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Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study

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Species pairs of whitefish (*Coregonus* sp.) found in postglacial lakes are used to illustrate the benefits of combining molecular and ecological approaches in studies of adaptive radiation. A detailed mitochondrial DNA phylogeographic analysis revealed that this species complex is composed of five major phylogenetic groups identifying races that survived the Pleistocene glaciations in distinct refugia. It also provided evidence for parallel evolution of sympatric ecotypes, involving both allopatric and sympatric origins. This strongly indicated the role of natural selection in driving their divergence. A comparative analysis of niche partitioning supported the hypothesis that the persistence of differential ecological opportunity throughout their ontogeny may be the selective force promoting the extent of specialization reached by whitefish ecotypes. The possibility that these same ecological processes are also responsible for determining the extent of their reproductive isolation was supported by a negative correlation between the extent of gene flow, estimated from microsatellite loci, and that of morphological specialization between ecotypes in different lakes. Previous experimental studies, however, revealed that embryonic mortality rates were 2 to 5 times higher in hybrid compared to pure crosses of ecotypes from distinct glacial races. This indicates that both genetic and ecological mechanisms may jointly act to determine speciation rate in whitefish. It is hoped that this study will not only stimulate further the interest of evolutionary ecologists for molecular genetics, but also that of molecularists for ecology. Promoting the fusion of such apparently remote fields of research may represent the most important achievement of molecular ecology as a discipline.

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INTRODUCTION

The development of molecular techniques over the last decade has provided evolutionary and conservation biologists with new tools to address fundamental and applied research issues (Haig, 1998; Parker *et al.*, 1998). The rate at which new molecular techniques are being developed, however, still outstrips their efficient incorporation into such studies (Parker *et al.*, 1998), and the use of molecular markers in ecology and conservation is still periodically questioned (e.g. France, 1998). On the other hand, the recent literature shows that studies fully integrating both ecological and genetic information are now emerging (see Hughes, 1998).

The study of adaptive radiation perhaps represents one of the best research opportunities to illustrate the benefits of a multidisciplinary approach in addressing complex biological problems (Givnish & Sytsma, 1997). A comprehensive understanding of how populations differentiate to exploit different resources and ultimately become reproductively isolated involves the realization of three main research objectives: (i) elucidating the evolutionary history of populations under scrutiny, (ii) identifying processes responsible for their phenotypic, ecological and genetic differentiation, and (iii) identifying the mechanisms involved in the development and maintenance of their reproductive isolation. The simultaneous realization of these research objectives necessarily requires the integration of both molecular and ecological approaches. Molecular systematics is most suited for generating a phylogeny that has been derived independently of the traits involved in the radiation studied (Givnish, 1997). The analysis of neutral loci is also necessary to test the null hypothesis of no genetic differentiation, and quantify the amount of gene flow among ecologically differentiated forms. Ecological studies are required to assess the link between morphological and resource-use specialization, or experimentally quantify the importance of ecological processes driving population divergence (e.g. Grant & Grant, 1996; Schluter, 1995).

In this paper, we report on a study of adaptive radiation and speciation in the whitefish species complex, *Coregonus* sp. Whitefish refers here to the Palearctic and Nearctic populations that have been divided into two major nomenclatural species complexes: the European whitefish *C. lavaretus* L. and the North American lake whitefish *C. clupeaformis* Mitchill. These two groups may be either considered as a single polytypic taxon or split into numerous ones (Bernatchez, 1995). Our objective here is to present this system as an illustration of the potential benefits of unifying genetic and ecological approaches to address a fundamental problem in evolutionary biology. We first summarize previously published data documenting the evolutionary history of whitefish using molecular systematics. We then present results of a comparative analysis of phenotypic differentiation, trophic niche partitioning and genetic divergence between ecotypes of different sympatric species pairs. These

provide further evidence for the role of resource-based natural selection in promoting both phenotypic divergence and the development of reproductive isolation.

EVOLUTIONARY HISTORY OF WHITEFISH POPULATIONS

Phylogeographic structure

We inferred whitefish circumpolar phylogeographic structure from mitochondrial DNA (mtDNA) variation (Bernatchez, Dodson & Boivin, 1989; Bernatchez & Dodson, 1990, 1991, 1994; Bernatchez *et al.*, 1996; Pigeon *et al.*, 1997). Over 1800 specimens representing 95 populations throughout the range of distribution and numerous phenotypic forms have been analysed. One hundred and thirty-three mtDNA genotypes have been identified thus far (Fig. 1). These are clustered into major clades, each diagnosed by two or three apomorphies. Clades were generally supported at high bootstrap levels, and sequence divergence were low, varying between 0.40% and 1.20%. All groupings corresponded to a geographic pattern of distribution (Fig. 2). Based on the extent of their nucleotide divergence and distribution, these mtDNA clades identify races that evolved in isolation during the Pleistocene, and indicated that secondary intergradation remained limited. A salient result of this analysis was the geographic partitioning of two mtDNA clades between northern and central Europe. As both regions harbour populations exhibiting a parallel pattern of phenotypic differentiation, this provides evidence for a postglacial replicate adaptive radiation within both groups (Bernatchez & Dodson, 1994).

Origins of sympatric ecotypes

The mtDNA tree was also used to elucidate the mode of speciation and patterns of phenotypic evolution of North American sympatric ecotypes (Bernatchez & Dodson, 1990; Bernatchez *et al.*, 1996; Pigeon *et al.*, 1997). The Yukon territory is an area of overlap between two mtDNA clades, one endemic to Beringia, and the other of a more recent Eurasian origin (Figs 1, 2). In one of the lakes (Little Teslin) harbouring two sympatric ecotypes, the more pelagic form is fixed for the Eurasian clade, whereas the Beringian clade predominates in the more benthic one. This indicated that their sympatric occurrence originated from the secondary contact of two monophyletic groups of whitefish that evolved allopatrically. A more pronounced admixture of mtDNA and nuclear genes between ecotypes in a nearby lake (Squanga) suggested that variable amounts of gene flow occur between ecotypes in different environments (Bodaly *et al.*, 1991; Bernatchez *et al.*, 1996). In a third lake (Dezadeash) from a separate catchment, the presence of the Eurasian clade only provided evidence, along with congruent results based on isozymes, for a distinct evolutionary origin for ecotypes of L. Dezadeash compared to those found in the other two lakes.

Evidence for multiple modes of speciation and parallel evolution was also documented among the sympatric dwarf and normal ecotypes from north eastern North America (Pigeon *et al.*, 1997). Lakes of the St. John river catchment form a zone of secondary intergradation between glacial races characterized in allopatry by diagnostic mtDNA clades (Fig. 3). In L.Cliff both ecotypes are fixed for alternate

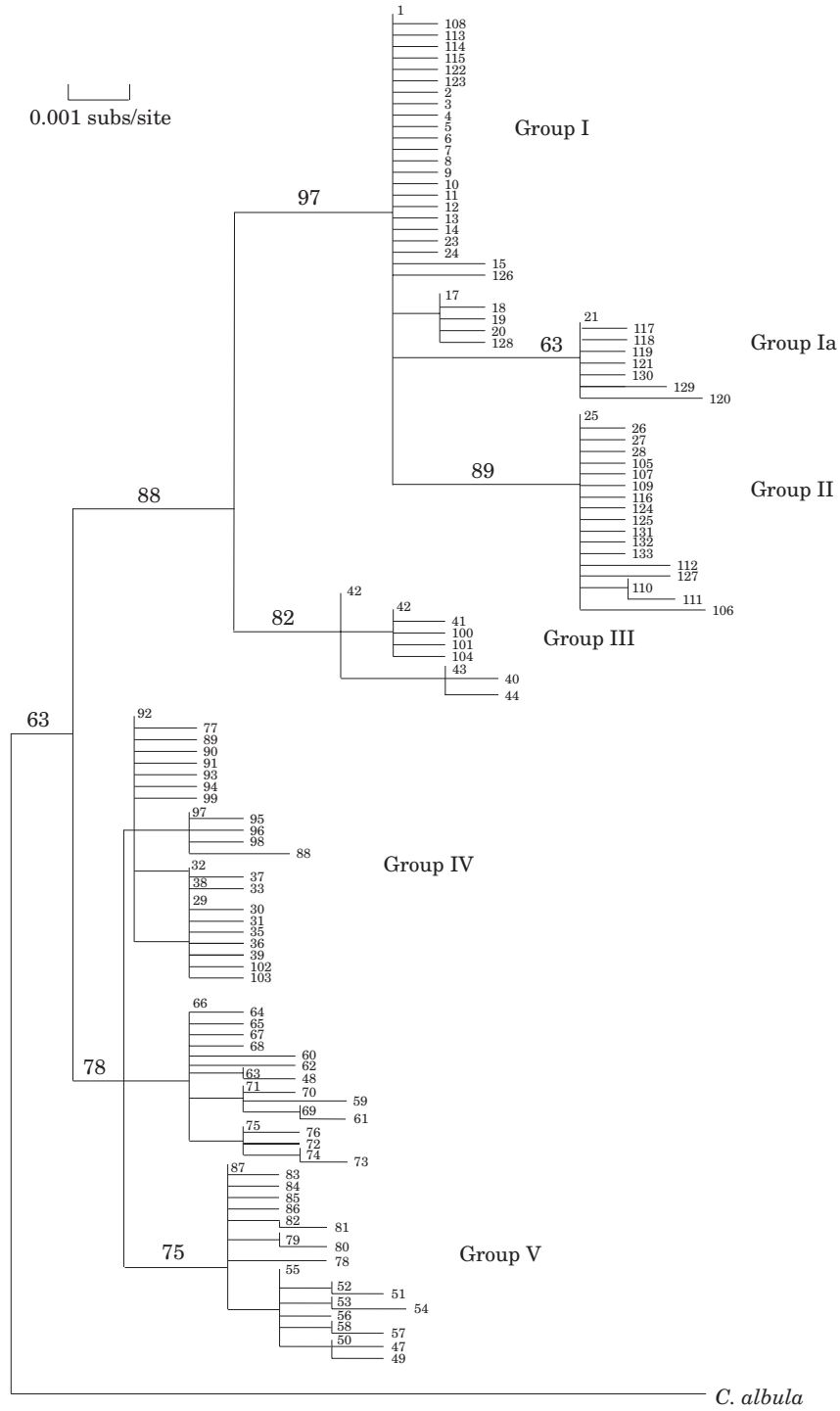


Figure 1. Phylogeographic structure in whitefish. Rooted majority rule consensus tree clustering 133 genotypes into five major (I to V) monophyletic groups. Bootstrap estimates (%) are given along branches.

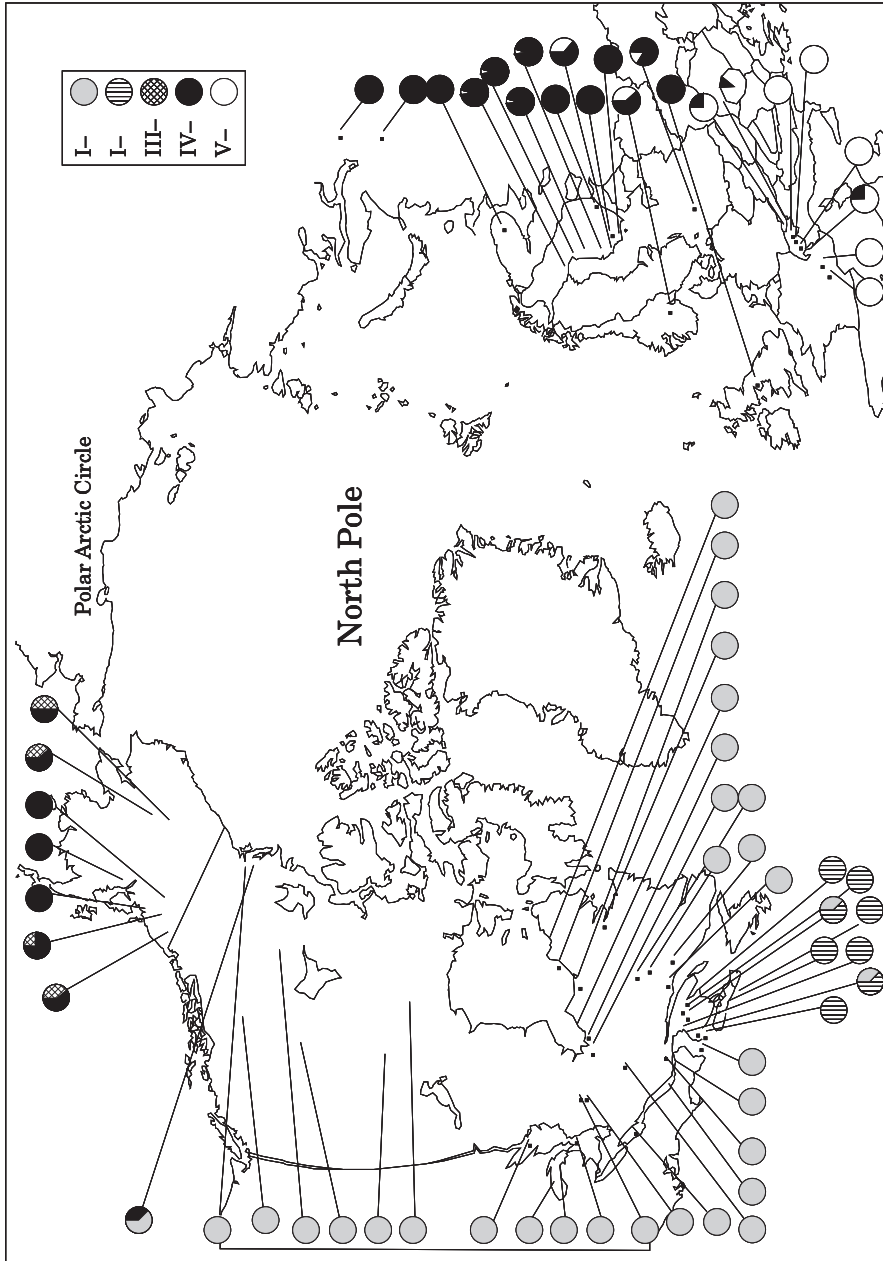


Figure 2. Geographic distribution of the five major phylogenetic groups. (Figs 1 & 2 modified from Bernatchez & Dodson, 1994.)

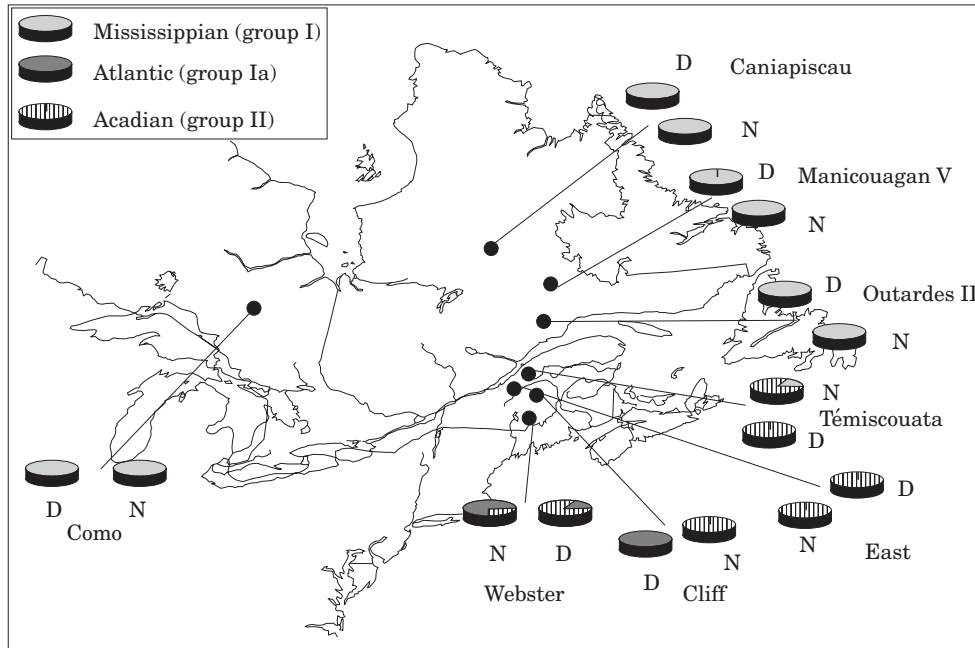


Figure 3. Geographic distribution of mtDNA groups defined in Figure 1 and representing Mississippian (group I), Atlantic (group Ia) and Acadian (group II) glacial races among dwarf (D) and normal (N) whitefish ecotypes from eight north eastern North American lakes. (Modified from Pigeon *et al.*, 1997.)

mtDNA clades, as a result of separate invasions by previously allopatric ancestors. Both mtDNA clades and nuclear genes (Kirkpatrick & Selander, 1979) intermix to various degrees in other lakes, indicating that variable amounts of introgressive hybridization have occurred in different environments. In contrast, a scenario of allopatric origin appeared very unlikely for the sympatric ecotypes found north of the St. Lawrence river in Québec (Fig. 3), since only the Mississippian glacial race (Group I) recolonized this area in postglacial times (Bernatchez & Dodson, 1991). The existence of similar dwarf/normal phenotypic dichotomy in these phylogenetically distinct assemblages provided clear evidence that similar phenotypic patterns evolved in parallel in separate, yet closely related lineages.

Much less information is available regarding the origin of sympatric forms found in European lakes (but see Vuorinen *et al.*, 1986). A recent study of most sympatric cases found in central Alpine lakes based on microsatellites has been informative in that respect (Douglas, Brunner & Bernatchez, 1999). Approximately 30 individuals from each of 20 ecotypic populations representing nine lakes were examined over six microsatellite DNA loci. All sympatric forms were significantly discriminated with regard to allele frequencies, and non-null pair-wise fixation indices. In all cases, smaller genetic distances, as well as lake-specific alleles shared by sympatric ecotypes provided stronger support for the hypothesis of replicate adaptive radiation from a unique ancestral population within a lake than to that of multiple invasions.

Generalities in north temperate fishes

The use of molecular systematics and phylogeographic analysis provided important knowledge about the patterns, and indirectly, the processes of adaptive radiation of whitefish sympatric forms. Along with similar results obtained in studies of other species complexes in north temperate postglacial lakes (e.g. Taylor & Bentzen, 1993; McVeigh, Hynes & Ferguson, 1995; Taylor, Foote & Wood, 1996; Taylor, McPhail & Schluter, 1997), several generalizations on the evolution of phenotypic and genetic divergence in those environments may be made. First, because the environments where they are found cannot be more than 18 000 years old, these species complexes show that both adaptive radiation and reproductive isolation have proceeded rapidly in environmental settings encountered in postglacial lakes. Secondly, replicate evolution of similar ecotypic forms is a common phenomenon among north temperate species complexes. This suggests that natural selection is the main evolutionary force driving the divergence of these forms for two reasons: (i) the traits differing among sympatric forms are highly repeatable across taxa and obviously related to function because of close correlation with dietary patterns, and (ii) random processes, such as genetic drift, are much less likely to generate such parallelism in functional traits than natural selection. Thirdly, a unique mode of speciation is not involved in the divergence of north temperate fish species. Results obtained on the whitefish species complex are of particular interest as they indicate that both allopatric and sympatric modes of speciation may be involved in the evolution of different species complexes within a same evolutionary lineage. Such observations, along with recent theoretical and empirical evidence that sympatric speciation is possible in many ecological contexts (Howard, 1998), indicate that debating which mode of speciation represents a general mechanism may be less crucial than research attempting to elucidate which biogeographical events and ecological processes have favoured population divergence, and ultimately reproductive isolation.

ECOLOGICAL DIVERGENCE AND REPRODUCTIVE ISOLATION OF WHITEFISH ECOTYPES

Introduction

According to the theory of adaptive radiation, divergent natural selection is the ultimate cause of diversification, and resource competition is one of the most important agents of such selection (reviewed in Schluter, 1996a). There is now ample evidence that phenotypic diversification in adaptive radiations of north temperate freshwater fishes may be the outcome of such processes (reviewed in Robinson & Wilson, 1994). The persistence of differentiated forms also requires the persistence of ecological opportunities, such as accessible resources free of other competitors. In the case of limnetic-benthic fish species pairs, the existence of distinct niches is likely to be the rule as both planktonic and benthic food production is a general, although variable, feature of north temperate lakes. The absence of potential competitors is also generally reported as a necessary condition for the persistence of these sympatric forms. However, there are counter examples of lakes in which potential competitors are absent, but without evidence for diversification (McPhail, 1994). This indicates that other ecological processes yet to be identified contribute

to the persistence of sympatric species pairs in a given environment. One hypothesis is that there may be lake to lake variation in the extent of potential for trophic niche partitioning. Most studies directly addressing the fundamental issues of adaptive radiation in fish have focussed on ecological interactions occurring at one given life history stage, generally the adult fish. Yet, ontogenic shifts in trophic niche and competitive interactions are well documented in the fish literature (Persson & Greenberg, 1990; Sandlund, Næsje & Jonsson, 1992; Snorrason *et al.*, 1994). Consequently, a comprehensive view of the role of competition and/or ecological opportunity in driving population divergence must take such possible changes into consideration.

While there is ample evidence that phenotypic diversification in an adaptive radiation is the outcome of divergent natural selection related to differential resource use, the role of such ecological forces in favouring and maintaining reproductive isolation, i.e. ecological speciation, is much less understood (discussed in Schluter, 1996b). A major argument favouring the role of niche-based divergent selection in the evolution of reproductive isolation is related to the fitness cost of producing hybrids of intermediate phenotype, which is expected to compromise their efficiency of resource exploitation (Schluter, 1993). The theory of adaptive radiation thus predicts that selection will drive the development of mechanisms either favouring mating among members of a given population, or limiting reproduction between populations.

Evidence to date that such mechanisms operate in fish species pairs, are largely derived from studies on *Gasterosteus* (Schluter, 1993, 1995; Rundle & Schluter, 1998). No tests of ecological speciation have been performed in other fish species pairs, because of the logistical constraints of performing experimental studies in natural conditions. A possible alternative is to compare the strength of reproductive isolation among species pairs evolved within a same time frame, but phenotypically and ecologically differing in the extent of their potential for occupying distinct trophic niches. As it may be a direct outcome of the strength of their reproductive isolation, the amount of gene flow occurring between sympatric populations can be used as a surrogate in such a test.

In this context, we performed a comparative study of morphological differentiation, trophic niche partitioning, and genetic divergence between dwarf and normal whitefish ecotypes to investigate the possible role of natural selection in driving population divergence, and ultimately, speciation. We first compared the level of morphological and trophic niche differentiation between dwarf and normal ecotypes from different lakes to test the hypothesis that the extent of specialization reached between sympatric ecotypes correlates with the persistence of different trophic resources in a given environment. Secondly, we compared the extent of trophic specialization and genetic divergence between forms in different lakes to investigate the possible role of natural divergent selection on the development of reproductive isolation.

Methods

Morphological analysis

Nine meristic and 19 morphometric variables were measured on 35–75 individuals of each dwarf and normal ecotypes from L. Cliff and L. East (detailed in Chouinard

et al., 1996). Morphological measurements were log-transformed and standardized to the mean fork length using the allometric method of regression against total length. To examine morphological differentiation between spawning groups, meristic and size-adjusted morphometric data sets were separately analysed by univariate *t*-tests and multivariate discriminant function analysis (DFA). The data were initially examined for differences between sexes which were not significant.

Niche partitioning

The overall strategy consisted in comparing the spatio-temporal distribution and trophic use of dwarf and normal whitefish ecotypes from L. Cliff and L. East through their ontogeny, from hatching to the adult stage. Background information on each lake is presented in Chouinard *et al.* (1996) and Chouinard & Bernatchez (1998). Larvae were studied only in L. Cliff (see below). The horizontal and vertical distribution, as well as trophic use was followed from hatching until juvenile metamorphosis, a period of approximately six weeks (detailed in Chouinard & Bernatchez, 1998). Larvae from each form could be diagnostically identified by alternate fixation of mtDNA genotypes in L. Cliff (Pigeon *et al.*, 1997).

Juvenile and adult fish were sampled using 63 m × 1.8 m gill nets comprising nine panels graded from 12.5 to 75 mm stretched mesh size. Sampling was conducted in 1994 in each lake before (mid- to late June) and following (mid- to late August) thermal stratification. At each sampling station, nets were set out at the surface, mid-water (10 m) and bottom (15–20 m), and checked every 4 h for 96 consecutive hours. All fish were counted, measured, weighed, and stage of gonad development was estimated to classify whitefish as dwarf or normal (Chouinard *et al.*, 1996).

Stomach contents of all normal and subsamples of the more abundant dwarf ecotype were preserved in 4% formaldehyde. Prey items were identified, enumerated and taxa were combined into different functional categories according to size and habitat criteria (see Results). The diet of each fish was first quantified as the weight proportion of each food category after prey size/type correction into equivalent weight units (Culver *et al.*, 1985; Tremblay & Magnan, 1991; Magnan, unpubl. data). Weight-adjusted diet compositions were further adjusted for the relative occurrence of each prey type prior to generating a standardized index of importance for each food category. These indices were then used to estimate the extent of trophic niche overlap between ecotypes in each lake according to Schoener (1970). Schoener's (1970) resource overlap index varies between 0 and 1, 0 corresponding to a completely distinct diet and 1 to complete diet overlap with respect to food category composition. Coefficients for spatial distribution were also quantified from the relative frequencies of each ecotype found at different depths based on Schoener's (1970) equation. The heterogeneity in horizontal distribution of each ecotype was statistically assessed using randomization chi-square tests (Roff & Bentzen, 1989) available in the REAP package (McElroy *et al.*, 1992).

Genetic analysis

Forty adult specimens of each dwarf and normal ecotypes from each lake were analysed at eight microsatellite loci. Six of these (*Bwf-1*, *Bwf-2*, *Coel-22*, *Coel-23*, *C2-157*, *C4-157*) are dinucleotide repeats specifically developed for *Coregonus* (Bernatchez, unpub.; Patton *et al.*, 1997; Turgeon, unpub.), whereas the other two are duplicated trinucleotide repeats originally developed from another salmonid, *Oncorhynchus mykiss*

(Morris, Richard & Wright, 1996). PCR were set up individually for each locus, as described in Brunner, Douglas & Bernatchez (1998). PCR products were separated on 5-6% acrylamide sequencing gels and autoradiographed. Electrophoresis, 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 the GENEPOP computer package, version 3.1 (Raymond & Rousset, 1995). GENEPOP was also used to estimate departures from Hardy–Weinberg equilibrium over all loci. This uses the Markov chain method (Guo & Thompson, 1992) to obtain unbiased estimates of Fisher's exact test through 1000 iterations, in order to test the alternative hypotheses of deficiency or excess of heterozygotes (Rousset & Raymond, 1995). This procedure was also used to perform homogeneity tests of allele frequency distribution at each locus in order to test the null hypothesis of no genetic differentiation between ecotypes within 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 & Cockerham, 1984) using Fstat, version 1.2 (Goudet *et al.*, 1996). Genetic differentiation was also estimated using mutational differences among alleles and assuming a strict stepwise mutation model by computing pairwise standardized R_{ST} with the program R_{ST} Calc (Goodman, 1997). The 95% confidence intervals on both θ and R_{ST} estimates were defined by the bootstrapping procedure over loci (number of replicates = 1000) available in both programs. For both θ and R_{ST} estimates, departure from the null hypothesis (θ and R_{ST} estimates = 0) was also statistically assessed by permutation procedures (1000 iterations). The amount of gene flow between ecotypes within each lake was estimated both from the relationships relating Nm to θ or R_{ST} ($F_{ST} = 1/(4Nm + 1)$) and 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).

Results

Morphological differentiation

The size of the dwarf ecotype and the juvenile and adult stages of normal ecotypes were very similar in both L. East and L. Cliff (Table 1). There were no significant differences ($P > 0.05$) between ecotypes from each lake, based on univariate analysis of the 19 morphometric variables and discriminant function analysis (Eigenvalue = 0.17 and 0.09 in Cliff and L. East respectively). Only one (adipose fin length) and two (pectoral fin and caudal peduncle length) variables were discriminant ($F > 1.00$) in L. East and L. Cliff, respectively.

Meristic variables of both ecotypes were also very similar in L. East. Of particular importance is the complete overlap ($P > 0.05$) in gill raker counts of both ecotypes (mean dwarf = 25.6, normal = 25.9, Fig. 4). Significant count differences were observed in univariate tests for two variables (number of pectoral fin rays and suprapelvic scales), and the discriminant function analysis indicated a significant difference

TABLE 1. Sample sizes and fork lengths (mm) for whitefish dwarf ecotype (D), juvenile (J), and adult (A) of the normal ecotype for Lakes Cliff and East at two sampling periods

	June			August		
	Dwarf	Normal (J)	Normal (A)	Dwarf	Normal (J)	Normal (A)
Cliff Lake	484	16	45	566	22	31
Mean	196	163	375	208	187	372
Range	130–268	135–294	345–414	151–266	163–272	308–425
SD	33	47	16	23	30	23
East Lake	553	54	38	763	23	21
Mean	157	170	382	164	208	340
Range	122–250	135–298	308–485	110–254	139–92	304–377
SD	21	38	43	24	51	22

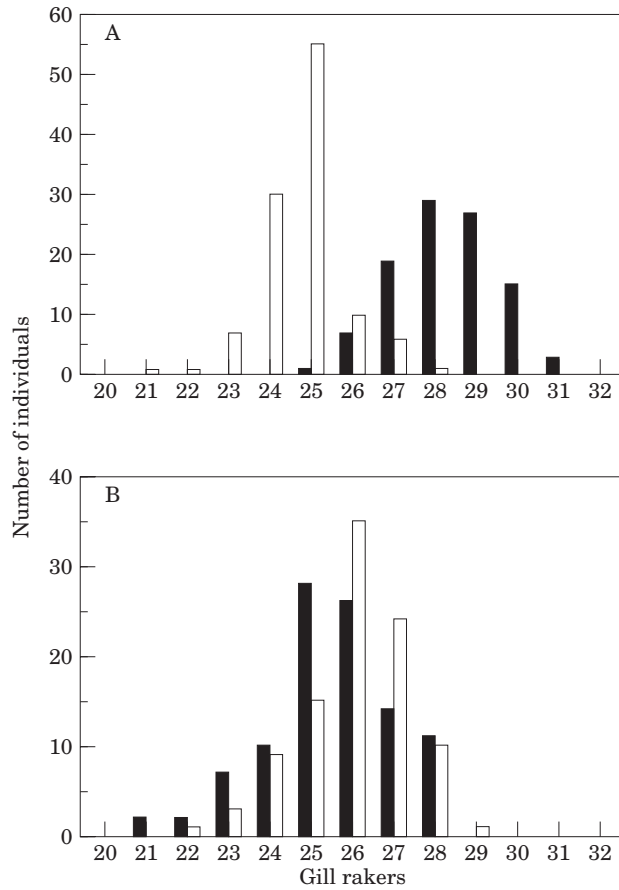


Figure 4. Absolute frequency distribution of gill raker counts between dwarf (■) and normal (□) ecotypes from Cliff (A) and East (B) lakes.

between ecotypes (Eigenvalue = 0.22). These differences nevertheless resulted in broad overlap of discriminant scores between both ecotypes (dwarf centroid = 0.5, normal = -0.65, Fig. 5).

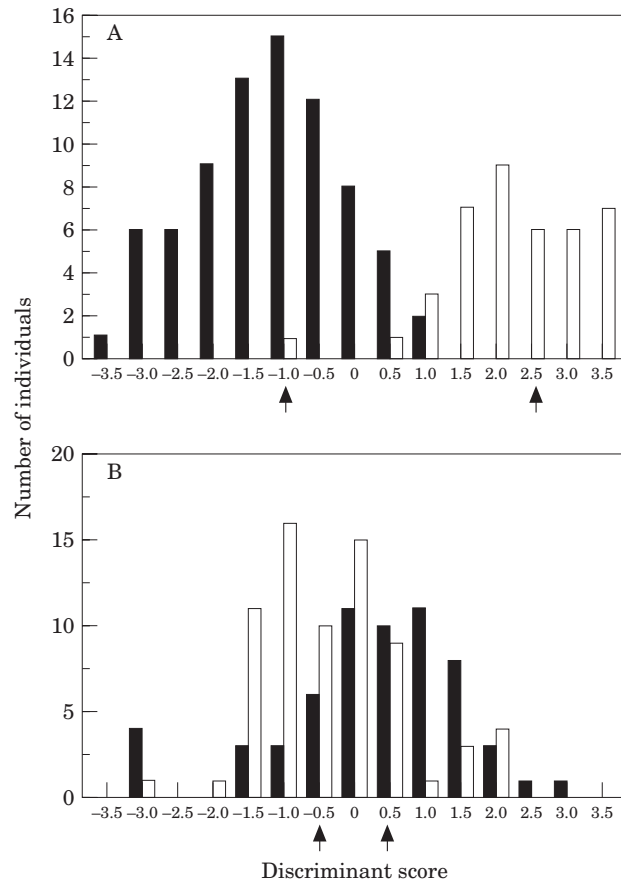


Figure 5. Discriminant analysis of dwarf (■) and normal (□) whitefish ecotypes from Cliff (A) and East (B) lakes based on nine meristics variables. Centroids of discriminant scores of each group are indicated by arrows.

In contrast, the meristic variables of dwarf and normal ecotypes in L. Cliff were very distinct. Univariate tests revealed significant differences for all but three variables, and all were considered discriminant ($F > 1.0$). There was very little overlap in gill raker counts, which averaged 24.9 in normal and 27.6 in dwarf ecotypes ($P < 0.000001$, Fig. 4). This resulted in a highly significant difference of the discriminant function analysis (Eigenvalue = 3.0), and very limited overlap in discriminant scores (dwarf centroid = -1.0, normal = 2.5, Fig. 5).

Niche partitioning

Results of larval niche partitioning in L. Cliff are detailed in Chouinard & Bernatchez (1998). Briefly, an almost completely similar patterns in horizontal distribution, vertical migration and trophic use was observed between ecotypes at the different periods sampled, resulting in nearly maximal Shoener's niche overlap index for both spatial (range 0.914–0.992) and trophic (range 0.965–0.997) uses. Since there was no evidence for niche partitioning between L. Cliff ecotypes which

TABLE 2. Schoener's index of niche overlap (D) calculated from differences in vertical distribution (Depth), and their weighted importance of main classes of food items (Diet) between dwarf ecotype (DW), juvenile (NJ) and adult (NA) stages of normal whitefish ecotype from Lakes Cliff and East for two sampling periods

	June			August		
	DW–NJ	DW–NA	NJ–NA	DW–NJ	DW–NA	NJ–NA
Depth						
L. Cliff	0.769	0.956	0.813	0.827	0.726	0.899
L. East	0.891	0.578	0.591	0.781	0.661	0.443
Diet						
L. Cliff	0.998	0.008	0.009	0.387	0.305	0.820
L. East	0.011	0.011	0.443	0.968	0.215	0.229

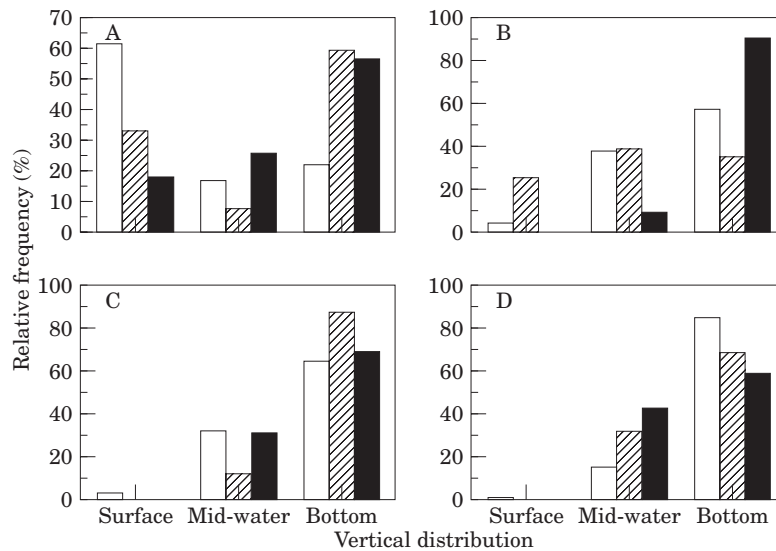


Figure 6. Relative abundance (%) of dwarf ecotype (□), juvenile (▨), and normal (■) stages of normal ecotype at three vertical depth strata (0 m, 10 m, 15–20 m) for two periods (June and August) in East (A, B) and Cliff (C, D) lakes.

are more differentiated at later stages both in morphology and trophic use than in L. East (see below), the study of larval niche partitioning was not undertaken for that lake.

For juvenile and adult fish, the observed distribution of ecotypes translated into high indices of spatial niche overlap in both lakes (Table 2). There was no general trend in the pattern of vertical distribution for both dwarf and normal ecotypes (Fig. 6). In L. East, the dwarf ecotype mainly occupied the limnetic zone, and both juvenile and adult fish of the normal ecotype occupied the benthic zone in June. In August, all three groups significantly ($0.0001 < P < 0.006$) differed in their vertical distribution, but not in the predicted manner. Both dwarf and normal adult fish were predominantly found in the benthic zone, whereas normal juveniles were more

evenly distributed throughout the water column. In L. Cliff, both ecotypes were more abundant in the benthic zone at all times (Fig. 6).

The ecotypes differed more importantly in their trophic use (Fig. 7). A strong dichotomy between adult of both ecotypes was observed in both lakes in the predicted manner. Dwarf fish fed almost exclusively on zooplankton (except in August in L. Cliff). Zoobenthos and fish preys predominated the diet of normal adult fish in L. Cliff at both periods. In L. East, the diet of normal adult fish was almost exclusively composed of benthic prey (large zoobenthos and molluscs) in June. In August, however, both of these prey types were completely absent, and small zoobenthos represented approximately 50% of their diet, the other half being composed of terrestrial insects and zooplankton. Overall differences in diet composition between adult dwarf and normal ecotypes translated in low indices of trophic niche overlap ($0.008 < D < 0.305$) in both lakes (Table 2).

A more pronounced overlap in trophic use was observed between the dwarf ecotype and juvenile fish of the normal ecotype. In L. Cliff, both groups fed almost exclusively on zooplankton in June, whereas in August, juvenile normals switched almost exclusively to small and large benthic prey. This resulted in a low index of trophic niche overlap at that time ($D = 0.390$). A reversed situation was observed in L. East. There was almost no overlap in diet in June, the normal juveniles essentially feeding on molluscs and large zoobenthos. In August, a shift from benthic prey to zooplankton was observed for normal juveniles. This resulted in a nearly maximal overlap in trophic niche ($D = 0.968$) between juvenile normal and dwarf ecotypes. Another difference observed between the two lakes was in the average weight of stomach contents, which was generally stable in time for a given group within lake, but lower in L. East than in L. Cliff (Fig. 7).

Genetic differentiation

In L. Cliff, no evidence for either significant ($P > 0.05$) excess or deficit of heterozygotes was detected by multi-locus probability tests (Fisher's method). We therefore cannot reject the null hypothesis of random mating for the two ecotypes in that lake. In L. East, heterozygote deficits were observed both for dwarf ($P = 0.0082$) and normal ($P = 0.0021$) ecotypes (Table 3). This could perhaps indicate the presence of undetected hybrids within each sample. No significant differences (Wilcoxon: $P > 0.05$) in number of alleles and gene diversity were observed among all samples. This confirms that subsequent estimates of genetic differentiation and derived N_m values are not strongly biased by variance in intra-population diversity.

Both homogeneity tests of allele frequency distribution and estimates of genetic differentiation confirmed that dwarf and normal ecotypes are genetically distinct populations in each lake. Highly significant ($P < 0.001$) heterogeneity in allele frequency distribution was observed for a majority of loci in both cases. Similarly, both θ and R_{ST} estimates were significantly different from zero. The extent of genetic differentiation between dwarf and normal ecotypes was more pronounced in L. Cliff than in L. East. Thus, all eight loci showed significant differences in allele frequency distribution in L. Cliff, whereas this was the case for only four in L. East. Both θ or R_{ST} estimates were approximately five times higher in L. Cliff than L. East, with non-overlapping 95% confidence intervals (Table 4). Consequently, gene flow estimates derived from those parameters, as well as from the private allele method, were much lower in L. Cliff than in L. East. The stronger differentiation observed

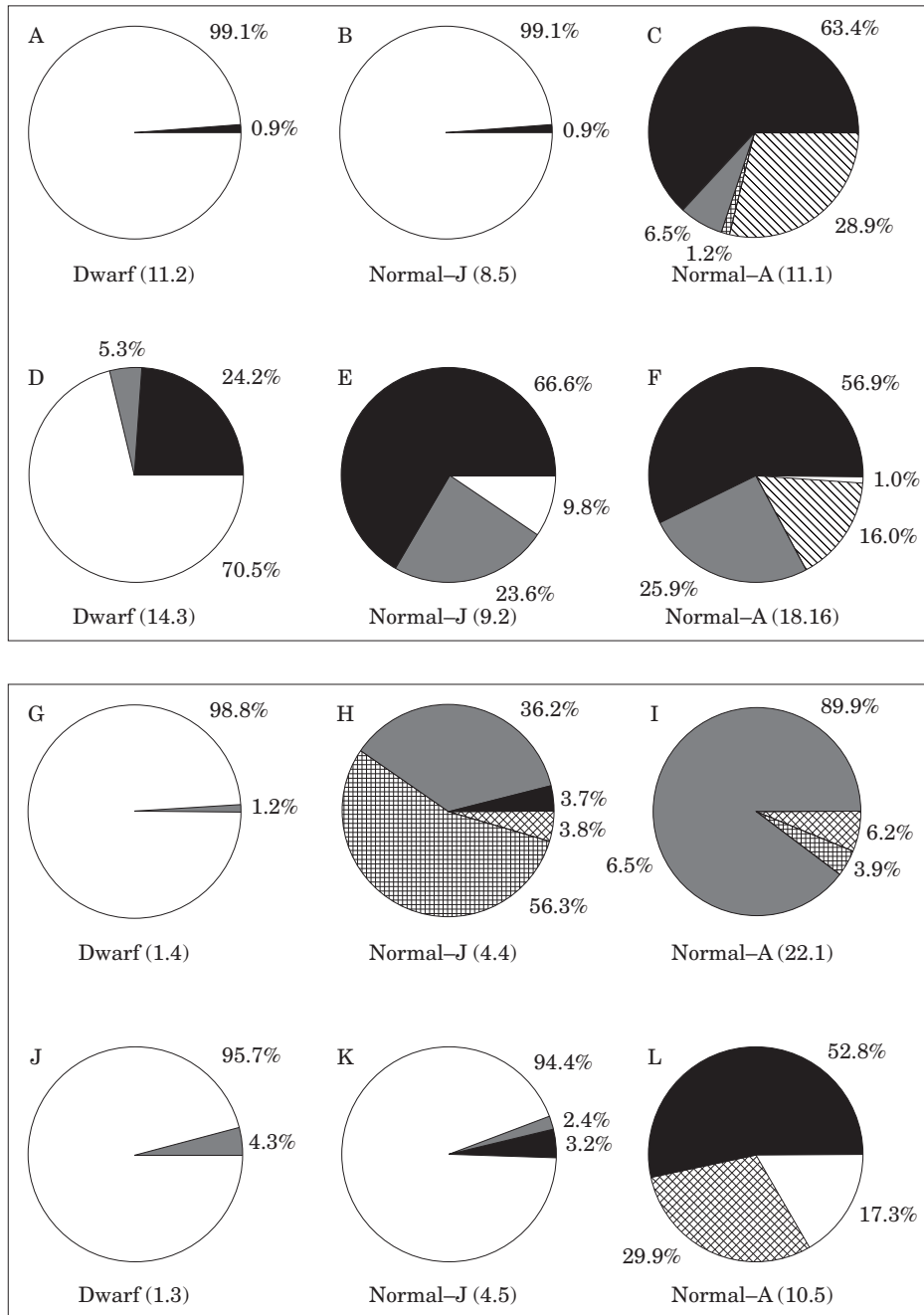


Figure 7. Diet composition expressed as the weighted importance (%) of prey categories for dwarf ecotype, juvenile (J) and adult (A) stages of normal ecotype in Cliff (A-F) and East (G-L) lakes for two periods, June (A-C, G-I) and August (D-F, J-L). (□) zooplankton; (■) small zoobenthos (<0.1 mg dry weight); (■) large zoobenthos (>0.5 mg dry weight); (▨) molluscs; (▩) fish, (▧) terrestrial insects. Average wet weight (g) of stomach contents of each population is given in parenthesis.

TABLE 3. Sample size (N), number of alleles (A), most common allele, allele range (in base pairs), observed heterozygosity (H_o) and gene diversity (H_e) for eight microsatellite loci analysed for dwarf and normal ecotypes in Lakes East and Cliff

	<i>Bwf-1</i>	<i>Bwf-2</i>	<i>C2-157</i>	<i>Cocl-22</i>	<i>Cocl-23</i>	<i>C4-157</i>	<i>Pupup-300</i>	<i>Pupup-600</i>
L. East (dwarf)								
N	39	40	40	39	40	39	40	33
A	8	6	9	6	5	7	2	13
Common	220	157	147	125	266	293	339	590
Range	212–216	147–159	121–167	105–127	260–270	285–301	336–339	524–62
H_o	0.589	0.659	0.600	0.589	0.560	0.333	0.351	0.909
H_e	0.577	0.640	0.770	0.897	0.566	0.534	0.451	0.888
L. East (normal)								
N	40	40	40	37	40	35	40	35
A	6	4	10	6	6	5	2	17
Common	220	157	145	123	266	293	339	590
Range	212–224	147–159	121–167	105–127	260–270	285–301	336–339	524–62
H_o	0.512	0.146	0.625	0.757	0.575	0.657	0.350	0.943
H_e	0.573	0.161	0.815	0.635	0.578	0.757	0.475	0.886
L. Cliff (dwarf)								
N	40	40	40	36	40	39	40	39
A	9	3	9	4	4	6	3	18
Common	220	157	147	123	266	293	339	590
Range	212–228	153–161	133–167	117–127	260–266	281–301	333–339	563–63
H_o	0.925	0.175	0.675	0.750	0.750	0.487	0.350	0.897
H_e	0.750	0.165	0.707	0.555	0.525	0.666	0.425	0.894
L. Cliff (normal)								
N	37	40	40	40	40	38	38	38
A	4	4	7	3	4	3	3	8
Common	214	157	147	123	262	297	336	590
Range	210–220	147–157	147–169	121–127	260–266	293–301	333–339	560–62
H_o	0.540	0.475	0.600	0.700	0.650	0.395	0.420	0.710
H_e	0.446	0.461	0.486	0.567	0.600	0.436	0.507	0.646

TABLE 4. Estimates of genetic differentiation and gene flow estimates (Nm) between dwarf and normal ecotypes based on allelic θ and molecular (R_{ST}) variance with their 95% confidence intervals obtained by bootstrapping over loci. Confidence intervals not available for gene flow estimates derived from the private allele method

Lake	θ	R_{ST}	Nm (θ)	Nm (R_{ST})	Nm (private)
L. Cliff	0.225	0.314	0.86	0.545	1.29
	0.132–0.304	0.273–0.371	0.82–1.89	0.67–0.92	na
L. East	0.045	0.060	5.56	4.17	4.08
	0.015–0.087	0.035–0.120	2.87–16.66	2.09–7.08	na

by considering mutational information likely reflects the modal distribution of alleles, and the variable extent of overlap of these modes between lakes. This is particularly well illustrated at two selected loci (Fig. 8).

Discussion

Phenotypic divergence and trophic specialization

According to the hypothesis that phenotypic divergence between sympatric dwarf and normal whitefish ecotypes is mainly driven by competitive interactions, we

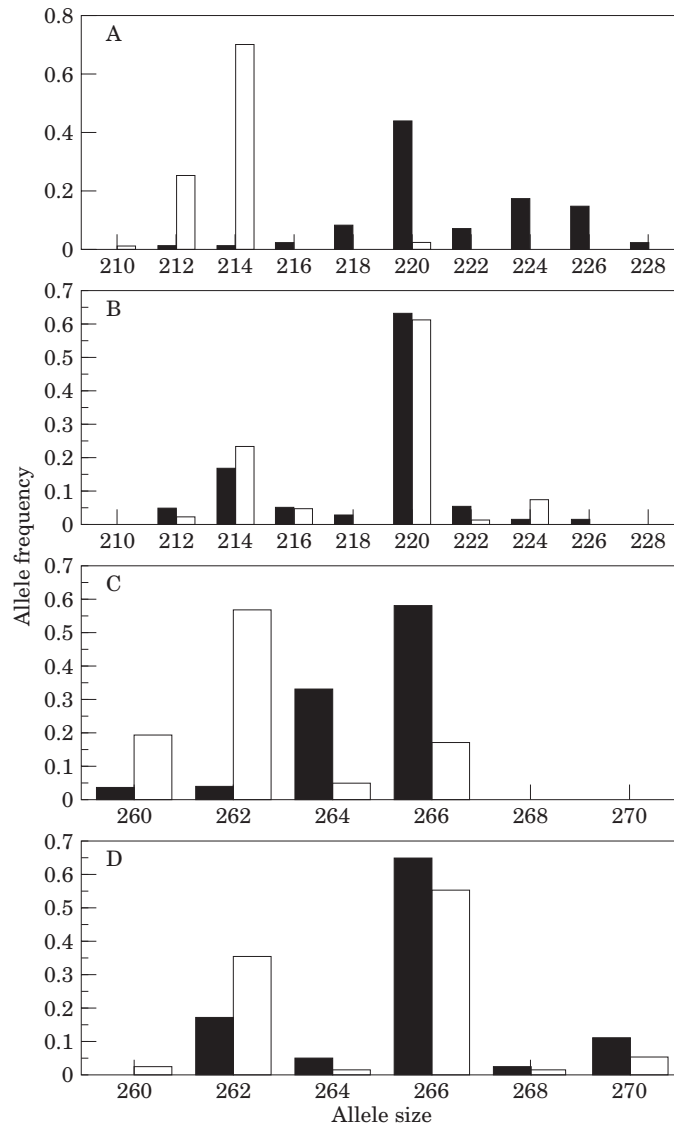


Figure 8. Relative frequency of allele (in base pairs) distribution between dwarf (■) and normal (□) ecotypes in Cliff (A, C) and East (B, D) lakes at two selected loci, *Bwf-1* A, B, *Coel-23* C, D.

predicted that both forms should be differentiated by phenotypic traits that can be functionally related to distinct resource use. Our results both partly supported and contradicted this prediction. First, unlike most cases of sympatric fish species pairs (reviewed in Robinson & Wilson, 1994), we found very limited evidence for morphometric differences (size-adjusted) between forms within each lake. L. East dwarf and normal fish were also almost indistinguishable in meristic traits, despite the fact that they represent genetically distinct populations. In contrast, ecotypes from L. Cliff. were strongly differentiated for meristic traits, and the number of gill rakers, the trait most likely functionally related to trophic use in whitefish (Svärdson,

1979; Lindsey, 1981), was the most discriminant. Thus, a higher number of gill rakers in the dwarf ecotype supports the hypothesis that these fish are more adapted to feeding on small particles, such as zooplankton, than the normal ecotype. The only two discriminant morphometric traits (length of pectoral fin and caudal peduncle) in the DFA are also functionally related to the efficiency of trophic uses (Gatz, 1979; Webb, 1984; Malmquist *et al.*, 1992), further supporting the hypothesis that ecotypes in L. Cliff differ in functional traits related to use of trophic resources.

Because both East and Cliff lakes were postglacially recolonized at the same period (10 000–12 000 years ago), the time available for their diversification cannot explain the difference in the extent of phenotypic specialization between ecotypes in each of them. Given the controlling influence of trophic-resource availability on patterns of morphological diversification (Robinson & Wilson, 1994), an alternative explanation is that the ecological opportunity for specializing on a particular resource may be more persistent in L. Cliff than L. East through ontogeny. This would not appear to be related to inter-lake differences in habitat availability, as an important overlap in the spatio-temporal distribution of dwarf and normal ecotypes was observed at all life-history stages in each lake. Although not strictly quantified, it also seems unlikely that differences in ecological opportunity between lakes would be in the form of overall differences in prey diversity, as numerous taxa for the two basic functional prey categories (zooplankton vs benthic organisms) composed the diet of whitefish in each lake (data available upon request).

Instead, differences in ecological opportunity could take the form of inter-lake variation in the production of different prey categories throughout the growing season. Reduction of secondary production and particularly of benthic organisms, towards the end of the growing season is a common but variable feature of most north temperate lakes (discussed in Tremblay & Magnan, 1991). Several observations support the hypothesis of differential food production in both lakes studied. First, much lower average stomach weights were observed in L. East compared to L. Cliff. Secondly, benthic prey still largely predominated the diet of both juvenile and normal stages of ecotype in L. Cliff in August. In L. East, benthic prey was almost absent in the diet of juvenile fish and represented only 50% of the diet of adult fish at the end of the summer. Because allopatric (always of normal ecotype) populations of lake whitefish are primarily benthic feeders (Scott & Crossman, 1973), it is possible to argue that the seasonal reduction of benthic prey in the diet of normal ecotype fish in L. East is more related to resource depletion than selection for alternate prey. This, however, remains to be firmly assessed by a detailed temporal analysis of prey production.

Assuming depletion of benthic production in L. East, normal ecotype fish from L. East would need to periodically switch to alternate planktonic prey. The theory of adaptive radiation would predict in such a situation that a trade-off in feeding efficiency throughout ontogeny would occur, less favouring the evolution of a specialized phenotype. The intermediate numbers of gill-rakers observed for both forms in L. East compared to L. Cliff corroborates this prediction.

In summary, these results provided additional support for the general view that the phenotypic divergence of sympatric fish species pairs is driven mainly by divergent natural selection. They also tend to support the more specific hypothesis that the extent of such divergence is correlated with the persistence of ecological opportunities for occupying distinct trophic niches throughout their ontogeny.

Divergent natural selection and reproductive isolation

The hypothesis that ecological forces are important in driving speciation, predicts that reproductive isolation should have evolved more rapidly between more phenotypically and ecologically differentiated forms, since variation in such traits reflects the intensity of divergent natural selection in different environments. The extent of genetic divergence quantified between dwarf and normal ecotypes in Cliff and East lakes from the analysis of variation at microstallite loci supported this view. Evidence for restricted gene flow observed in both lakes further demonstrated that dwarf and normal ecotypes of whitefish are reproductively isolated (Kirkpatrick & Selander, 1979; Chouinard *et al.*, 1996). The extent of differentiation, however, was much more pronounced in L. Cliff. than in L. East. The absence of shared alleles at several loci (see also Bernatchez & Dodson, 1990), likely indicated a complete reproductive isolation in L. Cliff, whereas the pattern in L. East indicates that moderate gene flow is still occurring. These results can thus be interpreted as reflecting a different level of speciation reached by distinct ecotypes in each lake. Thus, the correlation observed in whitefish ecotypes between the extent of genetic divergence and that of trophic specialization is congruent with the prediction that their reproductive isolation evolved as a consequence of the same ecological forces promoting phenotypic divergence.

Admittedly, however, these results are still not sufficient to strictly rule out alternative explanations to such processes. This must await a positive correlation between the extent of trophic specialization and that of reproductive isolation generalized to more than two environments, and ultimately to other species complexes. Similarly, the hypothesis that the extent of trophic specialization reached between ecotypes is related to the potential of using alternate resources throughout their ontogeny in different environments must be further investigated in additional lakes. Finally, critical experiments designed to test the hypothesis of a trade-off in fitness versus trophic specialization still needs to be performed in whitefish.

CONCLUSIONS

The goal of this paper was to synthesize knowledge on the adaptive radiation of whitefish as an illustration of the potential interest and benefits of unifying genetic and ecological approaches to address a fundamental problem in evolutionary biology. Although still preliminary on many issues, our results to date nevertheless show that the understanding of crucial aspects of the whitefish adaptive radiation, such as the origin and mode of divergence, and the extent of genetic divergence between species pairs could not have been gained without molecular genetic information. Similarly, insights into the potential role of differential trophic use opportunities in driving phenotypic divergence could only be obtained by ecological studies. Even more importantly, the fully integrated use of both approaches to estimate the congruence between the extent of gene flow and that of trophic specialization provided us with a unique way of documenting the role of ecological opportunities in driving speciation processes. This research program is certainly not the only one making an integrative use of both molecular genetics and ecological approaches in the study of adaptive radiation, or other important evolutionary issues (see Givnish & Sytsma, 1997). Nevertheless, it is our perception that the dichotomy between the two disciplines

still represents the rule rather than the exception in the discipline of evolutionary ecology. It is hoped that the results of this research program, along with other similar ones, will not only stimulate further the interest of evolutionary ecologists for molecular genetics, but also that of molecular geneticists for ecology. Promoting the fusion of such apparently remote fields of research may represent the most important achievement of molecular ecology as a discipline.

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