est is part of the St. John River drainage and is located 120 km east of Quebec City (47°15'N, 69°30'W), near the town of Mont-Carmel (Fig. 1). The lake is 9.1 km long with a surface area of 726 ha, and has a mean and a maximum depth of 14.7 and 36.7 m, respectively. The fish community is dominated by lake whitefish, and the two main predators are burbot (*Lota lota*) and lake trout (*Salvelinus namaycush*). Lake cisco (*Coregonus artedi*) is absent from the lake. Lake whitefish were captured at the bottom, surface, and midwater from 15 to 19 June 1993, using 63 × 1.8 m gill nets comprising nine panels graded from 1.25 to 7.6 cm mesh size (stretched measure) set out overnight. Fish were measured and weighed, and stage of gonad development was estimated according to Nikolskii (1963). Lake whitefish that had reached stage 3 of gonad development were considered potential spawners for the fall of the same year (Morin et al. 1982). Maturing fish of the two spawning size groups were kept at −80°C prior to genetic and morphological analyses. For convenience, in the text we designate the two spawning size groups as dwarf and normal forms.

Mitochondrial DNA analysis

Total DNA was extracted from either the eggs or the liver of 41 normal and 38 dwarf lake whitefish as described previously by Bernatchez et al. (1992). Two segments of the mitochondrial genome encompassing the ND-5/6 region (2.4 kilobase pairs) and the cytochrome b/control region (D-loop) (2.1 kilobase pairs) were

amplified by the polymerase chain reaction (PCR) as described in Bernatchez et al. (1995) and Bernatchez and Osinov (1995). These two fragments were pooled and digested with the following 10 restriction enzymes: AluI, CfoI, AvaII, DdeI, MboI, HincII, HaeIII, RsaI, BanI, Bsp1286I. Resulting fragments were separated on 1.2% agarose gel run for 5 h at 85 V, visualized by staining with ethidium bromide and photographed under uv light. Distinct single endonuclease patterns were identified by a specific letter and used in combination to describe composite mtDNA haplotypes. To assess the correspondence of mtDNA haplotypes identified by PCR − restriction fragment length polymorphism (RFLP) with those defined in previous lake whitefish mtDNA studies, we performed a restriction analysis over the whole mtDNA genome on new variants with the enzymes and methodology used in Bernatchez and Dodson (1991, 1994). This also allowed us to assign new mtDNA haplotypes to one of the lake whitefish glacial races defined by means of phylogeographic analyses (Bernatchez and Dodson 1991).

The genetic differentiation of lake whitefish spawning groups was evaluated from the analysis of frequency distribution of haplotypes using χ^2 randomization tests (Roff and Bentzen 1989), with 1000 randomizations generated using the MONTE program of the REAP software package (McElroy et al. 1992). Intragroup diversity of mtDNA lineages was quantified by the nucleon diversity index (h) of Nei and Tajima (1981). The intergroup component of total mtDNA variance was quantified by F_{st} estimates using the analysis of molecular variance model (AMOVA) of Excoffier et al. (1992), and considering mtDNA haplotypes as multiple alleles of a single locus.

Allozyme analysis

Protein electrophoresis was carried out on cellulose acetate using tissue homogenates as described by Hebert and Beaton (1989). The tissue used for each enzyme locus examined is provided in Bodaly et al. (1991b). Twenty-nine normal and 35 dwarf lake whitefish were screened at 14 loci coded from five enzyme systems, all being potentially polymorphic, as reported in previous surveys among northeastern American lake whitefish populations (Kirkpatrick and Selander 1979; Bodaly et al. 1991b, 1992). These included aldolase (ALD-1*, ALD-2*, EC 4.1.2.13), glycerol-3-phosphate dehydrogenase (G3PDH-1*, G3PDH-2*, G3PDH-3*, EC 1.1.1.8), lactate dehydrogenase (LDH-A1*, LDH-A2*, LDH-B1*, LDH-B2*, LDH-C*, EC 1.1.1.27), phosphoglucomutase (PGM-1*, PGM-2*, EC 5.4.2.2), and malate dehydrogenase (sMDH-B1*, sMDH-B2*, EC 1.1.1.37). Nomenclature of loci and alleles follows the recommendations of Shaklee et al. (1989) and Bodaly et al. (1991b).

Allele frequencies were estimated from direct allele counts. Levels of genetic variation were characterized from estimates of Hardy−Weinberg expected mean heterozygosity per locus (H_{exp}), proportion of polymorphic loci (P; 99% criterion), and mean number of alleles per locus (n_a) (Nei 1978). The fit of observed genotype frequencies within groups with expected Hardy−Weinberg equilibrium were estimated by χ^2 tests, as was the significance of differences in allele frequencies between groups. The level of population subdivision was quantified from F_{st} estimates.

Morphological analyses

Nine meristic variables were counted and 19 morphometric variables (distance measurements) were taken on 25 male and 25 female fish from each group (Table 1). Distances were standardized to the mean fork length using the allometric method of regression against total length (Thorpe 1976; Reist 1985; Claytor and MacCrimmon 1987). All measurements were log-transformed before data analysis. To examine morphological differentiation between spawning groups, meristic and size-adjusted morphometric data sets were analysed univariately using t tests and multivariately using discriminant function analyses. The data were initially examined for differences between the sexes to ensure that such differences did not

bias the results. All statistical analyses were performed using STATISTICA, version 4.5 (Statsoft).

Results

Definition of lake whitefish spawning groups

In total, 511 lake whitefish were captured from 15 to 19 June 1993. The frequency distribution of the percentage of maturing fish as a function of size clearly revealed two modes (Fig. 2). Thus, more than 90% of fish measuring between 150 and 200 mm (fork length) reached stage 3 of gonad development, as did all fish above 350 mm. The percentage in intermediate size classes was much lower, with no maturing fish observed in the 240- to 300-mm size group. Fish retained a priori to represent dwarf and normal spawning groups were selected in the 120- to 200-mm and 350- to 550-mm size classes, respectively (Fig. 2).

Genetic differentiation between dwarf and normal spawning groups

Only 3 (AvaII, HaeIII, BanI) of the 10 restriction enzymes used for mtDNA analysis were polymorphic (Table 2). These defined three composite haplotypes that were only slightly differentiated, differing by one or two restriction sites. By correspondence with haplotypes described in previous mtDNA studies, the first one corresponded to haplotype 25, the most abundant one found thus far within the Acadian race of lake whitefish (Bernatchez and Dodson 1991, 1994). Haplotypes 106 and 107 were new variants not observed before and were also representatives of the mtDNA phylogenetic group associated with the Acadian race. Thus, both haplotypes were identical with 25 (data not shown), based on restriction analysis on the whole mitochondrial genome as performed in earlier studies (Bernatchez and Dodson 1991). In the present PCR-RFLP analysis, haplotype 107 differed from haplotype 25 by a single AvaII site loss, whereas haplotype 106 differed by one site gain at HaeIII and one additional site loss at BanI (Table 2).

Evidence for reproductive isolation between dwarf and normal spawning groups was provided by highly significant heterogeneity in the distribution of the three mtDNA haplotypes ($\chi^2 = 10.00$, df = 4, $p < 0.003$). The new haplotype, 106, largely dominated (81%) the normal-size group, which also possessed haplotype 107, not observed in the dwarf group. Genetic distinctiveness between the two groups was further indicated by different nucleon diversity indices (Table 2). While mtDNA genetic differentiation was statistically significant, the component of the total mtDNA variance explained by intergroup diversity was low, as indicated by a significant F_{st} value of 0.091 ($p = 0.012$).

Low nuclear gene diversity was reflected in isozyme analysis within both dwarf and normal-size groups, as indicated by heterozygosity estimates, proportions of polymorphic loci, and the mean numbers of alleles per locus (Table 3). It is noteworthy that these values are possibly inflated, since enzymes were selected a priori on the basis of potential polymorphism. Only 2 of the 14 enzyme loci screened were polymorphic, namely LDH-B2* and G3PDH-3*, for which two alleles each were observed (Table 2). Genotype frequencies at G3PDH-3* were in agreement with Hardy−Weinberg expectations ($p > 0.05$), whereas significant deviation from

Table 1. Code, description, and reference of each of the morphometric and meristic variables used in the analysis of morphological differentiation between dwarf and normal forms of lake whitefish.

Code	Description	Reference
Morphometric variables		
POL	Preorbital length: tip of the snout to the anterior fleshy margin to the orbit	Lindsey 1962
OOL	Orbital length: anterior fleshy margin of the orbit to the posterior fleshy margin	Lindsey 1962
PSL	Postorbital length: posterior fleshy margin of the orbit to the most posterior projection of the bony operculum	Lindsey 1962
TTL	Trunk length: posterior end of the operculum to the origin of the dorsal fin	Lindsey 1962
DOL	Dorsal fin length: origin of the dorsal fin to the posterior edge of the fin behind the final ray	Lindsey 1962
LUL	Lumbar length: end of the dorsal fin to the origin of the anal fin	Lindsey 1962
ANL	Anal fin length: origin of the anal fin to the posterior edge of the fin	Lindsey 1962
CPL	Caudal peduncle length: end of the anal fin to the end of the body (structural base of the caudal rays as indicated by a crease when the tail rays are flexed)	Lindsey 1962
FKL	Fork length	Lindsey 1962
MXL	Maxillary length	Lindsey 1962
MXW	Maxillary width	Lindsey 1962
MBL	Mandibule length	Hubbs and Lagler 1974
PCL	Pectoral length: most basal part of the first ray distally to the end of the fin when it is laid flat against the body	Bodaly 1979
PVL	Pelvic length: most basal part of the first ray where it forms a crease with the body distally to the most posterior point of the fin when laid flat against the body	Bodaly 1979
BDD	Body depth: vertical depth from the dorsal origin to the ventral surface of the body	Hubbs and Lagler 1974
HDD	Head depth: vertical depth through the pupil of the eye from the dorsal surface of the cranium to the ventral edge of the gular region	Hubbs and Lagler 1974
ADL	Adipose length: distance from the point where skin and scales meet at the anterior end of the fin posteriorly to the free margin of the fin	Bodaly 1979
CPL	Caudal peduncle length	Bodaly 1979
IOW	Interorbital width	Lindsey 1962
Meristic counts		
ULS	Scales above the lateral line	Lindsey 1962
SPS	Suprapelvic scales	Lindsey 1962
LLS	Lateral line scales	Lindsey 1962
DRC	Dorsal ray count	Lindsey 1962
ARC	Anal ray count	Hubbs and Lagler 1974
PVC	Pelvic ray count	Hubbs and Lagler 1974
PEC	Pectoral ray count	Hubbs and Lagler 1974
UGR	Upper gill raker count	Bodaly 1979
LGR	Lower gill raker count	Bodaly 1979

Table 2. Sample sizes (N), frequency distributions of composite mtDNA haplotypes, and nucleon diversity indices (h) for dwarf and normal forms of lake whitefish in Lac de l'Est.

Haplotype		Dwarf ($N = 38$)		Normal ($N = 41$)	
		Frequency	N	Frequency	N
25	AAAAAAAAAA	0.38	14	0.13	5
106	AAAAAACACA	0.62	24	0.81	33
107	AABAAAAAAA	0.00	0	0.07	3
h		0.484		0.330	

Note: Letter codes designate restriction patterns for *Alu*I, *Cfo*I, *Ava*II, *Dde*I, *Mbo*I, *Hinc*II, *Hae*III, *Rsa*I, *Ban*I, and *Bsp*1286I. The numbers designating haplotypes are in accordance with previous mtDNA studies (Bernatchez and Dodson 1994; Bernatchez et al. 1996).

equilibrium ($p < 0.05$) was observed at *LDH-B2** in the dwarf group.

Population differentiation between dwarf and normal-size lake whitefish groups was indicated by significant heterogeneity in allelic frequency distribution at *LDH-B2** (Table 3). Allelic distribution between the two groups was not significantly different at *G3PDH-3**. As for mtDNA, population subdivision at these two loci was low and significant for *LDH-B2**, ($F_{st} = 0.08$, $p = 0.004$), but not significant at *G3PHD-3** ($F_{st} = 0.004$, $p = 0.19$).

Morphological differentiation

No differences ($p > 0.05$) were found between the sexes for either the dwarf or the normal-size groups when the effects of form were held constant. Consequently, the sexes were pooled for further analyses. All morphological analyses

Fig. 2. Frequency distribution of maturing fish as a function of size (fork length) for lake whitefish captured in June 1993 in Lac de l'Est ($N = 511$).
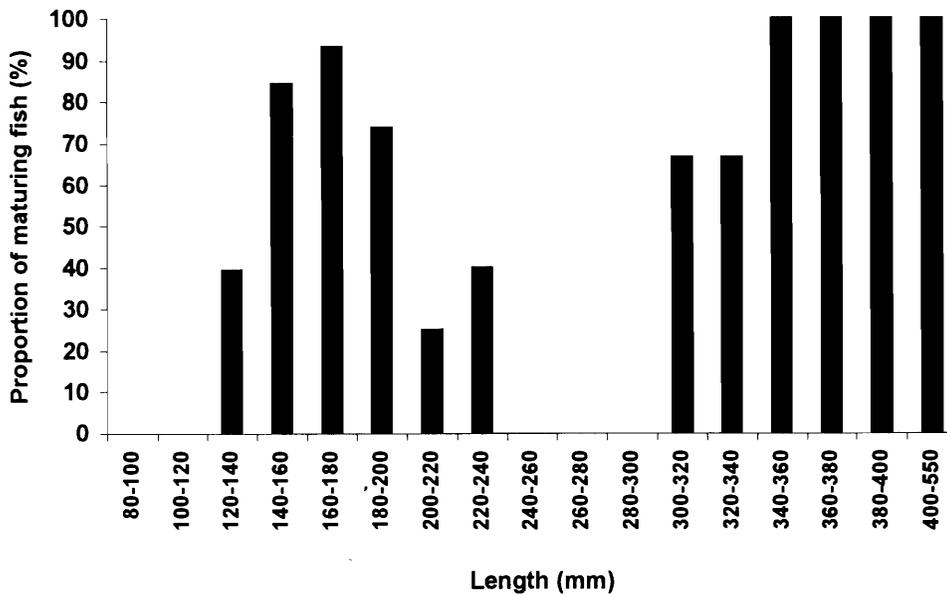

Table 3. Sample sizes (N), allele frequency distributions, average expected heterozygosities (H_{exp}), proportions of polymorphic loci ($P_{0.99}$), and mean numbers of alleles per locus (n_a) calculated at two polymorphic loci for dwarf and normal forms of lake whitefish in Lac de l'Est.

Locus and allele	Dwarf ($N = 35$)	Normal ($N = 29$)	p
*LDH-B2**			
a	0.59	0.36	0.008
b	0.41	0.64	
*G3PDH-3**			
a	0.44	0.34	0.420
b	0.56	0.66	
H_{exp}	0.069	0.065	
$P_{0.99}$	0.143	0.143	
n_a	1.14	1.14	

Note: Levels of significance (p) in frequencies of allelic distribution are provided for the two polymorphic loci.

Table 4. Univariate comparison of 18 morphometric variables between dwarf and normal forms of lake whitefish in Lac de l'Est.

Variable	Standardized mean (log)		Significance (t test)
	Dwarf	Normal	
POL	0.9998	0.9928	ns
OOL	1.0178	1.0205	ns
PSL	1.3326	1.3237	ns
TTL	1.6687	1.6644	ns
DOL	1.3623	1.3620	ns
LUL	1.5878	1.5824	ns
ANL	1.3880	1.3834	ns
CPL	1.5170	1.5121	ns
MXL	1.0573	1.0544	ns
MDL	1.1519	1.1423	ns
MXW	0.6204	0.6150	ns
PCL	1.5377	1.5402	ns
PVL	1.5193	1.5241	ns
BDD	1.6181	1.6094	ns
HDD	1.2620	1.2572	ns
CPD	1.1643	1.1552	ns
ADL	1.1641	1.1412	ns
IOW	1.0624	1.0609	ns

Note: Data are given for a standardized size of 219 mm. ns, not significant.

revealed a strong similarity between the dwarf and normal-size forms of lake whitefish in Lac de l'Est. At a common standardized size of 219 mm, there were no significant differences between the forms, based on univariate analysis of the 19 morphometric variables studied (Table 4). Similarly, the discriminant function analysis indicated no significant difference ($p > 0.05$) between the two groups. Adipose length was the only variable considered discriminant ($F > 1.00$). Consequently, the accuracy obtained for the discriminant function by a posteriori classification was very low for both forms, with 58% of fish classified correctly in each case, which is basically identical with that expected by chance alone (50%). This implies an almost complete overlap in body morphometry between the forms.

Counts at the nine meristic variables were also very similar, although they revealed more accentuated differentiation between the two forms. Thus, significant count differences ($p < 0.05$) were observed in univariate tests for two variables, namely the numbers of pectoral rays and suprapelvic scales, which were both slightly lower in the dwarf group (Table 5). Discriminant analysis also indicated a significant difference ($p < 0.05$) between the forms. These results refute the hypothesis of morphological identity of the two spawning groups. The two variables contributing most to the discriminant axis were those exhibiting significant univariate

Fig. 3. Discriminant analysis of nine meristic variables.
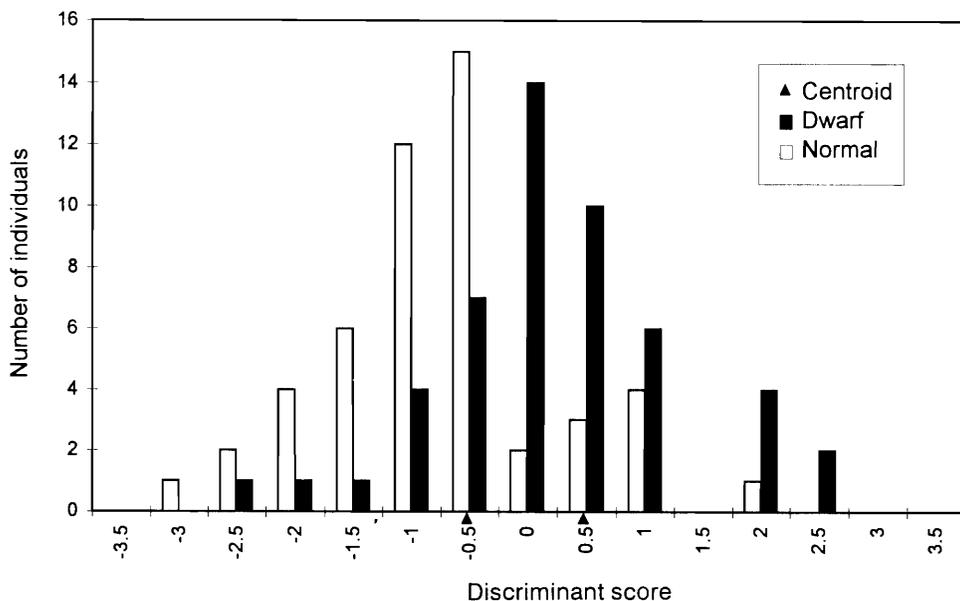
Table 5. Univariate comparison of nine meristic variables between dwarf and normal forms of lake whitefish in Lac de l'Est.

Variable	Mean		Significance (*t* test)
	Dwarf	Normal	
LLS	81.90	81.24	ns
SPS	9.24	9.56	*
ULS	10.42	10.62	ns
DRC	11.78	11.62	ns
ARC	13.28	13.20	ns
PEC	14.56	14.96	*
PVC	11.12	11.32	ns
UGR	9.98	9.96	ns
LGR	15.56	15.94	ns

Note: ns, not significant; *, $p < 0.05$.

differences. Four other variables contributed significantly ($F > 1.00$) to the discriminant function analysis (in decreasing order): pelvic ray count, upper gill raker count, lateral line scales, and scales above the lateral line. Consequently, the accuracy of a posteriori classification using the discriminant function was moderate but much higher than for morphometrics, being 80 and 72% for the normal and dwarf forms, respectively. Nevertheless, a substantial overlap of the two groups was evident in the plot of discriminant scores (Fig. 3).

Discussion

Reproductive isolation and genetic differentiation

The distinction of two spawning size groups of lake whitefish in Lac de l'Est was accompanied by genetic divergence between them. Thus, highly significant differences ($0.001 < p < 0.01$) were observed in the frequency distribution of mtDNA haplotypes and alleles at one of the two polymorphic enzyme loci. Significant differences at several meristic varia-

bles also most likely implied genetic differences, since such traits are known to have a heritable component in fishes (Gardner et al. 1988; Lindsey 1988; Skúlason et al. 1989; Skúlason and Smith 1995). Furthermore, field observations indicated that the two spawning groups apparently use the same spawning locations at the same time (A. Chouinard, unpublished data), which reduces the probability of differential exposure to environmental conditions during their early development. Altogether, these results confirm the hypothesis that lake whitefish in Lac de l'Est which mature at small (dwarf) and normal sizes are structured into two distinct gene pools. However, low F_{st} values at mtDNA and enzyme loci, coupled with the apparent lack of spatial and temporal spawning segregation, suggest that reproductive isolation is incomplete and that gene flow is still occurring between the two forms.

Among all possible prezygotic and postzygotic reproductive isolation barriers (Avise 1994), three are more likely to explain restricted gene flow between dwarf and normal-size lake whitefish in Lac de l'Est despite the apparent absence of habitat and temporal reproductive isolation. First, ecological selection against hybrids imputable to reduced foraging efficiency or other ecological attributes compared with both parental populations could be inferred, as previously documented in threespine stickleback, *Gasterosteus aculeatus* (Hatfield 1995). Second, the fact that sympatric adults of dwarf and normal-size lake whitefish differ in size by one order of magnitude suggests that assortive mate choice may be an important mechanism maintaining reproductive isolation in the face of the potential to hybridize. For instance, assortive mating through body size has been shown to act as a strong premating isolating mechanism between the kokanee and sockeye salmon, two life-history types of *Oncorhynchus nerka* (Foote 1988). Third, while both populations apparently spawn in shallow littoral zones, gill-netting catches on spawning grounds suggest that normal-size lake whitefish may preferentially use deeper sites than dwarf fish do. Such microhabitat segregation of spawning sites has recently been

reported in sockeye salmon (Varnavskaya et al. 1994). At this time, the relative importance of these potential reproductive barriers in restricting gene flow between dwarf and normal-size populations is unknown, and their elucidation will require more detailed studies of the spawning biology of lake whitefish in Lac de l'Est.

The extent of genetic differentiation between normal-size lake whitefish populations from Lac de l'Est was also relatively weak compared with that observed among most sympatric pairs of lake whitefish investigated thus far. In an isozyme study of lake whitefish from Second Musquacook Lake, part of the St. John River drainage, Kirkpatrick and Selander (1979) reported more pronounced differences in electromorph frequencies at three of four polymorphic loci than we observed at a single locus. More recently, Bernatchez and Dodson (1990) observed an alternate fixation of mtDNA haplotypes (F_{st} = 1.00) between dwarf and normal-size lake whitefish populations in Cliff Lake, also part of the St. John River drainage. In a study of reproductive isolation and origins among sympatric trophic ecotypes in southern Yukon, higher levels of genetic differentiation were reported for two of the three lakes investigated, Deazadeash and Little Teslin, whereas sympatric forms were less differentiated in Squanga Lake (Bodaly et al. 1988; Bernatchez et al. 1996). Finally, a level of differentiation comparable to that reported here was reported between dwarf and normal forms in Como Lake, Ontario, where significant differences in mtDNA haplotypes and slight allelic variation at two enzyme loci were observed (Vuorinen et al. 1993). Altogether, these studies indicate that there is a continuum in the extent of genetic differentiation between members of sympatric pairs of lake whitefish, and that dwarf and normal-size spawning forms in Lac de l'Est represent the lower end of it.

Origins of sympatric forms

Two major modes of divergence have generally been invoked to explain the origins of sympatric forms in freshwater fishes (Foote et al. 1989; Bernatchez and Dodson 1990; Hartley et al. 1992; McPhail 1993; Taylor and Bentzen 1993a, 1993b). Under the allopatric model, sympatric pairs are the result of secondary contact between evolutionary distinct lineages. This model is best supported by the demonstration that each population of a given sympatric pair (or flock) shares exclusive genetic characters with other allopatric populations. Under the alternative model of sympatric divergence, forms in a given lake radiated from a single ancestral population. The test of incipient monophyly, whereby populations within lakes share uniquely derived characters observed nowhere else, provides the best support for this model (Smith and Todd 1984). Both modes of allopatric and sympatric divergence have been reported in lake whitefish. In Cliff Lake, the dwarf and normal-size forms segregated for distinct mtDNA lineages that were representative of two glacial races of lake whitefish, thus confirming their allopatric origin (Bernatchez and Dodson 1990, 1991). More recently, a similar scenario best explained the existence of high and low gill-raker forms of lake whitefish in Little Teslin and Squanga lakes (Bernatchez et al. 1996). In contrast, sympatric divergence was genetically supported for Deazadeash Lake and Como Lake forms of lake whitefish, although an allopatric origin could not be entirely ruled out in these cases

(Bodaly et al. 1992; Vuorinen et al. 1993; Bernatchez et al. 1996).

The present study also favoured the origin of genetically distinct dwarf and normal-size forms of lake whitefish in Lac de l'Est through sympatric divergence. Thus, all specimens from this lake possessed a mitochondrial genome typical of the Acadian race of lake whitefish, thus refuting the scenario of secondary contact between glacial races, as observed in Cliff Lake (Bernatchez and Dodson 1990). Furthermore, both populations were dominated by a mtDNA haplotype observed nowhere else, despite extensive surveys (Bernatchez and Dodson 1991, 1994), which provides evidence for incipient monophyly. The weak differentiation of this haplotype from others representative of the Acadian race is also plausible, with its postglacial appearance in Lac de L'Est through mutational events. These results add support to the view that sympatric diversification may be a common, although not generalized, explanation for the origin of sympatric pairs in northern fishes (Smith and Todd 1984; Foote et al. 1989; Taylor and Bentzen 1993a, 1993b).

Lack of morphological specialization

What makes dwarf and normal-size spawning groups of lake whitefish in Lac de l'Est of particular interest is that despite significant genetic differences between them, they were almost completely indistinguishable morphologically. These results are in contrast with most, if not all, studies of sympatric pairs in fishes, which revealed a clear distinction in morphometric and (or) meristic variables between forms (Svärdson 1979; Bodaly 1979; Galat and Vucinich 1983; McPhail 1984; Lavin and McPhail 1986; Fortin and Gendron 1990; Edge et al. 1991; Vuorinen et al. 1993; Taylor and Bentzen, 1993a, 1993b; Snorrason et al. 1994; Leslie and Grant 1994; Junquera and Perez-Gándaras 1994). For instance, more than 95% of a posteriori classification accuracy was reported for sympatric forms of lake whitefish in Como Lake, despite the fact that they could barely be differentiated at the molecular level (Vuorinen et al. 1993).

It is generally hypothesized that competition in exploiting different trophic resources is a major force promoting morphological diversification among closely related groups (Schluter and McPhail 1993; Robinson and Wilson 1994). A corollary of this hypothesis is that sympatric pairs should differ primarily in morphological characters that are functionally related to competition for resources (Smith and Todd 1984). In fish, morphological traits such as eye length, jaw length, head depth, and total gill-raker count are recognized as important to feeding performance and reflect trophic ecological differences (McPhail 1984). In support of the competition hypothesis, sympatric pairs generally differ primarily in such characters, even in the absence of detectable distinction at the molecular level (Magnuson and Ferguson 1987). Generally speaking, fish with larger eyes and more gill rakers are planktivorous, while those with fewer gill rakers and longer jaws are macrophagous and (or) piscivorous (McPhail 1984; Gardner et al. 1988; Skúlason et al. 1989; Taylor and Bentzen 1993a, 1993b). In lake whitefish, differences in these traits are associated with differential utilization of limnetic and benthic trophic niches (Bodaly 1979). None of the morphological characters that could potentially be related to trophic ecology differed between the dwarf and

normal spawning groups of lake whitefish in Lac de l'Est. While our results indicated the existence of two genetically differentiated sympatric populations in this lake, they did not support the hypothesis that these represent ecotypes which are morphologically specialized for distinct trophic niches.

Several reasons can potentially explain the apparent lack of specialization in trophic morphology between the sympatric lake whitefish populations in Lac de l'Est. It could be argued that there has been insufficient time since divergence for significant diversification in morphology to have evolved. However, all north-temperate lakes harbouring sympatric pairs of fishes were postglacially recolonized at about the same time, approximately 10 000 − 12 000 years ago (Hocutt and Wiley 1986). Therefore, the time available for morphological diversification of sympatric populations in Lac de l'Est was apparently no less than that for other sympatric pairs which are much more differentiated. Clearly, time of divergence alone is insufficient to explain the extent of trophic specialization in sympatric pairs. Given the controlling influence of trophic-resource availability on patterns of morphological diversification (Schluter and McPhail 1993; Bush 1994; Robinson and Wilson 1994), an alternative hypothesis is that selective pressures have been sufficient to initiate the process of reproductive isolation, whereas limited resource availability acts as an opposite force controlling the level of morphological diversification that can be reached. Such a scenario best explained the variance in levels of reproductive isolation and trophic specialization observed among sympatric pairs of lake whitefish from southern Yukon (Bernatchez et al. 1996). In Lac de l'Est, the absence of a potential competitor of dwarf lake whitefish, the lake cisco, could have been sufficient to initiate sympatric divergence. However, the intensification of divergence may be limited by the lack of trophic diversity. We are currently testing this hypothesis by comparing the ontogeny of trophic ecology in sympatric populations of Lac de l'Est with that of other sympatric pairs which have reached more pronounced morphological specialization and reproductive isolation.

In summary, this study established that sympatric dwarf and normal-size spawning groups of lake whitefish in Lac de l'Est represent two genetically distinct populations despite the potential for gene flow between them. This, coupled with their low level of morphological diversification, suggests that they represent an early stage of population divergence, making them of particular interest for understanding the gene−environment processes involved in the early steps of speciation events.

Acknowledgments

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