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Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill)

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Abstract

In this study, we investigate the relative role of historical factors and evolutionary forces in promoting population differentiation in a new case of sympatric dwarf and normal ecotypes of the rainbow smelt (*Osmerus mordax* Mitchill) in Lac Saint-Jean (Québec, Canada). Our first objective was to test the hypothesis that the evolution of sympatric smelt ecotypes in Lac Saint-Jean has been contingent upon the secondary contact between two evolutionary lineages in postglacial times. Secondly, the Q_{ST} method was applied to test the null hypothesis that the extent of phenotypic differences relative to that of neutral marker variation would be similar in comparisons involving populations within and among ecotypes. Thirdly, we applied a quantitative-genetic method as an exploratory assessment as to whether the amount of gene flow observed between populations could affect divergence in adaptive traits under specific conditions. This study revealed a unique situation of dwarf and normal smelt ecotypes that are, respectively, characterized by selmiparous and iteroparous life histories and the occurrence in each of two genetically distinct populations that synchronously use the same spawning habitat in two tributaries. Historical contingency has apparently played little role in the origin of these populations. In contrast, an important role of divergent natural selection in driving their phenotypic divergence was suggested. While divergent selection has apparently been strong enough to maintain phenotypic differentiation in the face of migration, this study suggests that gene flow has been sufficiently important to modulate the extent of adaptive differentiation being achieved between ecotypes, unless the extent of stabilizing selection acting on smelt ecotypes is much more pronounced than usually reported in natural populations.

Keywords: adaptive radiation, ecotypes, gene flow, microsatellite, population divergence, selection

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Introduction

Elucidating the causes of population divergence, and ultimately speciation, is a central objective of evolutionary biology (Schluter 2000; Barton 2001). The importance of understanding evolutionary processes for conservation biology is also increasingly acknowledged (Rosenzweig 2001; Palumbi 2001; Western 2001). Of particular relevance is the deciphering of the relative role of historical contingency (e.g. biogeographical context), and that of the deterministic interactions between evolutionary processes (e.g. antagonistic interactions between natural selection and gene flow) in shaping diversity (e.g. Losos *et al*. 1998; Taylor & McPhail 2000; Mayr 2001; Hendry *et al*. 2002).

Over the last several years, north temperate freshwater fish have gained popularity as model systems to assess processes that promote evolutionary novelties (reviewed in Robinson & Schluter 1999). Of particular interest is the occurrence of sympatric and parapatric ecotypes that are morphologically similar but remain partially reproductively isolated (Taylor 1999). This phenomenon predominates in salmonid fish, but also exists in other families, including Gasterosteidae, Osmeridae, Centrarchidae and Catostomidae. It has commonly been reported that similar patterns of niche differentiation and associated patterns of

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body shape, behaviour and life history traits are shared across these groups (e.g. Schluter & McPhail 1993; Robinson & Wilson 1994; Schluter 1996; Smith & Skùlason 1996). This has led to the general acceptance that their diversification has been driven by the same selective processes, namely that resource-based natural selection is the primary force driving the divergence and reproductive isolation of these populations (e.g. Robinson & Schluter 1999; Schluter 2000).

A critical look of the existing literature, however, indicates that patterns and causes of diversification in north temperate sympatric fish ecotypes may be more diversified than commonly reported. For instance, members of the two most studied cases of 'limnetic-benthic' sympatric pairs, the three-spine stickleback (*Gasterosteus* sp.) and whitefish (*Coregonus* sp.), differ substantially in their patterns of differentiation. Sympatric species of stickleback strongly segregate in habitat use between the shallow littoral and deeper pelagic zone (Taylor *et al*. 1997), whereas habitat partitioning is much more variable, and sometimes non-existent in whitefish, despite pronounced differences in trophic resource use (Bodaly 1979; Bernatchez *et al*. 1999). Average adult size and age at maturity are relatively similar between sympatric stickleback when contrasted to a 10-fold difference in adult size and nonoverlapping age at maturity in sympatric whitefish ecotypes (Fenderson 1964). The role of evolutionary forces in shaping population divergence in north temperate fish has also often been assumed rather than empirically investigated, except for a few taxa (e.g. see Schluter 2000). This may potentially lead to erroneous interpretations, for instance when inferring the role of selection in phenotype–environment associations (Turgeon *et al*. 1999). To date, empirical tests for the role of divergent natural selection in promoting phenotypic divergence between sympatric fish ecotypes have been performed for stickleback (reviewed in Schluter 2000), pumpkinseed *Lepomis* sp. (Robinson *et al*. 1996) and whitefish (Bernatchez 2003) only. There is also little consensus regarding the role of historical contingency as a necessary factor towards the initiation or development of population divergence. For instance, the hypothesis that sympatric divergence is the most plausible explanation for stickleback evolution was accepted until recently (Taylor & McPhail 1999), yet the most recent analyses supported the hypothesis that their sympatric evolution was contingent upon double invasions of postglacial lakes by ancestral marine populations (Taylor & McPhail 2000). This is concordant with the observation for whitefish, where secondary contact between distinct evolutionary lineages appears necessary to explain the occurrence of sympatric ecotypes in several lakes, but not in others (Bernatchez & Dodson 1990; Pigeon *et al*. 1997; Douglas *et al*. 1999). These observations implicate the necessity of empirically documenting patterns and causes of population diversification among other species complexes to contribute towards a better understanding of the mechanisms that generate diversity in northern fish.

In this study, we investigate the relative role of historical factors vs. evolutionary forces (natural selection, genetic drift and gene flow), in promoting population differentiation in the rainbow smelt (*Osmerus mordax* Mitchill). In order to achieve this, we performed a combined analysis of life history, morphological and genetic differentiation in a new case of sympatric dwarf and normal smelt ecotypes in Lac Saint-Jean (48°45′N, 72°15′W, Québec, Canada, Fig. 1). This lake, a large oligotrophic reservoir (1053 km²) with

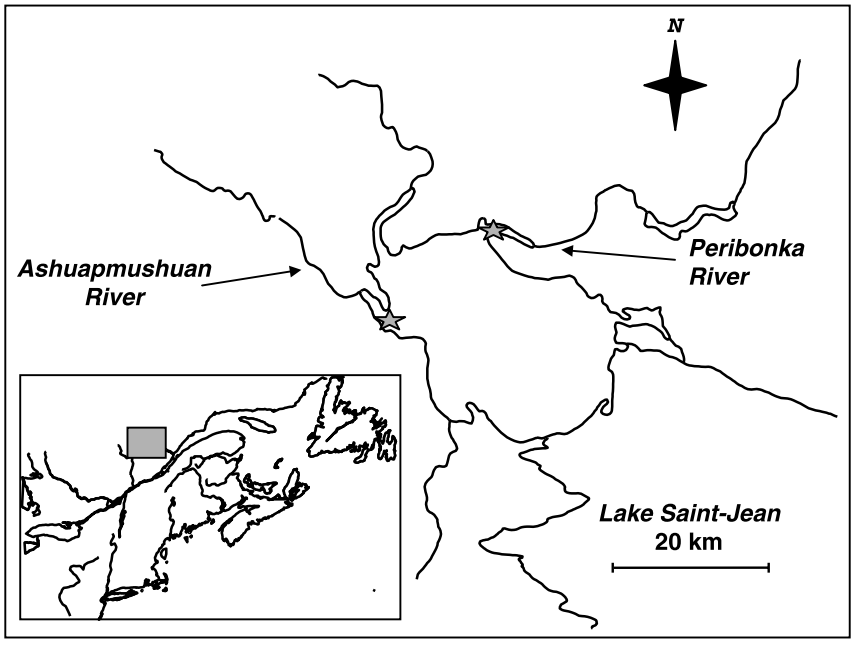

Fig. 1 Location map of Lac Saint-Jean (shaded area in the inset map), showing sampling locations (stars) near the mouth of the Ashuapmushuan and Peribonka rivers.

mean and maximal depths of 12 m and 63 m, respectively, was formed following the retreat of the Laflamme Sea between 10 300 and 8700 years BP (Elson 1969). Colonization of the lake by smelt most likely occurred during the same time frame (Bernatchez 1997).

Previous phylogeographical studies of mitochondrial DNA variation have shown that two distinct lineages of smelt evolved in allopatry for several hundred thousands of years, and subsequently came into contact in the nearby St Lawrence estuary where they remained reproductively isolated (Baby *et al*. 1991; Taylor & Bentzen 1993a; Bernatchez & Martin 1996; Bernatchez 1997). A first specific objective of this study was therefore to test the hypothesis that the evolution of sympatric smelt ecotypes in Lac Saint-Jean has been contingent upon the secondary contact between two evolutionary lineages in postglacial times.

Lac Saint-Jean is also special for the occurrence of both dwarf and normal smelt that synchronously use the same riverine spawning habitats. However, this observation has never been formally documented. We therefore tested for the occurrence of ecotypes in the lake and further assessed the possible role of divergent selection in maintaining differences between ecotypes by contrasting the extent of gene flow and phenotypic differentiation within and between spawning habitats. Theory predicts that divergence at quantitative traits should be similar to that of allele frequencies at nuclear loci, if they are evolving neutrally and have an additive genetic basis (Wright 1951). Under the influence of migration, mutation and genetic drift, the among-population proportion of total genetic variance in phenotypic traits is therefore expected to equal that of nuclear marker loci (Lande 1992). As an indirect method for detection of natural selection, one can therefore compare the extent of population differentiation at quantitative traits (Q_{ST}) with that quantified at neutral molecular markers (F_{ST}) (Spitze 1993; Merilä & Crnokrak 2001; McKay & Latta 2002). The prediction is that divergent selection will cause Q_{ST} to be larger than the differentiation observed on the basis of neutral marker variation. The Q_{ST} method was therefore applied to test the null hypothesis that the extent of phenotypic differences relative to that of neutral marker variation would be similar in comparisons involving populations within and among forms.

Materials and methods

Study site and sampling

Sampling was conducted during spawning times in May 1998 and 1999, using 30×1.8 m gillnets, comprising three panels of 1.25, 2.00 and 2.50 cm stretched mesh size. Nets were set out overnight at the mouth of the two main tributaries where smelt are known to spawn: the Ashuapmushuan and Peribonka rivers (Fig. 1). Fish were measured, weighed, aged from scale analyses, and their stage of gonad development was estimated according to Nikolskii (1963). Only fish that had reached stage 4 and more (near spawning conditions or spent) were kept for subsequent analyses (Table 1). White muscle biopsies were preserved in 95% ethanol for DNA analyses, whereas entire fish were individually frozen for subsequent morphological analysis.

Morphological analysis

Fish were filmed with fins extended on the left side against a light-blue background using a Sony camcorder equipped with a 50-mm macro-lens. Video images were analysed using the software OPTIMAS (version 2.0). Bi-dimensional coordinates were obtained for 15 homologous landmarks to calculate 10 morphometric variables as detailed in Turgeon *et al*. (1999). Four additional morphometric variables were measured using a digital calliper (0.01 mm): the inter-orbital width, mandible length, maxillary width and maxillary length. Three gill-raker variables were measured with a micrometer under a dissecting scope on the first left gill-raker arch: length of the lower (LGL) and the upper gill arch (UGL) and the mean length of the first and third most posterior rakers of the lower arch (GRL). Rakers were counted on the lower (LGR) and the upper (UGR) arch, and the total count (TGR) was considered for analysis. The intergill-raker space was estimated as (LGL + UGL)/((LGR + UGR) − 1) (Turgeon *et al*. 1999). The univariate residual method was used to adjust each morphometric character for size heterogeneity among individuals (Reist 1985; Fleming *et al*. 1994). We first tested for the between group homogeneity of the regression relationship with an ANCOVA. As no significant differences in slope was detected

Table 1 Sample name and sample size used for the analyses of life history traits, morphology, mitochondrial DNA (mtDNA) and microsatellite loci

		Sample size		
Sample name	Life history	Morphology	mtDNA	Microsatellite
Ashuapmushuan-98 (Dwarf)	30	51	61	87
Ashuapmushuan-98 (Normal)	54	54	60	82
Peribonka-98 (Dwarf)	30	51	60	62
Peribonka-98 (Normal)	50	70	70	67
Peribonka-99 (Normal)			60	60

in the between group relationship, the overall pooled sample linear regression line describing the relation between each variable and the fork length were established, and the shape variates was defined from the regression residuals. Maxillary angle, total gill-raker count and size-adjusted morphometric data were analysed multivariately using the discriminant function analysis. All statistical analyses were performed using STATISTICA (1994) software, version 7.0 (Statsoft).

Genetic analysis

Total DNA was extracted from the muscle tissues using a standard proteinase K phenol-chloroform protocol (Sambrook *et al*. 1989). The glacial origin (Atlantic or Acadian lineage, *sensu* Bernatchez (1997)) of each fish was established by a PCR-RFLP analysis of the mitochondrial genome (mtDNA) performed using two restriction endonucleases that generated diagnostic fragment patterns between lineages (Pigeon *et al*. 1998). Briefly, a 2.4-kb segment encompassing the ND5 and ND6 subunits of NADH dehydrogenase was amplified using primers developed by Cronin *et al*. (1993) and digested with *Apa*I and *Dde*I. MtDNA fragments were separated on 1.2% agarose gels at 90 V run for 5 h. Restriction fragments were revealed by ethidium bromide staining. Smelt known to possess a mtDNA haplotype characteristic of either the Atlantic or Acadian lineage (Bernatchez 1997) were run on each gel as positive controls.

The extent of genetic differentiation and gene flow between ecotypes on each spawning ground was assessed using six microsatellite loci. Four of these were developed in this study and two were developed for the Eulachon (*Thaleichthys pacificus*) by McLean & Taylor (2001) (Table 2). Rainbow smelt specific loci were developed as detailed in Wirth *et al*. (1999). Microsatellite polymorphism was analysed using the fluorescent dye detection method. One primer of each locus was 5′-labelled with three different fluorescent dyes; HEX (yellow) for *OSMO-Lav 12* and *OSMO-Lav 157*, TET (green) for *OSMO-Lav 45* and *TPA 14* and 6-FAM (blue) for *OSMO-Lav 16* and *TPA 26*. PCR was carried out in a 10-mL reaction volume containing 1 unit of Taq polymerase, 1.0 mL of reaction buffer (10 mM Tris-HCL [pH 9.0], 1.5 mM $MgCl_2$, 0.1% TritonX-100, 50 mM KCl), 750 mmol of dNTPs, between 25 and 50 ng template DNA and 0.03 pmol of each primer. A PCR duplex (for *OSMO-Lav 12–OSMO-Lav 16*, and *TPA 14–TPA 26*) and simplex (for *OSMO-Lav 45* and for *OSMO-Lav 157*) was performed in a Perkin-Elmer 9600 thermocycler (version 2.01) with the following profile: an initial denaturing step of 5 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at annealing temperature and 30 s at 72 °C. Samples were heated to 95 °C for 5 min and chilled on ice prior gel loading. Electrophoresis procedures were conducted on a denaturing 5% polyacrylamide gel with an ABI 377 automated sequencer/gene scanner for 2 h at 3000 V. Allelic size was determined (using GENESCAN software version 2.1; AppliedBiosystems 1996a) by reference to the internal sizing standard and by comparison with the same standard sample of known allelic size that was run on each gel. The final scoring of allelic size and tabulation of data for each locus were conducted with the GENOTYPER software, version 2.0 (AppliedBiosystems 1996b).

Genetic polymorphism was quantified by the number of alleles per locus (A), observed heterozygosity (H_O), and unbiased gene diversity (H_E) using the GENETIX software, version 4.02 (Belkhir *et al*. 2000). Samples were tested for conformity with Hardy–Weinberg equilibrium under the alternative hypotheses of heterozygote excess or heterozygote deficiency using the score test (U-test) described in Rousset & Raymond (1995). Global tests across loci and across samples were also performed using GENEPOP version 3.1d (Raymond & Rousset 1995). The null hypothesis of no

Table 2 Microsatellite primer sequences, annealing temperature, characteristic of the main continuous repeat region in each cloned allele, and cloned allele size for rainbow smelt primers (*OSMO-Lav*). Details for the *Tpa*-loci are provided in McLean & Taylor (2001)

Name	Motif	Primer sequence (5′–3′)	Cloned allele (pb)	Ta (°C)
OSMO-Lav 12	$(GT)_{37}$	1: CTG TAA TAT TCC ACTGCT GC 2: CAA GTA GAC AGT AGG GAGA	164	55
OSMO-Lav 16	$(GT)_{15}$	1: GGA TCT TGG ATG AGA ACA T 2: GGC TCT TTC ATT ACA CAG G	92	55
OSMO-Lav 45	$(CT)_{33}$	1: CTG TTG ATA GAT TGG CAT C 2: CCC ATT CAA TTA GAC AGT G	201	55
OSMO-Lav 157	$(GT)_{33}$ AAGAGA$(GT)_7$	1: CTT GCT TAT GTA AAG GTG GG 2: GAT CCA CCA GTT CTC ACA	246	55
Tpa-14		1: AGA GGC GCA GAT GAA GAG 2: CAC GTT GCC GTG GTA ATG C		57
Tpa-26		1: AGG ACT GGC GTG GGA AAT 2: CTG CAC TGC TGT CTG GAG AA		57

difference in allelic frequency distribution at each locus between all sample pairs was tested using the Markov chain method to obtain unbiased estimates of Fisher's exact test through 1000 iterations (Guo & Thompson 1992) available in GENEPOP 3.1d. Probability values over all loci were obtained by the Fisher method (Sokal & Rohlf 1995). The extent of genetic differentiation between samples was estimated from the estimator θ (Weir & Cockerham 1984) of F_{ST} using *F*-stat 1.2 (Goudet *et al*. 1996). As it has been shown that the performance of differentiation statistics varies under specific contexts (Balloux & Goudet 2002), we also computed population divergence estimates using allelic size variance (R_{ST}) according to Goodman (1997). The 95% confidence intervals were estimated by bootstrapping over loci (1000 replicates) and probability values were determined by 1000 permutations. Statistical significance in the above tests was adjusted for multiple comparisons using sequential Bonferroni adjustments (Rice 1989). Gene flow was assessed by the effective number of migrants per generation (N_em) between forms within a river and within forms among rivers using two methods: (i) Wright's formula: $F_{ST} = 1/(1 + 4N_em)$; and (ii) Slatkin's private allele method (Slatkin 1985) as implemented in GENEPOP 3.1d. Although those estimators are based on several assumptions that may not be met in Lake Saint-Jean (e.g. symmetry of migration rates and equal population size) (Whitlock & McCauley 1999), they still provide a useful basis for relative comparisons, especially when gene flow is pronounced (Slatkin 1987; Neigel 2002).

Q_{ST}–F_{ST} *comparisons*

The hypothesis that the differentiation between smelt ecotypes is driven by divergent natural selection was tested by comparing the extent of differentiation at phenotypic traits (Q_{ST}) with that of neutral expectations quantified at microsatellite loci (F_{ST}). Phenotypic variance was used as a surrogate for additive genetic variance (e.g. Kremer *et al*. 1997; Merilä *et al*. 1997; Bernatchez 2003; see also Discussion) for the five morphological traits that significantly differed between ecotypes (see Results) to test the null hypothesis that Q_{ST} estimated from these traits did not significantly differ from F_{ST} estimated at microsatellite data. Components of phenotypic variance were estimated by performing an analysis of variance using STATISTICA. The phenotypic variance was equal to twice the observational component of variance for individuals within populations and was used as a surrogate for $2\sigma^2_{GW}$. The phenotypic variance between populations was equated to the observational variance component for populations and was used as a surrogate for σ^2_{GB}. Q_{ST} values were calculated for each individual trait, averaged across traits, and contrasted with multilocus θ values. Values were considered significantly different when their 95% confidence intervals did not overlap.

Results

Life history trait variation

Field survey with gillnet fishing and electo-fishing of spawning fish indicates that normal and dwarf ecotypes synchronously use the same riverine spawning habitat in both the Ashuapmushuan and Peribonka river (Michel Legault, unpublished data). The spatial distribution and the gonad development synchronicity of spawning fish of both ecotypes within each river were similar. Furthermore, the spawning habitat location and the synchronicity of spawning of both ecotypes has been confirmed by a survey of newly larval drift. A strong multimodal distribution of spawning fish was observed in both the Ashuapmushuan and Peribonka rivers (Fig. 2). The first size mode included fish measuring between 60 and 100 mm in length (mean of 85 mm in the Peribonka and 83 mm in the Ashuapmushuan), and did not overlap with a second size mode that comprised fish measuring between 120 and 255 mm (mean of 187 mm in the Peribonka and 157 mm in the Ashuapmushuan). We refer to fish of the 60–100 mm mode and those > 120 mm, as group I and group II, respectively. A highly significant difference in size at a given age was observed between

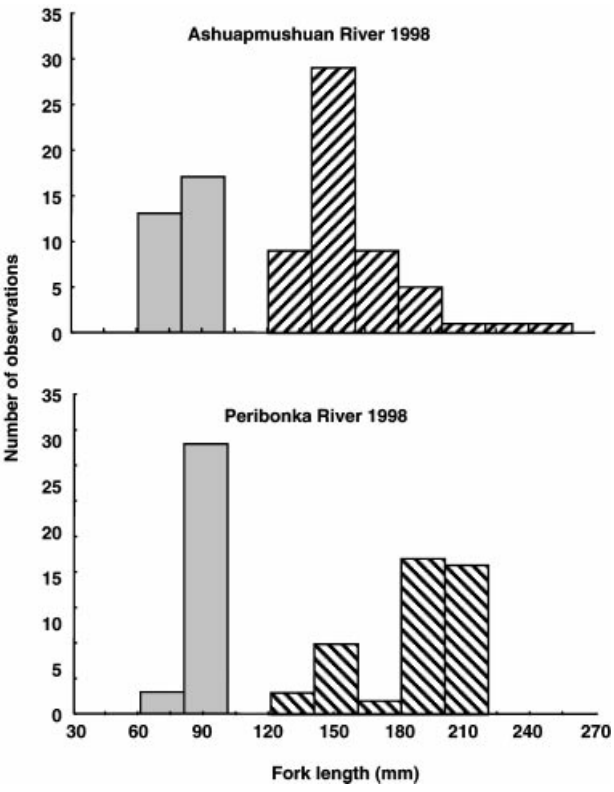

Fig. 2 Length frequency distribution for dwarf (grey) and normal (cross-hatched) smelt ecotypes captured in 1998 in the Ashupmushuan (top panel) and the Peribonka (lower panel) rivers.

the two groups. In both rivers, 100% of group I were 2-years-old, and these were significantly smaller than group II fish of the same age (Fig. 3). In the Peribonka river, mean size of group I was 85 mm (range: 79–93 mm), and that of 2-year-old fish in group II was 143 mm (range: 129–219 mm) ($t = -16.62$; $P < 0.0001$). In the Ashuapmushan river, the mean size of group I was 83 mm (range: 76–86 mm), and that of 2-year-old fish from group II was 140 mm (124–162 mm) ($t = 17.51$; $P < 0.0001$). In contrast to group I, group II fish included fish from several age classes in both rivers (three in the Ashuapmushuan and four in the Peribonka river; see Fig. 3). Overall, the mean age of group II spawning smelt was 2.48 years in the Ashuapmushan river and 3.06 years in the Peribonka river. In summary, there were two distinct life history forms of smelt that reproduced within each river: a semelparous and slow-growing, and an iteroparous and fast-growing form. By definition (*sensu* Chouinard *et al*. 1996), we are referring to these as dwarf and normal smelt ecotypes in the rest of the paper.

Morphological differentiation

The discriminant function analysis showed that the morphology of dwarf and normal smelt significantly differed within both rivers lending to a posteriori reclassification success of 98% in each river (Fig. 4). Five variables that showed significant *F*-values in both rivers were indications of the most important discriminant functions: the total number of gill-rakers, the maxillary angle, the eye area, the maxillary length and the intergill-raker spacing (Table 3). The most discriminating trait was the number of gill-rakers. In both rivers, dwarf smelt were consistently characterized by a higher number of gill-rakers, a more pronounced maxillary angle, larger eye area, a smaller mouth and less space between gill-rakers. Morphological differences were much less pronounced between either dwarf or normal populations from different rivers. When separately analysing samples of the two ecotypes, the reclassification success of a posteriori test was 40% and 48% between populations of dwarf and normal smelt, respectively.

Genetic differentiation

Mitochondrial DNA (mtDNA). A total of 309 out of 312 fish analysed possessed mtDNA typical of Atlantic lineage as defined by Pigeon *et al*. (1998). These fish were characterized by the following fragment patterns: *Apa*I (1700 bp and 700 bp); *Dde*I (800 bp, 400 bp, 325 bp, 275 bp, 250 bp and 150 bp). The other three fish (all normal smelt from the Peribonka river) showed fragment patterns typical of the Acadian lineage of smelt: *Apa*I (one fragment 2400 bp); *Dde*I (700 bp, 500 bp, 325 bp, 275 bp, 250 bp and 150 bp).

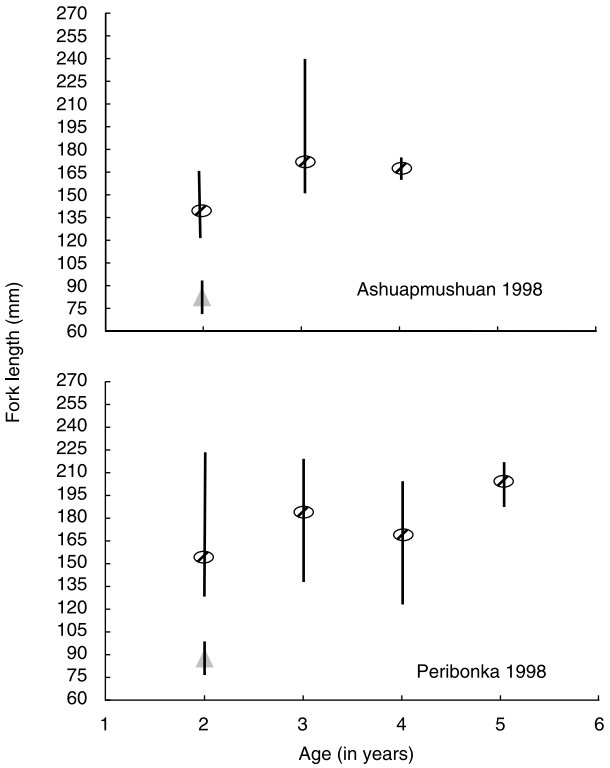

Fig. 3 Relationships between length (in mm) and age for dwarf (grey triangles) and normal (cross-hatched ovals) smelt ecotypes captured in 1998 in the Ashupmushuan (top panel) and the Peribonka (lower panel) rivers. Vertical bars show the range of values observed in each group.

Microsatellite loci

No obvious dichotomy in allelic composition was observed between dwarf and normal smelt (Table 4). For instance, the allelic size range (A_R), the most common allele at each locus (A_C) and its frequency (F_C) were as similar between dwarf and normal samples as between samples of the same form. The pattern of allelic size distribution did not reveal evidence of multimodal size distribution for any loci that could also indicate distinct historical origin (e.g. Lu *et al*. 2001) of smelt from different ecotypes (Fig. 5).

Hardy–Weinberg (H-W) expectations for genotype frequencies in each sample and across all loci were rejected for three of the five samples. Departures from equilibrium were caused by *OSMO-Lav 45*, which was the only locus that significantly deviated from the H-W expectation (significant deficit) in all three samples. This locus was the most variable, and had the widest allelic size range (Table 4). It is therefore more likely that the overall deficits in heterozygotes observed in three samples was caused by the nonamplification of the largest alleles and/or the occurrence of null alleles at *OSMO-Lav 45* than population admixture (Wahlund's effect) or other biological causes.

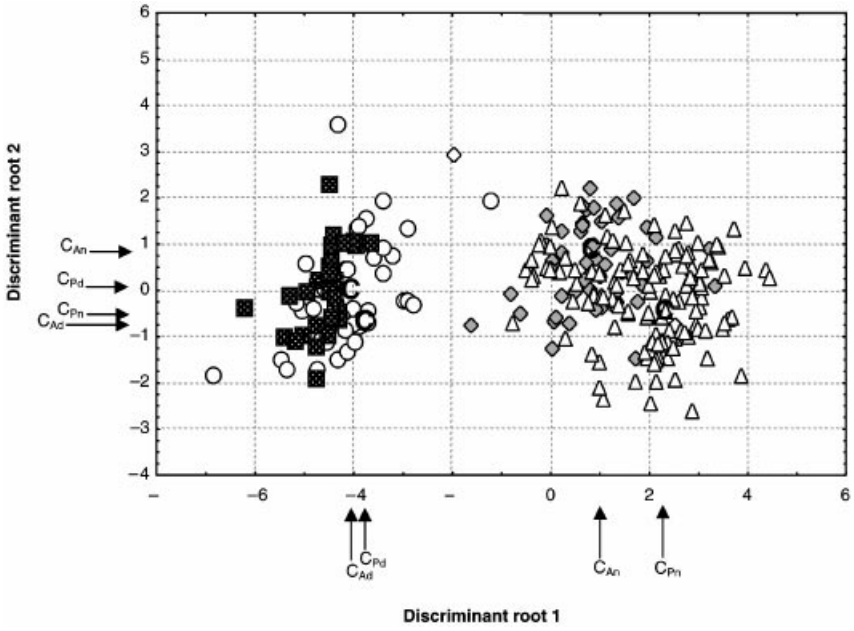

Fig. 4 Distribution of Lac Saint-Jean smelt individuals in the multivariate space defined by the first and second discriminant root function describing variation of morphological trait. ○: Peribonka-98Dwarf; ■: Ashuapmushuan-98Dwarf; △: Ashuapmushuan-98Normal; ◇: Peribonka-98Normal. CAd, CAn, CPd and CPn refer to x and y coordinates of the centroid position (C) for each population.

Table 3 Summary of the discriminant function analysis for morphometric and meristic variables of dwarf and normal smelt ecotypes. *F*-values larger than one are considered as contributing to the discriminant function. Asterisks indicate significant *P*-values ($\alpha = 0.05$). Size for morphometric traits are standardized to 180 mm fork length

	Ashuapmushuan				Péribonka			
	Standardized size		*F*-values	Test wise *P*-values	Standardized size		*F*-values	Test wise *P*-values
Morphometric traits	Dwarf	Normal			Dwarf	Normal		
Head length	28.52	29.81	2.951	0.088	28.43	29.74	1.056	0.206
Head depth	25.64	25.54	0.945	0.965	25.56	25.49	1.564	1.134
Snout length	24.57	24.32	0.838	0.645	23.94	24.26	0.857	0.702
Eye area	20.21	17.23	13.082	< 0.001*	19.84	16.85	15.072	< 0.001*
Eye height	2.31	2.53	1.652	0.254	2.22	2.25	0.952	0.599
Pectoral fin length	27.45	27.12	1.887	0.171	27.89	28.02	1.165	0.355
Maxillary length	18.15	20.21	7.034	< 0.001*	17.94	20.35	6.023	< 0.001*
Body depth	30.12	30.56	0.883	0.451	31.21	31.16	0.895	0.424
Peduncular height	15.89	15.10	0.969	0.408	16.23	15.98	1.000	0.353
Maxillary angle	27°	23°	13.378	< 0.001*	27°	23°	13.343	< 0.001*
Interorbital width	6.67	7.12	1.582	0.104	6.52	7.20	1.156	0.124
Mandible length	22.56	23.23	0.997	0.756	22.64	23.11	0.982	0.712
Maxillary width	2.81	2.94	1.149	0.285	2.88	3.06	1.254	0.288
Intergill-raker spacing	0.67	0.84	10.081	< 0.001*	0.64	0.86	11.128	< 0.001*
Meristic trait								
Gill-raker counts	32	31	19.549	< 0.001*	32	31	18.649	< 0.001*

All samples differed in their allelic composition at three or four loci, except the two temporal samples of normal smelt in the Peribonka River (Table 5). Consequently, homogeneity tests of allele frequency distributions revealed significant differences following sequential Bonferroni corrections in all pairwise sample comparisons, except between these temporal samples. This provided evidence that dwarf and normal smelt ecotypes within each river represented genetically distinct populations. The lack of significant differences between the temporal samples of normal smelt in the Peribonka River also indicated that the allelic composition was temporally stable, at least in that

Table 4 Allelic variability at six microsatellite loci in sympatric rainbow smelt ecotype of Lac Saint-Jean. Number of samples successfully used for genetic analysis (N), number of alleles at each locus (A), mean number of alleles at six loci (A_M) most common allele (A_C; in base pairs), frequencies of the most commun alleles (F_C), range of allele size (A_R), observed heterozygosity (H_O), gene diversity (H_E) at each locus and average heterozygosity across six loci (H_M). d indicates significant heterozygote deficit following the sequential Bonferroni correction (a = 0.05, k = 30) whereas D indicates significant heterozygote deficit across loci (global test, Fisher's method)

Sample	Ecotype		*Osmo 12*	*Osmo 16*	*Osmo 45*	*Osmo 157*	*Tpa 14*	*Tpa 26*	A_M	H_M
Ashuapmushuan-98	Normal	N	81	82	79	81	77	81		
		A	16	8	25	12	12	5	11	
		A_C	166	80	213	246	134	217		
		F_C	0.28	0.67	0.17	0.45	0.31	0.58		
		A_R	136–198	74–90	177–233	218–259	114–138	221		
		H_E	0.82	0.50	0.91	0.74	0.65	0.56		0.67
		H_O	0.84	0.63	0.60d	0.77	0.63	0.53		0.65D
	Dwarf	N	85	86	85	85	80	87		
		A	18	7	25	16	7	3	13	
		A_C	162	80	221	246	134	217		
		F_C	0.26	0.74	0.15	0.4	0.34	0.6		
		A_R	150–194	74–90	163–237	218–263	128–140	213–217		
		H_E	0.83	0.42	0.91	0.78	0.49	0.56		0.61
		H_O	0.80	0.47	0.86d	0.76	0.60	0.54		0.63D
Peribonka-98	Normal	N	66	65	65	67	64	63		
		A	16	6	25	16	6	5	12	
		A_C	166	80	203	246	134	217		
		F_C	0.33	0.73	0.10	0.41	0.50	0.50		
		A_R	154–188	74–84	165–239	218–263	114–140	213–221		
		H_E	0.80	0.43	0.93	0.76	0.59	0.58		0.68
		H_O	0.90	0.47	0.81	0.83	0.54	0.59		0.66
Peribonka-98	Dwarf	N	61	61	58	62	61	61		
		A	21	8	24	16	6	4	13	
		A_C	166	80	209	246	136	217		
		F_C	0.30	0.68	0.12	0.42	0.53	0.60		
		A_R	154–196	74–90	185–237	218–255	126–136	213–221		
		H_E	0.84	0.50	0.92	0.76	0.59	0.56		0.66
		H_O	0.88	0.63	0.75	0.83	0.54	0.52		0.65
Peribonka-99	Normal	N	60	58	59	58	59	57		
		A	16	9	24	15	6	5	12	
		A_C	166	80	203	246	134	217		
		F_C	0.30	0.73	0.11	0.48	0.53	0.59		
		A_R	154–190	74–92	175–231	230–259	122–140	213–217		
		H_E	0.85	0.44	0.93	0.75	0.58	0.58		0.67
		H_O	0.95	0.48	0.61d	0.81	0.54	0.63		0.65D

population. Heterogeneity in allele frequency translated into θ estimates that were significantly different from zero between dwarf and normal smelt within each river (Table 6). The extent of differentiation based on R_{ST} was comparable with that based on θ estimates, although R_{ST} values tended to be smaller, as reported by Balloux & Goudet (2002) in situations of high gene flow. An AMOVA (analysis of molecular variance; Excoffier *et al.* 1992) showed that the extent of genetic variance attributable to genetic differences between populations of different ecotypes from a same river (1.34%, $P < 0.0001$) was low and very similar to that observed between populations of the same ecotype from different rivers (1.15%, $P < 0.0001$). The modest levels of genetic differentiation observed between dwarf and normal smelt populations translated into high gene flow estimates, although absolute estimates varied among the two methods used (Table 6). The most important observation, however, was that the extent of gene flow between ecotypes within rivers was almost as pronounced as that observed between populations of a given ecotype between rivers. Thus, the ratio of between-populations-within-river/between-populations-within-ecotype varied between 0.76 and 0.81, depending on methods for estimating gene flow.

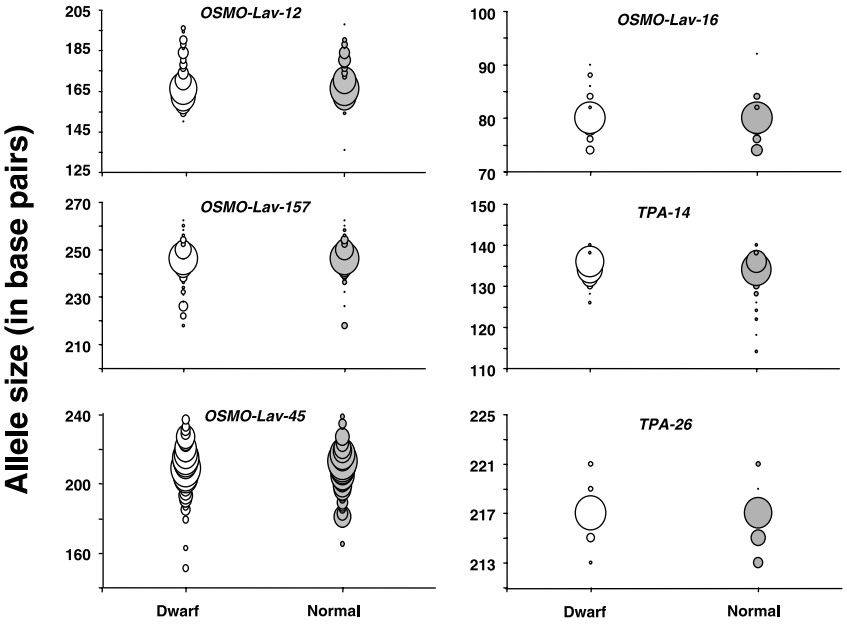

Fig. 5 Allele frequency histogram of microsatellite loci for all dwarf smelt (white circles) and all normal smelt (grey circles) combined. Allele designation is in base pairs and size of circles is proportional to allele frequency within each sample.

Table 5 Genic differentiation between dwarf and normal ecotypes of Rainbow smelt. Multilocus *P*-values were estimated by the Fisher's method using individual *P*-values calculated from each of the six loci. Numbers below the main diagonal indicate the number of loci that showed significant differences in the exact tests of differentiation. (Asterisks indicate significant sequential Bonferroni-adjusted *P*-value)

	Ashuapmushuan-98 (Dwarf)	Ashuapmushuan-98 (Normal)	Peribonka-98 (Dwarf)	Peribonka-98 (Normal)	Peribonka-99 (Normal)
Ashuapmushuan-98(Dwarf)		< 0.001*	0.002*	< 0.001*	< 0.001*
Ashuapmushuan-98(Normal)	4		< 0.001*	< 0.001*	< 0.001*
Peribonka-98(Dwarf)	3	4		0.002*	0.002*
Peribonka-98(Normal)	4	3	4		0.04
Peribonka-99(Normal)	4	4	4	0	

Table 6 Estimation of the genetic differentiation based on allelic (F_{ST}), molecular variance (R_{ST}) and gene flow estimates based on two different methods

	Ashuapmushuan Dwarf/Normal	Peribonka Dwarf/Normal	Dwarf Ashuapmushan/Peribonka	Normal Ashuapmushan/Peribonka
F_{ST}	0.024	0.013	0.009	0.025
% 95 interval	0.010–0.038	0.002–0.029	–0.012–0.022	0.011–0.048
P	< 0.001	< 0.001	0.065	< 0.001
R_{ST}	0.015	0.011	0.008	0.011
% 95 interval	0.002–0.024	0.004–0.022	–0.012–0.019	–0.007–0.029
P	0.005	0.01	0.01	0.01
Nm (Wright)	10 (0.010)	19 (0.019)	28 (0.028)	10 (0.010)
Nm (Slatkin)	21 (0.021)	42 (0.042)	62 (0.062)	18 (0.018)

Q_{ST}–F_{ST} *comparisons*

In both the Ashuapmushuan and Peribonka rivers, a more pronounced level of differentiation between dwarf and normal smelt ecotypes was observed for morphological traits (Q_{ST}) than at neutral markers (F_{ST}) (Fig. 6). The average Q_{ST} values observed in both rivers were not significantly different (Ashuapmushuan: 0.55, 95% C.I. = 0.23–0.87 mean = Peribonka: mean = 0.68, 95% C.I. = 0.43–0.92). We then assessed the differential influence of

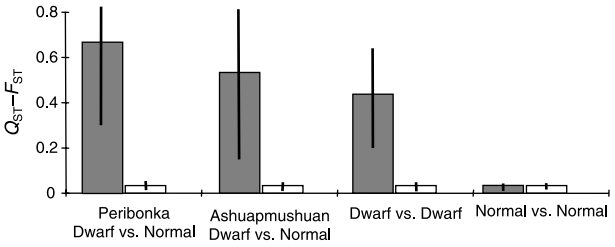

Fig. 6 Comparison of F_{ST} (white bars) and Q_{ST} (grey bars) values with their 95 confidence intervals (vertical bars) for comparisons involving the different ecotypes within each river and different populations of the same ecotype from different rivers.

divergent selection on dwarf and normal ecotypes. If the level of selection acting on both ecotypes has been similar, Q_{ST} estimates in either dwarf-dwarf comparisons or normal-normal comparisons should be comparable, whereas they should significantly differ under differential selective regimes. The result for the dwarf-dwarf comparison was comparable to those obtained between ecotypes; the Q_{ST} (mean = 0.45, 95% C.I. = 0.25–0.65) was much higher than the F_{ST} estimate. In sharp contrast, F_{ST} and Q_{ST} (mean = 0.014, 95% C.I. = 0.006–0.022) did not differ significantly when comparing both populations of the normal ecotype.

Discussion

This study provided evidence for the occurrence of dwarf and normal ecotypes of rainbow smelt in Lac Saint-Jean that differ in both morphological and life history traits. Lac Saint-Jean smelt are also unique for the occurrence of two genetically distinct populations within each ecotype. Furthermore, populations of each ecotype synchronously use the same riverine spawning habitat, and this is replicated in two tributaries. Below, we discuss the possible role of historical contingency and that of the deterministic interactions between evolutionary forces in shaping this pattern of population diversity.

No evidence for the role of historical contingency in priming population divergence

Previous phylogeographical studies of mitochondrial DNA variation showed that two distinct lineages of smelt evolved in allopatry for several hundred thousands of years, and subsequently came into contact in the nearby St-Lawrence estuary where they remained reproductively isolated (Taylor & Bentzen 1993a; Bernatchez 1997). We therefore tested the hypothesis that the evolution of sympatric smelt ecotypes in Lac Saint-Jean has been contingent upon the secondary contact between these two evolutionary lineages in postglacial times. Our a priori expectation was to observe an association between distinct mtDNA clades and ecotypes. This was not supported since all but three fish from one of the populations were characterized by mtDNA typical of the Atlantic glacial lineage (Bernatchez 1997).

We cannot entirely rule out the possibility that the two glacial lineages of smelt have naturally colonized Lac Saint-Jean and that the signal of association between mtDNA clades and ecotypes was lost following stochastic lineage sorting and pronounced gene flow between the founding populations (e.g. Lu *et al*. 2001; Redenbach & Taylor 2002). This scenario, however, seems unlikely. First, following the completion of our study, we found that stocking of smelt was attempted at the end of the 19th century (Catellier 1911). The number of stocked fish and the success of this introduction have not been documented. However, these smelt originated from the St-Lawrence River North-shore where they are characterized by mtDNA clade of Acadian origin (Bernatchez 1997). It is therefore very likely that mtDNA traces of the Acadian smelt lineage found in Lac Saint-Jean are due to this past introduction. Secondly, the time of divergence between glacial lineages of smelt has been estimated to approximately 700 ky (Bernatchez 1997). A shorter time frame than this has been shown to be sufficient in generating modal differences in allelic sizes at microsatellite loci through stepwise mutational processes (Slatkin 1995) between evolutionary lineages in other northern temperate fish (e.g. Angers & Bernatchez 1998; Lu *et al*. 2001). Yet, we found no evidence for the occurrence of a bimodal distribution in allelic size in Lac Saint-Jean smelt for any of the loci used.

While the above observations refute the hypothesis of a secondary contact between glacial lineages, they do not rule out the possibility of a double invasion from a single glacial lineage, as recently documented in stickleback (Taylor & McPhail 2000). In this species, it was hypothesized that extant sympatric pairs result from two temporally separate invasions of lakes by ancestral anadromous ancestors following glacial recessions, about 12 ky and 10 ky ago. Microsatellite DNA diversity revealed low genetic variation in freshwater populations, supporting the hypothesis that they were derived by colonization of fresh water by a more diverse anadromous ancestral population. Furthermore, Taylor & McPhail (2000) observed higher allelic diversity and heterozygosity in limnetic than benthic populations, which supported the hypothesis that limnetic populations had evolved following the second submergence, and therefore their allelic composition had less time for divergence from that of the anadromous ancestors by genetic drift. As in sticklebacks, freshwater smelt populations are derived from anadromous ancestors (Bernatchez 1997) and show reduced allelic diversity relative to contemporary anadromous populations (this study; Lecomte *et al.*, unpublished data). However, the situation of Lac Saint-Jean differs from that of stickleback populations from

the Strait of Georgia region in that there is no geological support for an historical scenario of multiple submergences for Lac Saint-Jean. Unlike stickleback, smelt ecotypes were also nearly identical in allelic diversity and heterozygosity.

In summary, the available evidence offers more support for the hypothesis that smelt ecotypes from Lac Saint-Jean evolved from a single ancestral population and through processes of intralacustrine divergence. As such, these findings corroborate Taylor & Bentzen's (1993a) contention that historical conditions promoting unique ecological or genetic interactions are not necessary for the evolution of sympatric ecotypes in *Osmerus*. This is concordant with other north temperate fish, namely the kokanee-sockeye salmon (*Oncorhynchus nerka*), the Arctic charr (*Salvelinus alpinus*) and the North American ciscoes (*Coregonus* sp.) (Taylor *et al*. 1996; Brunner *et al*. 1998; Gíslason *et al*. 1999; Turgeon *et al*. 1999; Turgeon & Bernatchez 2001). In contrast, multiple colonizations have been associated with the occurrence of sympatric populations in the brown trout (*Salmo trutta*) (Hynes *et al*. 1996) and the whitefish population complex (*Coregonus* sp.) (Bernatchez & Dodson 1990). In the case of whitefish, however, either intralustrine divergence or multiple invasions have best explained the occurrence of sympatric ecotypes in other geographical areas of the species' range (Bernatchez *et al*. 1996; Pigeon *et al*. 1997; Douglas *et al*. 1999).

While these observations show that historical conditions are not a prerequisite for the evolution of sympatric ecotypes in north temperate lakes, it is noteworthy that the highest level of differentiation (implying more restricted gene flow) has been reported in situations where multiple invasions were inferred. For instance, F_{ST} values of 0.05 or less between sympatric ecotypes have typically been reported for cases of intralacustirne divergence, whereas F_{ST} values over 0.20 have been mainly reported between sympatric populations of stickleback, whitefish or trout believed to have evolved following multiple invasions (see details in the above references). Thus, although it may not be necessary to prime divergence, these observations raise the hypothesis that the development of reproductive isolation between sympatric north temperate fish ecotypes may have been accelerated in situations where multiple invasions have occurred. This is also predicted by the theory of reproductive reinforcement that infers that divergence may be facilitated if partial ecological/genetic incompatibility favouring premating isolation has already existed between founding populations that came into contact (e.g. Liou & Price 1994).

Support for the role of divergent natural selection in maintaining population divergence

If not contingent upon historical conditions that promoted unique ecological interactions, then it follows that smelt population divergence and reproductive isolation have been promoted by either stochastic processes or ecological determinism (Losos *et al*. 1998). In the following section, we argue that available evidence supports the hypothesis of a major role for divergent natural selection in explaining the pattern of smelt population diversity we observed in Lac Saint-Jean.

First, genetically distinct dwarf and normal smelt ecotypes differ in morphological traits that have been shown to be associated with the use of distinct trophic niches in other smelt populations (Taylor 2001) and other taxa (Taylor 1999; Bernatchez 2003). As observed in dwarf smelt, limnetic ecotypes of other species complexes are typically characterized by one or more of the following traits; a higher number of gill-rakers, a smaller mouth, larger eye area, and less space between gill-rakers (Robinson & Schluter 1999). It is noteworthy that Taylor & Benzten (1993ab) reported that trophic ecotypes of smelt that evolved from an Acadian lineage ancestor (as opposed to the Atlantic lineage in this study) also primarily differed by the same traits that we documented here. This provides a first indication that smelt ecotype divergence has been driven by divergent natural selection since this is the only process expected to produce parallel patterns of diversification in functional traits among phylogenetically independent lineages (Rundle *et al*. 2000).

Dwarf and normal smelt from Lac Saint-Jean also strikingly differed in life history strategies, with the dwarf ecotype characterized by a smaller length at a given age, a younger mean age at maturity, a shorter lifespan and a semelparous life cycle, compared to the iteroparous normal ecotype. Life-history theory makes specific predictions as to how trade-offs between growth, survival and reproduction may maximize the fitness of organisms (Roff 1992; Crespi & Teo 2002). Namely, it is predicted that selection will favour early maturation if gain in reproduction, that can be obtained by delaying maturation (fecundity generally increases with body size in fish), is smaller than the increased risks of dying before reproducing. Clearly, differences in age structure between both ecotypes indicate that the probability of survival is lower for the dwarf than the normal ecotype. Higher mortality rate in dwarf smelt could hypothetically be related to higher predation pressure, as recently reported for limnetic and benthic whitefish ecotypes (Kahilainen & Lehtonen 2002). An additional, and perhaps more important factor, however, may be that higher metabolic rates and reduced bioenergetic conversion efficiency (defined as growth rate/consumption rate ratio) in dwarf smelt may prevent them to funnel enough energy into growth and reproduction at older ages. For instance, Forseth *et al*. (1994) and Tucker & Rasmussen (1999), respectively, documented for the Arctic charr and Atlantic salmon (*Salmo salar*) that fish with higher metabolic rates and lower conversion efficiencies within a

cohort also matured earlier. More recently, Trudel *et al*. (2001) documented in *Coregonus* that higher metabolic rate and lower bioenergetic conversion efficiency were associated with slower growth and younger age at sexual maturity in the dwarf ecotype of whitefish. This pattern was paralleled in lake cisco (*C. artedi*), a coregonine species also functionally adapted to occupy the limnetic trophic niche. If the observations of Trudel *et al*. (2001) for *Coregonus* also apply to other limnetic forms, differential metabolic rates and bioenergetic conversion efficiencies could be a key factor in explaining the evolution of distinct life history strategies between smelt ecotypes. Although this remains to be investigated, a first indication that dwarf smelt may be bioenergetically less efficient than normal smelt was provided by the observation of their much smaller size at 2-years-old.

Further support for the role of natural selection in driving divergence between smelt ecotypes was provided by comparing the extent of differentiation at neutral markers (F_{ST}) and morphological traits (Q_{ST}). Thus, dwarf and normal smelt spawning in both the Peribonka and Ashuapmushuan rivers were morphologically more differentiated than expected under the neutrality hypothesis, which is generally interpreted as evidence for divergent natural selection (Spitze 1993; Lynch *et al*. 1999; Merilä & Crnokrak 2001; Morgan *et al*. 2001). These results therefore suggest that despite the fact that they synchronously spawn in the same tributary, gene flow has not been sufficient to override the effect of selection in promoting phenotypic divergence between the dwarf and normal smelt ecotypes.

The Q_{ST} results should however, be interpreted cautiously in this study, given that phenotypic variance was used in this study whereas the strict use of the Q_{ST} method implies that knowledge of additive genetic variation for quantitative traits must be obtained. Yet, the method has been applied using phenotypic variance (e.g. Kremer *et al*. 1997; Merilä *et al*. 1997; Bernatchez 2003), under the assumption that it is an accurate surrogate for additive genetic variance. Indeed, several investigators have emphasized the similarity between genetic and phenotypic variance (e.g. Roff & Mousseau 1987; Cheverud 1988; Roff 1995, 1996). The measures of phenotypic covariance have also been shown to be as nearly successful as those of genetic covariance in predicting the direction of divergence between species (e.g. Schluter 1996). Merilä (1997) and Kremer *et al*. (1997) also performed sensitivity analyses showing that only a very large environmental component of phenotypic variance would affect conclusions of Q_{ST} analyses. Consequently, results of Q_{ST} studies based on either phenotypic or genotypic variance do not differ in their general patterns of F_{ST}–Q_{ST} relationships (Lynch *et al*. 1999; Schluter 2000; Merilä & Crnokrak 2001).

If the high Q_{ST}/F_{ST} ratio observed in comparing dwarf and normal populations was an artefact caused by the use of phenotypic variance instead of additive genetic variance, then one should expect this to bias Q_{ST} estimates in the same manner in any comparison. On the contrary, we observed that the mean Q_{ST} value for the dwarf-dwarf comparison was much higher than the F_{ST} estimate, whereas mean F_{ST} and Q_{ST} did not differ significantly when comparing both populations of the normal ecotype. These results suggest that phenotypic differences between smelt ecotypes are driven by divergent natural selection that is predominantly acting on the dwarf ecotype. The hypothesis that selection may be acting more strongly on dwarf smelt is corroborated by the observations that this ecotype appears to be at an 'ecological disadvantage' relative to the normal ecotype, both in terms of growth and survival. This pattern is also congruent with the results of a similar study recently performed in the dwarf and normal ecotypes of whitefish. Bernatchez (2003) showed that Q_{ST} value for gill-raker counts between dwarf and normal populations largely exceeded F_{ST}, which supported the hypothesis that differences in gill-raker count between sympatric morphs is under the influence of divergent selection. Similarly, departure from neutral expectations was pronounced in dwarf-dwarf comparisons, whereas Q_{ST} for gill-raker count did not significantly differ from F_{ST} in normal-normal comparisons, indicating that this trait was not strongly influenced by divergent selection in normal populations.

On the role of gene flow in constraining phenotypic divergence

The above results suggest that gene flow has not been sufficient to override the directional effect of selection on phenotypic divergence. This, however, does not exclude the possibility that gene flow has been acting as a constraining force against more pronounced (or 'optimal') phenotypic divergence (e.g. King & Lawson 1995; Garcia-Ramos & Kirkpatrick 1997). A comparison with the extent of phenotypic differentiation observed in other cases of smelt trophic ecotypes suggests that phenotypic divergence between Lac Saint-Jean smelt ecotypes could be partially hampered by gene flow. For instance, trophic ecotypes from Lake Utopia (New Brunswick, Canada) are morphologically more differentiated than those from Lac Saint-Jean (see Table 2 of Taylor & Bentzen 1993b). Concomitantly, a recent microsatellite DNA analysis with the same loci used in this study indicated that the extent of gene flow between Lake Utopia ecotypes was also more restricted than observed in Lac Saint-Jean (mean $F_{ST} = 0.090$ vs. 0.015 in this study) (Curry *et al*. 2003).

Hendry *et al*. (2001) have recently developed a quantitative-genetic approach to examine the equilibrium difference between two populations that are experiencing different selective regimes and that are linked by gene flow.

Empirical tests using this model showed that adaptive divergence is sometimes constrained by gene flow in natural populations. A rigorous application of this approach requires the quantification of several parameters that are not available for smelt. Consequently, we applied the method of Hendry *et al*. (2001) only as an exploratory assessment to see whether the amount of gene flow observed between smelt populations in Lac Saint-Jean could affect divergence in adaptive traits under specific conditions. More specifically, we compared the extent of observed divergence in gill-raker count between dwarf and normal smelt to that predicted by the model of Hendry *et al*. (2001), given the observed levels of gene flow, phenotypic variance, and assuming different strength of stabilizing selection within populations. We focus the discussion on gill-raker count because it was the most differentiated trait between ecotypes and therefore, evidence for a constraining effect at this character should extend to less differentiated ones.

The analysis is based on equation 7 in Hendry *et al*. (2001):

$$D^* = (D_\theta G)/[G1 - m + (\omega^2 - P)m],$$

where D* is the equilibrium difference between populations in adaptive traits (here gill-raker counts), D_θ is the optimal difference for this trait, P is the phenotypic variance (average value for dwarf and normal smelt = 0.8830), G is the additive genetic variance (assumed to be 0.7P based on heritability estimates of gill-raker counts in other fish; Hatfield 1997; Bernatchez 2003), *m* is the proportion of migrant per generation, and ω^2 is the strength of stabilizing selection within populations. Approximation of *m* was obtained as follows. We first considered the mean $N_e m$ value of 14.5 (based on Wright's method) between dwarf and normal (Table 6). This value is lower than that derived from Slatkin's private allele method and is therefore conservative. We then estimated N_e using the equilibrium relationships $H_E = 1 - (1 + 8N_E\mu^{-0.5})$ of Ohta & Kimura (1973), where H_E is the averaged gene diversity observed in dwarf and normal ecotypes ($H_E = 0.655$), and μ is the mutation rate (assumed to be 5×10^{-4} according to Estoup & Angers 1998). This generated an N_e estimate of approximately 1800, and therefore a *m* estimate of 0.0085. Considering that: (i) the maximal theoretical time to reach equilibrium is approximately $2N_e$ generations and further reduced with increasing gene flow (Crow & Aoki 1984); (ii) smelt have a generation time of about 2 years; and that (iii) the species colonized Lac Saint-Jean between 10 300 and 8700 years BP (Elson 1969; Bernatchez 1997), it is very likely that these populations are near equilibrium conditions (predicted time to equilibrium without gene flow *c*. 7200 years). Three absolute values of selection ($\omega^2 = 4P$, $36P$, and $100P$) were selected in order to compare our predictions with those reported by Hendry *et al*. (2002) between lake and stream populations of stickleback in the Mysty system (British Columbia, Canada). These would correspond to relatively strong, moderate, and weak stabilizing selection in nature (Kingsolver *et al*. 2001). Thus, Kingsolver *et al*. (2001) reported that absolute values of quadratic selection gradients (γ) quantified so far were exponentially distributed, 95% of the significant values ranged between zero and 2.5, with a median value of 0.10. Using the approximate relationship between ω^2 and γ ($\omega^2 = -1/\gamma$; Arnold *et al*. 2001), the ω^2 considered here translate into absolute quadratic selection gradient values of 0.287, 0.031, and 0.011, respectively. Under these conservative conditions, we estimated that gene flow between the dwarf and normal smelt ecotypes would constrain adaptive divergence in gill-raker counts to 97% of its optimum if stabilizing selection is strong, 72% of its optimum if selection is moderate, and 47% if selection is weak. These values are in the range of those reported by Hendry *et al*. (2002).

In summary, this study empirically documented patterns and likely causes of population diversification in rainbow smelt populations revealing a unique situation of dwarf and normal smelt ecotypes in Lac Saint-Jean. These populations are: (i) characterized by the occurrence of two genetically distinct populations in each ecotype; (ii) synchronously using the same spawning habitat; and (iii) this phenomenon is replicated in two tributaries. Our results also showed that historical contingency has apparently played little role in the origin of these populations. In contrast, they provided evidence for an important role of divergent natural selection in driving their phenotypic divergence. However, whether or not natural selection is also important in promoting their reproductive isolation remains to be investigated. This is because we have no evidence that the phenotypic traits that evolved under divergent natural selection are also involved in promoting pre or post-mating reproductive isolation (e.g. Rundle *et al*. 2000). Finally, while divergent selection has apparently been strong enough to maintain phenotypic differentiation in the face of gene flow, this study suggests that gene flow has been sufficiently important to modulate the extent of adaptive differentiation being achieved between ecotypes, unless the extent of stabilizing selection acting on smelt ecotypes is much more pronounced than usually reported in natural populations.

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This study is the main part of R. Saint-Laurent's MSc thesis on the evolutionary biology of rainbow smelt supervised by L. Bernatchez. Michel Legault is a freshwater biologist with various interests in conservation and management. L. Bernatchez and M. Legault have been collaborating for 10 years in a research programme that aims at understanding population structure and dynamics of salmon and smelt from Lac Saint-Jean. Louis Bernatchez's major interests are in the understanding of patterns and processes of molecular and organismal evolution, as well as their significance to conservation.
