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Genetic and morphological variation between two forms of lacustrine brook charr

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Genetic data suggest that the littoral and pelagic forms of brook charr *Salvelinus fontinalis* in Lake Bondi are two populations with partial reproductive isolation and non-random mating. Genetic differentiation between the two groups was supported by differences in allele frequencies and by deviation from Hardy–Weinberg equilibrium when the two groups were pooled; no such deviation was observed when fish were divided into littoral and pelagic groups. In contrast to Lake Bondi, no clear evidence of genetic differentiation was observed in Lake Ledoux. Discriminant function analyses of morphological characters support the existence of littoral and pelagic groups in Bondi and Ledoux Lakes. In Lake Bondi, the two groups differed significantly in two shape variables (pelagic fish had shorter dorsal fins, and longer body length posterior to the dorsal fin than littoral ones) whereas in Lake Ledoux, the groups differed in four shape variables (pelagic fish had shorter pectoral fins, shorter dorsal fins, and a shorter and higher caudal peduncle than littoral ones). Discriminant analyses of these characters were effective in reclassifying fish into their appropriate groups in both populations, with an efficiency of 78% for juveniles in Lake Bondi and 69% for adults in Lake Ledoux. Differences in morphology between the two forms are consistent with adaptations required to forage in each zone, i.e. benthic form in the littoral zone and planktivorous form in the pelagic zone.

Key words: trophic polymorphism; genetic differentiation; microsatellite analysis; morphological variation; trophic morphology; shape characters; functional morphology; *Salvelinus fontinalis*.

INTRODUCTION

In several freshwater lakes, single-species populations of fish are composed of both open-water types (limnetic or pelagic forms) and shallow-water types (benthic or littoral forms) (reviewed in Robinson & Wilson, 1994). Furthermore, sympatric pairs have been described with respect to adult size as normal and dwarf morphotypes (Hindar & Jonsson, 1982; Bernatchez & Dodson, 1990; Taylor & Bentzen, 1993*b*). In general, these forms share resources by developing specific local adaptations to different habitats, such as variations in spatial distribution and feeding habits (e.g. Robinson *et al.*, 1993). Genetic differences have also been found between several sympatric pairs of fish (reviewed in Hindar, 1994; Bernatchez *et al.*, 1996; Pigeon *et al.*, 1997), although it appears

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that reproductive barriers have only begun to develop and are still incomplete in many cases. Because they represent an ongoing process of population differentiation, these young sympatric forms may represent good model systems for the understanding of factors involved in the early stages of local adaptations and reproductive isolation.

Brook charr *Salvelinus fontinalis* (Mitchill) exhibit interindividual differences in spatial distribution and diet in several lakes of the Canadian Shield (Québec, Canada). Venne & Magnan (1995) found that juvenile brook charr (0+) of Lake Bondi were divided spatially into two groups. The littoral group was found between 0 and 2 m depth while the profundal group (equivalent of open-water types) was found between 3 and 6 m depth. Bourke *et al.* (1997) also observed interindividual differences in habitat use in adult brook charr in two other Québec lakes using radio-telemetry. Interindividual differences in habitat preference of fish were related to functional differences in body morphology and coloration; the pectoral fins of benthic and generalist individuals were significantly longer than those of pelagic ones. Furthermore, the coloration of the lower flank of benthic and generalist individuals was silver-grey while that of pelagic ones was red, which was attributed to a diet rich in zooplankton in the latter group (Bourke *et al.*, 1997). The analysis of stomach contents data of 3776 brook charr captured in 69 lakes of the Canadian Shield showed that, in allopatry, the proportion of benthic and pelagic specialists fit remarkably well with those based on interindividual preferences in spatial distribution, identified through radio-telemetry (Bourke *et al.*, 1999), suggesting that the presence of the two forms is typical to these lakes. Bourke *et al.* (1999) also indicated that the proportion of each form in a given lake is related to the intensity of interspecific competition with creek chub *Semotilus atromaculatus* (Mitchill) and white sucker *Catostomus commersoni* (Lacépède). These studies therefore suggested that brook charr inhabiting oligotrophic lakes of the Canadian Shield might often exhibit trophic polymorphisms, where some individuals are littoral and others are pelagic. These results, however, suggested a polymorphism more subtle than the contrasting ones reported in other sympatric forms (e.g. Hindar & Jonsson, 1982; Walker *et al.*, 1988; Bernatchez & Dodson, 1990). While this trophic polymorphism may have evolved in response to competition for food (Crowder, 1984; Magnan, 1988; Magnan & Stevens, 1992; Lacasse & Magnan, 1992), it is unclear whether resource competition is sufficient to maintain this polymorphism and ultimately produce reproductive isolation. There is evidence for partial reproductive isolation in brook charr: Bourke *et al.* (1997) showed that two groups belonging to the same population may select different spawning grounds, which indicates a potential for reproductive isolation; and the results of O'Connor & Power (1973) supported the hypothesis of homing in brook charr, in one lake containing two spawning grounds.

The present study tested the hypothesis of genetic differentiation between littoral and pelagic brook charr from two lakes of the Canadian Shield (Bondi and Ledoux), by microsatellite analysis, the most powerful method for fine-scale studies of genetic diversity in fishes (O'Reilly & Wright, 1995). Angers *et al.* (1995) and Angers & Bernatchez (1998) showed the usefulness of microsatellites for assessing microgeographical differentiation among recently diverged brook charr populations. The study also determined if differences in diet and spatial

distribution between the two forms of brook charr were related to resource-based polymorphism (e.g. in characters related to swimming ability and manoeuverability and in structure of the feeding apparatus).

MATERIALS AND METHODS

STUDY LAKES

Bondi and Ledoux Lakes, located 1·6 km apart, in the Mastigouche Reserve, north of Trois-Rivières, Québec, Canada (46°40′ N, 73°20′ W), are characteristic small oligotrophic temperate lakes with surface area 23·3 and 11·9 ha, mean depth 8·0 and 5·5 m, conductivity 17·1 and 21·2 μS cm^{-1}, respectively, and typical dissolved oxygen, thermal stratification, and Secchi disk transparency (Lacasse & Magnan, 1992). Northern redbelly dace *Phoxinus eos* (Cope) is the only other fish in the lakes.

FISH SAMPLING

Young-of-the-year (YOY) brook charr in Lake Bondi and adults (3+ and older) in Lake Ledoux were collected on 25–26 June and between 8–21 August 1996. These age classes corresponded to those studied by Venne & Magnan (1995) in Lake Bondi (YOY) and Bourke *et al.* (1997) in Lake Ledoux (3+ and older). It would have been preferable to sample YOY and adult fish in both lakes but sampling limitations were imposed in the study lakes for conservation purposes. In Lake Bondi, fish were caught with multifilament (9·9 m long × 1·8 m deep) and monofilament gillnets (7·5 m and 15 m long × 1·8 m deep; filament diameter of 0·13 mm), both with stretched meshes of 15·9 mm. In Lake Ledoux, fish were captured with multifilament gillnets (32 m long × 2 m deep) with stretched meshes of 60, 76, 90, and 100 mm (filament diameters of 0·17, 0·20, 0·20, and 0·25 mm, respectively).

The nets were set perpendicular to the shore, covering the littoral (<2 m) and the pelagic (>3 m for YOY and >4 m for adults) areas, without overlapping. In Lake Bondi, gillnets were set by day at 11 stations distributed around the lake while in Lake Ledoux they were mainly set by night at four stations. In both lakes, the nets were removed every 2 or 3 h to keep fish in good condition for further analyses. Adipose fins were clipped and preserved in 95% ethanol for genetic analyses.

GENETIC ANALYSES

Microsatellite analysis

Total DNA was extracted according to Bernatchez *et al.* (1992). Microsatellite polymorphism was analysed by specific polymerase chain reaction (PCR) at five loci using primers developed for *S. fontinalis* (Sfo-8, Sfo-12, Sfo-18, and Sfo-23; Angers *et al.*, 1995) and *Salmo trutta* L. (MST-85; Presa & Guyomard, 1996). PCR conditions for amplifying loci were as detailed in Angers *et al.* (1995). Electrophoresis on polyacrylamide sequencing gels, fixation, drying, and autoradiography followed standard procedures. Alleles were sized by comparison with the standard M13 sequence and co-migration of allele standards of known sizes were run on all gels.

Data analysis

Genotypic phase disequilibrium was computed first to verify the physical independence of loci. Significant values were estimated by Fisher's exact test approximated by the Markov chain method available in version 3.0 of GENEPOP (Raymond & Rousset, 1995). Genetic polymorphism was measured as the number of alleles per locus (A), and the observed heterozygosity (H_o) and expected heterozygosity (H_e) (Nei, 1987) using GENEPOP v.3.0. These estimates were computed globally for each lake and subsequently for each group within lake. To ensure that the loci were sensitive enough to detect a population differentiation, brook charr from the physically isolated lakes were compared. Heterogeneity in allele frequencies between pooled brook charr from both

lakes was estimated using the Markov chain method to obtain unbiased estimates of the exact test through iterations (1000 in this study) (Guo & Thompson, 1992).

The degree of population genetic structure was quantified using an analysis of variance (ANOVA) framework. Gene diversity analyses were computed first using Weir & Cockerham's (1984) analogous F statistic (θ), which estimates the correlation between pairs of alleles through an ANOVA using allele frequencies only. The population subdivision was also estimated using the molecular variance model of Excoffier *et al.* (1992). This procedure estimates standard variance components and an array of allelic correlation measures, which also take into account the variance in allele size between pairs of alleles. The mutational differences among alleles were entered as a matrix of Euclidean squared distances. These two methods were applied for every locus and all loci pooled. The molecular variance analysis was performed using version 1.0 of the program ARLEQUIN (Schneider *et al.*, 1997).

GENEPOP v. 3.0 was then used to assess departure from Hardy–Weinberg (HW) proportions within lakes. As HW disequilibrium was observed in both lakes, indicating non-random mating (see Results), F_{is} values were computed for every locus and over all loci to test specifically whether departures from HW equilibrium were caused by an excess or a deficiency of heterozygotes. Overall F_{is} estimates were positive for both Lake Bondi and Lake Ledoux, which indicates non-random union of gametes within these lakes (see Results). Consequently, more detailed analyses were performed to evaluate the extent of the genetic differences between forms from each lake. Genetic differentiation based on heterogeneity in allelic frequencies and F statistics were computed as described above. Significance levels were estimated by Fisher's exact test in the cases of five alleles per locus or less and by the Markov chain method when the number of alleles was higher, to obtain unbiased estimates of the exact test through 1000 iterations (Guo & Thompson, 1992).

MORPHOLOGICAL MEASUREMENTS

In the laboratory, the left side of all fish was photographed against a horizontal light-blue background using a fixed Minolta X-700 35-mm camera equipped with a Minolta 50-mm macrolens and a ring flash. Fish were photographed with the pectoral and pelvic fins oriented approximately parallel to the main body axis in a natural position, and with the caudal and dorsal fins extended. Coloured pins were inserted in the anterior end of the anal and dorsal fins, as well as at the junction of the head and the body to ensure that body landmarks would be visualized easily. On each photograph, a millimetre ruler placed beside specimens provided scale.

Morphological structures associated with swimming and trophic characteristics were investigated to differentiate littoral and pelagic individuals in both lakes. To examine shape characters related to swimming (body and fin measurements), morphometric measurements were made by image analysis (high-performance colour CCD camera, Cohu model 8285; Targa Plus frame grabber; version 1.2 of Mocha software). Spatial x–y co-ordinates of 64 YOY and 60 adult body landmarks were recorded. From these landmark co-ordinates, both conventional (Hubbs & Lagler, 1947) and truss distances (Strauss & Bookstein, 1982) were obtained (Fig. 1). Measurements were also made of structures associated with feeding habits of 97 YOY and 61 adults: mouth width and length were measured to the nearest 0·01 mm using a digital caliper; and the length of the longest gill raker was measured at the bend of the first gill arch, using an ocular micrometre ($\pm$ 0·05 mm) mounted on a 10 $\times$ compound microscope.

It has been suggested that in morphometric studies, populations should be compared in terms of shape variates, free from the effects of size variation (Reist, 1985, 1986). The effect of size on body and fin measurements was removed by use of a regression technique (Fleming *et al.*, 1994). The data were transformed first to natural logarithms and standardized to a mean of 0 and standard deviation of 1. Each morphometric character was then transformed into a shape variate (regression residuals) by expressing it as the deviation of individuals from the pooled within-group regression line describing the relationship between the character and standard length (Reist, 1986). This deviation, orthogonal to standard length, should be approximately independent of size and reflect residual variation resulting from measurement error and biological deviation of

TABLE I. Number of alleles (A), range of allele size in base pairs, and overall expected heterozygosity (H_e) for the different microsatellite loci used in the study of brook charr populations of Bondi and Ledoux lakes

Loci	A	Size	H_e
Sfo-8	17	202–308	0·858
Sfo-12	8	197–273	0·546
Sfo-18	9	171–187	0·619
Sfo-23	20	143–221	0·855
MST-85	13	168–202	0·501

individuals from the predicted character–length relationship (Kuhry & Marcus, 1977). Fleming *et al.* (1994) compared the results from the residual method with those from the sheared principal components and Burnaby methods (Rohlf & Bookstein, 1987) and found that all methods led to similar conclusions.

To examine morphological differentiation between littoral and pelagic individuals from both lakes, shape variates were analysed with discriminant function analyses, which determined the most useful traits for separating population groups. Tolerance values were low when all shape variates were included in the model, indicating the presence of colinearity among these variables; therefore, a step-wise procedure was used to eliminate redundant ones. Cut-off significance values for variable selection, P-to-enter and P-to-remove, were both set to $P=0{\cdot}15$. Forward and backward methods of variable selection were compared. Final models obtained by the two methods differed little with regard to percentage of correct classification, magnitude of canonical correlations, and sign of canonical coefficients. Therefore, only the final model with the lower P value, obtained by forward selection for Lake Bondi and by backward selection for Lake Ledoux, was retained for interpretation.

Analysis of covariance (ANCOVA), with fish length as the covariate, was used to compare the mouth width, mouth length, and gill raker length of littoral and pelagic groups in both lakes (Packard & Broadman, 1987). Values were adjusted to a grand mean fish length of 86 mm (Lake Bondi) or 359 mm (Lake Ledoux). All statistical analyses were performed using SYSTAT for Windows, version 7.

RESULTS

GENETIC VARIATION

Overall genetic diversity

Genotypic phase disequilibrium was computed to verify the independence of loci within the littoral and pelagic groups in each lake (data not shown). When testing each pair of loci independently, a highly significant phase disequilibrium was detected between Sfo-23 and MST-85 ($P<0{\cdot}000001$) in the littoral group of Lake Bondi and between Sfo-8 and Sfo-18 ($P<0{\cdot}000001$) in the littoral group of Lake Ledoux. The pairs involved were not the same, which suggested physical independence between loci. All microsatellites were polymorphic, the total number of alleles per locus varied between eight and 20, and overall heterozygosity at each locus ranged between 0·501 and 0·858 (Table I). The intrapopulation diversity was also generally high, with expected heterozygosity varying between 0·367 and 0·891, depending on locus and population (Table II).

TABLE II. Sample size (n), total number of alleles (A), and observed (H_o) and expected (H_e) heterozygosity by locus for brook charr from Bondi and Ledoux lakes (pooled groups) and divided by groups (littoral and pelagic) within each lake; allele frequencies are available upon request

Locus/ allele	Interlake comparisons		Intergroup comparisons			
	Bondi	Ledoux	Bondi		Ledoux	
			Littoral	Pelagic	Littoral	Pelagic
n	87	61	46	41	32	29
Sfo-8						
A	12	14	11	9	11	12
H_o	0·805	0·869	0·739	0·878	0·875	0·862
H_e	0·818	0·889	0·824	0·803	0·882	0·900
Sfo-12						
A	7	6	7	7	5	6
H_o	0·609	0·344	0·630	0·585	0·313	0·379
H_e	0·625	0·398	0·587	0·661	0·393	0·410
Sfo-18						
A	6	7	5	4	7	6
H_o	0·494	0·606	0·478	0·512	0·656	0·552
H_e	0·504	0·692	0·476	0·537	0·672	0·684
Sfo-23						
A	14	17	12	10	12	16
H_o	0·713	0·853	0·739	0·683	0·813	0·897
H_e	0·792	0·891	0·812	0·775	0·898	0·881
MST-85						
A	9	10	7	8	9	9
H_o	0·299	0·689	0·348	0·244	0·688	0·690
H_e	0·367	0·644	0·401	0·329	0·612	0·681
Total						
Mean A	9·6	10·8	8·4	7·6	8·8	9·8
Mean H_o	0·584	0·672	0·587	0·581	0·669	0·676
Mean H_e	0·621	0·703	0·620	0·621	0·692	0·711

Interlake differentiation

The genetic differentiation between brook charr from the two lakes was highly significant at all loci based on allele frequencies (Table III). Thus microsatellite markers revealed a high degree of differentiation between these isolated populations. F statistics revealed an overall moderate but highly significant estimate based either on allelic or molecular variance analyses (see Table V). For a single locus, θ values ranged between 0·0255 (Sfo-8) and 0·1150 (Sfo-18), while Φ values varied between 0·0123 (Sfo-23) and 0·2483 (Sfo-18). Three of the five loci indicated higher estimates with allelic variance analysis. The estimate with pooled loci, however, was higher using molecular variance analysis.

Intralake differentiation

Allele frequencies were significantly different ($P<0·05$) between littoral and pelagic groups for two of the five loci in Lake Bondi (Table III). If the critical P is adjusted using the Bonferroni technique to ensure that the overall

TABLE III. Genetic differentiation based on statistical significance (P) of allele frequency distribution estimated for the two brook charr populations (Lake Bondi *v.* Lake Ledoux) and the two groups (littoral *v.* pelagic) within each lake

Loci	Interlake comparisons		Intergroup comparisons			
	Bondi	Ledoux	Bondi		Ledoux	
			Littoral	Pelagic	Littoral	Pelagic
Sfo-8	<0·000001		0·04556		0·18728	
Sfo-12	<0·000001		0·01200		0·95126	
Sfo-18	<0·000001		0·45944		0·08252	
Sfo-23	<0·000001		0·54336		0·25730	
MST-85	<0·000001		0·39886		0·63106	

TABLE IV. Allelic correlation within individuals (F_{is}; Weir & Cockerham, 1984) estimated from all fish pooled within Bondi and Ledoux lakes and between the two groups (littoral and pelagic) within each lake

Loci	Interlake comparisons		Intergroup comparisons			
	Bondi	Ledoux	Bondi		Ledoux	
			Littoral	Pelagic	Littoral	Pelagic
Sfo-8	0·016	0·023	0·104	− 0·094	0·008*	0·042
Sfo-12	0·026	0·136*	− 0·075	0·115	0·208*	0·075
Sfo-18	0·020	0·124	− 0·005	0·047	0·023	0·196
Sfo-23	0·101	0·044	0·090	0·120	0·097	− 0·017
MST-85	0·187*	− 0·070	0·133	0·260*	− 0·125	− 0·013
Global	0·070*	0·051*	0·049	0·090	0·042**	0·057

*P<0·05; **P<0·01.

probability of our analyses is maintained at 0·05, then the null hypothesis is not rejected in any of the comparisons, even though the results are enticing. No significant differentiation of allele frequencies was detected between the two groups in Lake Ledoux (Table III). Departures from HW equilibrium due to heterozygote deficiency (indicating departure from HW genotypic frequency expectations) were observed within both Lake Bondi and Lake Ledoux when all fish were pooled (Table IV). F statistics also revealed positive F_{is} estimates for each locus of both populations except for MST-85 of Lake Ledoux (F_{is}= − 0·70, P=0·985). However, only MST-85 (Lake Bondi, F_{is}=0·187) and Sfo-12 (Lake Ledoux, F_{is}=0·136) were statistically significant (P<0·05). By splitting the population of Lake Bondi into littoral and pelagic groups, the significant overall F_{is} (for pooled loci) disappeared. A different scenario was observed in Lake Ledoux, where F_{is} remained significant within the littoral group, mainly because of the HW disequilibrium at Sfo-8 and Sfo-12 (Table IV).

TABLE V. Degree of population genetic structure based on allelic (θ; Weir & Cockerham, 1984) and molecular (Φ; Excoffier *et al.*, 1992) variance assessed from the two brook charr populations (Bondi and Ledoux lakes) and the two groups (littoral and pelagic) within each lake

Loci	Interlake comparisons		Intergroup comparisons			
	Bondi *v.* Ledoux		Bondi		Ledoux	
			Littoral *v.* pelagic		Littoral *v.* pelagic	
	θ	Φ	θ	Φ	θ	Φ
Sfo-8	0·0255	0·1497	0·0077	0·0082	0·0000	0·0000
Sfo-12	0·0521	0·1594	0·0112	0·0105	0·0000	0·0000
Sfo-18	0·1150	0·2483	0·0000	0·0000	0·0394	0·1036
Sfo-23	0·0499	0·0123	0·0000	0·0126	0·0007	0·0335
MST-85	0·0764	0·0134	0·0012	0·0066	0·0000	0·0000
Global	0·0604*	0·1385*	0·0027	0·0094	0·0050	0·0000

*$P<0·000001$.

TABLE VI. Results of the discriminant function analyses used to compare shape characters of littoral and pelagic brook charr

Lake	Discriminant variables	Wilk's lambda	*F* value	Probability	Canonical correlation
Bondi	D4-5 D5-6	0·5935	20·8896	<0·00001	0·638
Ledoux	D12-13 D6-8 D7-9 D4-5	0·6873	6·3707	0·0003	0·559

D*x*–*y*, Distance between landmarks *x* and *y*

The genetic structure parameters computed for populations separated into littoral and pelagic groups were weak and not significant ($P<0·05$) for both θ and Φ estimates (Table V). The higher values observed in Lake Bondi were obtained by molecular variance analysis for both a single locus (Sfo-23; $\Phi=0·0126$) and pooled loci ($\Phi=0·0094$). In Lake Ledoux, only two of the five loci showed structure indices above zero (Table V). Once again, for a single locus, higher values were obtained from the analysis based on molecular variance (Sfo-18, $\Phi=0.1036$; Sfo-23, $\Phi=0·0335$). However, the overall analysis for pooled loci revealed higher values with allelic rather than molecular variance analysis.

MORPHOLOGICAL VARIATION

The discriminant function analysis yielded a highly significant difference between the two groups of YOY in Lake Bondi ($F=20·8896$, $P<0·0001$; Table VI). Inspection of canonical coefficients revealed that pelagic fish had shorter dorsal fins and longer body length posterior to the dorsal fin than did littoral

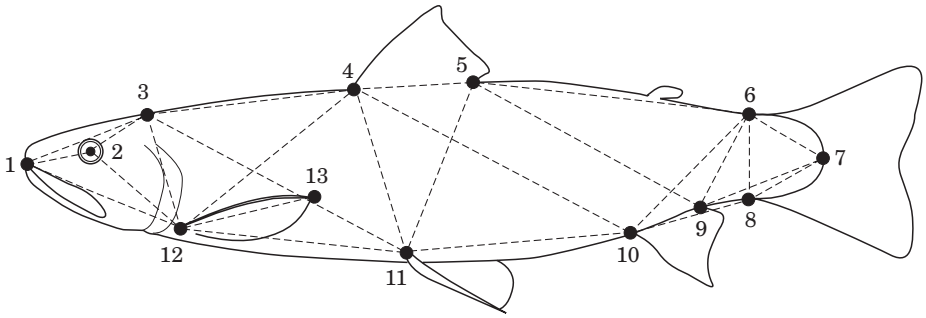

FIG. 1. Location of the 13 body landmarks (points) used to calculate the conventional and truss distances (dashed lines: 27 interpoint distances) included in the discriminant function analyses. Landmarks refer to: (1) anterior tip of snout at upper jaw, (2) centre of eye, (3) most posterior aspect of neurocranium, i.e. beginning of scaled nape, (4) origin of dorsal fin, (5) insertion of dorsal fin, (6) anterior attachment of dorsal membrane from caudal fin, (7) base of middle caudal rays, (8) anterior attachment of ventral membrane from caudal fin, (9) insertion of anal fin, (10) origin of anal fin, (11) origin of pelvic fin, (12) origin of pectoral fin, and (13) distal tip of pectoral fin.

ones (Fig. 1). The a posteriori classification accuracy of the discriminant function (jackknife procedure) was high for both groups: 28 of the 35 (80%) littoral individuals and 22 of the 29 (76%) pelagic ones were correctly reclassified, for an overall classification accuracy of 78%. In Lake Ledoux, the discriminant function analysis also yielded a highly significant difference between the two groups of adults ($F=6{\cdot}3707$, $P<0{\cdot}0003$; Table VI). Canonical coefficients revealed that pelagic fish had shorter pectoral fins, shorter dorsal fins, and a shorter and higher caudal peduncle than did littoral ones (Fig. 1). The a posteriori classification accuracy of the discriminant function was slightly lower than in Lake Bondi: 18 of the 28 (67%) littoral individuals and 24 of the 33 (71%) pelagic ones were correctly reclassified, for an overall classification accuracy of 69%. The frequency distributions of discriminant function scores [Fig. 2(a) and (b)] illustrate the overlap between pelagic and littoral groups for the two lakes. The spread of scores along the morphological space defined by the discriminant function is about the same for the two groups [Fig. 2(a) and (b)].

There were no significant differences in the adjusted mouth width, mouth length, and gill raker length of littoral and pelagic groups in either lake (Table VII).

DISCUSSION

GENETIC VARIATION

Microsatellite diversity in brook charr

The microsatellite polymorphism observed in this study is congruent with the few studies conducted on northern freshwater fish species using microsatellite markers. For example, in brook charr from the Parc National de la Mauricie, Angers & Bernatchez (1998) reported the number of alleles per locus varying between five and 18 and an overall heterozygosity ranging between 0·40 and 0·85. In landlocked Atlantic salmon *Salmo salar* L., of Lac Saint-Jean (Québec, Canada), Tessier *et al.* (1997) observed between two and 17 alleles per locus and an overall heterozygosity varying between 0·49 and 0·91. McConnell *et al.*

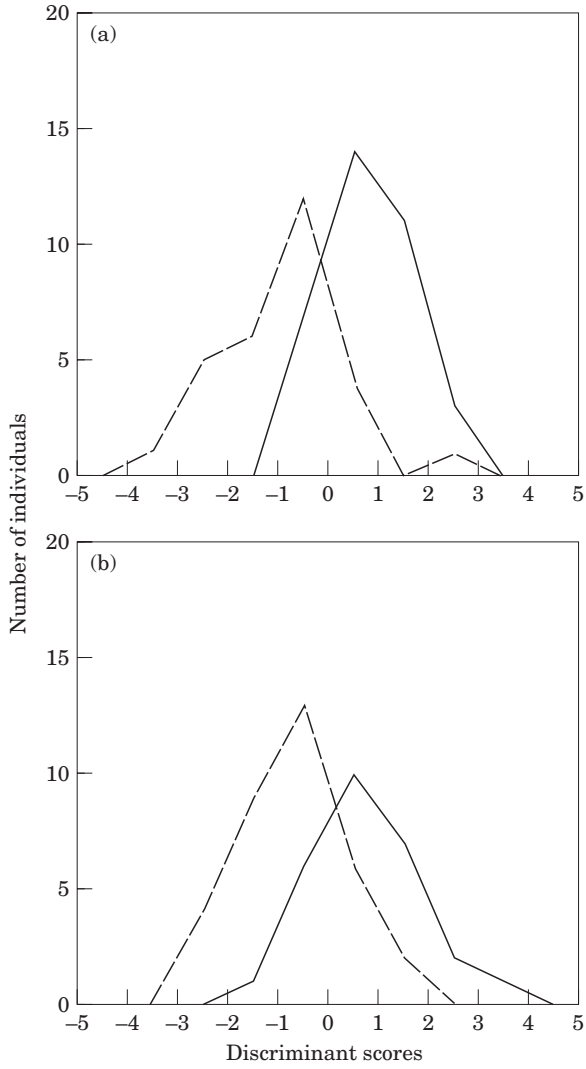

FIG. 2. Frequency distribution of discriminant scores for littoral and pelagic brook charr from Lake Bondi (a) and Lake Ledoux (b). ——, Littoral group; – – –, pelagic group.

(1995) also identified between three and 16 alleles per locus and heterozygosity estimates ranging between 0·30 and 0·89 for Atlantic salmon populations from Nova Scotia.

Microsatellites used in this study have proven their sensitivity by detecting large differences in allele frequencies between brook charr from each of these lakes at all loci. It appears that genetic differences probably reflect the effects of drift and mutation acting differently on these populations between which gene flow has been restricted during the last 15 000 years.

Genetic differentiation between forms

The genetic measurements suggested that the littoral and pelagic groups in Lake Bondi represent two partially reproductively isolated and non-randomly

TABLE VII. Adjusted means (± S.D.) of trophic characters of littoral and pelagic brook charr. Means were adjusted to a mean fish length of 86 mm in Lake Bondi and 359 mm in Lake Ledoux

Lake/ character	Littoral individuals	Pelagic individuals	P
Lake Bondi	(49)	(48)	
Mouth width (mm)	4·26 ± 0·34	4·27 ± 0·32	0·920
Mouth length (mm)	6·57 ± 0·41	6·48 ± 0·45	0·314
Gill raker length (mm)	1·09 ± 0·10	1·06 ± 0·10	0·105
Lake Ledoux	(28)	(33)	
Mouth width (mm)	19·05 ± 1·56	18·79 ± 2·09	0·590
Mouth length (mm)	26·91 ± 3·88	25·44 ± 1·93	0·060
Gill raker length (mm)	3·55 ± 0·33	3·50 ± 0·38	0·593

Number of fish is in parentheses.

mating populations. Genetic differentiation between the two groups was supported by differences in allele frequencies and by deviation from HW equilibrium when pooling the two groups; no such deviation was observed when fish were divided into littoral and pelagic groups. Detecting a departure from HW equilibrium is one of the first steps in detecting differences in genetic population structure (Raymond & Rousset, 1995). In verifying the origin of such a disequilibrium, the F statistics revealed a highly significant positive F_{is} estimate for pooled loci. This indicates an overall departure from HW genotypic frequency expectations due to heterozygote deficiency, indicating the non-random union of gametes within Lake Bondi and supporting the hypothesis that this lake is formed of diverse groups of fish (Venne & Magnan, 1995).

Alternative explanations could account for heterozygote deficiency, but these appear less probable in the present context. For instance, HW disequilibrium could be due to temporal variations in allele frequencies. However, it is difficult to imagine a scenario involving stochastic temporal variation because sampling of the YOY was done in the same year for both groups, which ensures that the same cohorts were used for the comparisons. Null alleles can also create an apparent heterozygote deficiency because they can be detected only in the homozygote; they have been reported regularly as an explanation for observed heterozygote deficiency (e.g. O'Reilly & Wright, 1995; García *et al*., 1997). Null alleles cannot be ruled out empirically, but appear unlikely in the present case. First, the heterozygote deficiency was caused by different loci in fish from the two lakes, which would imply detectable null alleles at some loci and the differential absence of these alleles depending on groups. Second, no null alleles were observed by Angers *et al*. (1995) and Angers & Bernatchez (1998), where the same loci were screened in 26 geographically close brook charr populations. Stochastic departures from HW equilibrium are also expected to occur in small populations. However, our results showed that the significantly positive F_{is} disappeared when the HW equilibrium was computed considering both groups separately. If the small population size was responsible for the heterozygote deficiency, the probability of obtaining significant F_{is} estimates would be even higher with split groups, since populations would be smaller. In addition,

stochastic disequilibrium would be expected to imply excesses or deficiencies of heterozygotes equally, which was not the case here. Thus, although these factors could still partly explain the observed heterozygote deficiency in Lake Bondi, it appears more plausible that they reflect mainly the mixture of two genetically distinct forms, littoral and pelagic.

In contrast to Lake Bondi, no clear evidence of genetic differentiation was observed in Lake Ledoux. First, no significant differentiation in allele frequencies was detected between the two groups. Second, when HW equilibrium was tested for littoral and pelagic groups separately, F_{is} remained significant within the littoral group. These results indicate that the intra-lake heterogeneity revealed by the overall heterozygote deficiency did not correspond to the classification based on spatial distribution of individuals (littoral or pelagic). Thus, one cannot conclude that fish from the two zones represent two genetically different populations. These results do not agree with those of Bourke *et al.* (1997), who reported different groups of adult brook charr in Lake Ledoux based on differences in habitat use and morphology. As our classification was based on the capture zone (littoral *v.* pelagic), the significant overall positive F_{is} estimate detected in the littoral group could be explained by the presence of pelagic individuals in this zone. Bourke *et al.* (1997) observed that littoral individuals were localized in the littoral zone 82% of the time and pelagic individuals in the pelagic zone 69% of the time, indicating some movement of fish from both groups between the two zones (pelagic individuals being less faithful to their zone than littoral ones). Such an admixture of individuals reduces the chance of detecting small differences in allele frequencies. On the other hand, we did not observe a genetic differentiation between the two groups when the classification of individuals was based on morphology. Further work will be needed to confirm the presence of two genetically different forms of brook charr in Lake Ledoux by basing the classification on either 0+ individuals, which are presumably less mobile, or on a higher number of adults to increase the power of discriminant analyses.

MORPHOLOGICAL VARIATION

The analyses of morphological characters of brook charr support the existence of littoral and pelagic groups in Bondi and Ledoux Lakes. The YOY of Lake Bondi differed significantly in two variables (pelagic fish had shorter dorsal fins, and longer body length posterior to the dorsal fin than littoral ones) whereas adults of Lake Ledoux differed in four variables (pelagic fish had shorter pectoral fins, shorter dorsal fins, and a shorter and higher caudal peduncle than littoral ones). Discriminant analyses of these characters were effective in reclassifying individuals into their appropriate groups in both populations.

To state that morphological differences between littoral and pelagic individuals represent adaptations to different resources implies that particular morphotypes have higher foraging ability and thus higher fitness, in their respective niche. In Lake Bondi, the efficiency of the pelagic form in searching and feeding on dispersed and mobile prey in open water may be improved by longer body length posterior to the dorsal fin and shorter length of the dorsal fin, which minimize drag and allow for efficient cruising (Gatz, 1979*a*, *b*; Webb,

1984). A shorter dorsal fin was also found in pelagic individuals of Lake Ledoux. This suggests a functional relationship between dorsal fin morphology and habitat preferences of brook charr. The pectoral fin of benthic individuals was longer than that of pelagic ones in Lake Ledoux. Long pectoral fins are related to slow and precise manoeuvering (Gatz, 1979*a*, *b*; Webb, 1982, 1984) required for living in structurally complex habitats and feeding on benthic organisms. A similar relationship between length of pectoral fin and foraging behaviour was found in other freshwater fish, such as pumpkinseed sunfish *Lepomis gibbosus* (L.) (Ehlinger, 1990) and Arctic charr *Salvelinus alpinus* (L.) (Malmquist *et al*., 1992), suggesting a strong functional relationship between pectoral fin size and feeding tactics in fishes. The morphological differences reported above thus appear to be specializations to exploit different habitats.

In contrast to characters related to locomotion, no significant differences were found in trophic characters between littoral and pelagic forms of brook charr. These results suggest that body traits linked to swimming performance are more important than traits related to feeding in these relatively unproductive lakes

MODE OF DIVERGENCE OF SYMPATRIC MORPHS

Lakes Bondi and Ledoux were formed after the sheets of ice that covered North America retreated about 15 000 years ago. These lakes were colonized subsequently by brook charr from glacial refugia (Lacasse & Magnan, 1994; Angers & Bernatchez, 1998). These recent, species-poor lakes offer two functional habitats, the littoral and pelagic zones. Soon after the colonization, the brook charr invaders may have experienced an ecological vacuum and weak, or no competition. As brook charr populations increased naturally, intraspecific competition intensified (Malmquist *et al*., 1992; Bourke *et al*., 1999). Van Valen (1965) suggested that under such circumstances, phenotypic variation between individuals should increase, reducing the stress of competition. Based on our knowledge of these lacustrine systems, the scenario of Van Valen (1965) could apply in Bondi and Ledoux Lakes. In fact, food segregation may have occured first through behavioural flexibility of individuals. Behavioural flexibility in fish probably plays an important role in adaptation to resource variability in lakes (e.g. Skúlason *et al*., 1993). Changes in foraging behaviour may have caused large effects on foraging efficiency (Dill, 1983; Ehlinger, 1989), and behaviour is amenable to more rapid change than morphology. For example, different foraging modes can be used to exploit planktonic and benthic organisms, and fish can switch rapidly between them when moving between habitats (Schluter, 1993). This behavioural change could have been followed by morphological differentiation and the use of different trophic niches. Reproductive isolation could be promoted then by disruptive selection (Wimberger, 1994; Skúlason & Smith, 1995).

Bourke *et al*. (1997) and results of the present study provided some evidence of reproductive isolation between littoral and pelagic individuals of brook charr. While there is evidence of genetic differentiation between forms in Lake Bondi, the genetic structure parameters (θ and Φ) revealed that the degree of divergence is more subtle than generally observed in other sympatric pairs of northern

freshwater fish (Schluter & McPhail, 1993; Taylor & Bentzen, 1993*a*,*b*; Snorrason *et al*., 1994; Chouinard *et al*., 1996; Bernatchez *et al*., 1996; Pigeon *et al*., 1997). Such low population genetic structure may suggest that reproductive isolation is incomplete and that gene flow is still occurring between the two forms.

Bernatchez *et al*. (1996) hypothesized that sympatric morphotypes found in lakes with greater opportunities for trophic niche partitioning should have evolved more specialized traits for occupying these niches and that selective pressures should favour the reinforcement of isolating mechanisms to maintain these differences. Similarly, the partial reproductive isolation detected in Lake Bondi could be a consequence of directional selective pressures imposed by the need to maintain trophic specialization between the two forms, as recently proposed to explain the correlation between morphological and genetic divergence among sympatric ecotypes in whitefish (Bernatchez *et al*., 1996; Chouinard *et al*., 1996; Pigeon *et al*., 1997). Taken together, the ecological, morphological and genetic data suggest that brook charr from Lake Bondi have developed adaptations associated with habitat division (littoral and pelagic), and directional selective pressure may have been sufficient to maintain trophic specialization and produce genetic differences between them. Such trophic specialization based on segregation by habitats appears very early in the life history of brook charr. YOY brook charr use either active or sit-and-wait foraging tactics in streams: fish foraging in running water are more sedentary and more aggressive than those foraging in still water (Grant & Noakes, 1988). McLaughlin & Grant (1994) also found significant differences in morphology of YOY brook charr foraging in slow *v*. fast running water. In Lake Bondi, Venne & Magnan (1995) found that YOY brook charr were divided spatially into littoral and profundal individuals which differred in growth, condition and feeding habits.

When considering all sympatric forms of north temperate freshwater fish that have been characterized genetically, there appears to be a continuum in the extent of genetic differentiation among sympatric populations (Hindar, 1994; Chouinard *et al*., 1996). With an interpopulation component of genetic variance <1%, brook charr forms from Lake Bondi seem to represent the lower end of this continuum. The process of divergence may be related to the level of ecological stability, discreteness of niches, and perhaps in some cases to the age of the freshwater system (Skúlason *et al*., 1993). According to this hypothesis, the two forms observed in Lake Bondi may represent an early phase of trophic specialization, where the pelagic specialization may have evolved in the absence of interspecific competition and been driven by both intraspecific competition and the availability of an empty planktonic trophic niche (Robinson & Wilson, 1994; Skúlason & Smith, 1995). Although the results reported here are limited to a single case, they suggest that brook charr inhabiting oligotrophic lakes may exhibit trophic polymorphism associated with slightly genetically differentiated and non-randomly mating groups of fish.

Genetic (Lake Bondi) and morphological (Bondi and Ledoux Lakes) differentiation between littoral and pelagic individuals in the present study support the results of Venne & Magnan (1995) and Bourke *et al*. (1997, 1999) indicating the presence of a subtle trophic polymorphism in brook charr, adapting the benthic form to the littoral zone and the planktivorous form to the pelagic zone. These

lacustrine systems seem to represent early stages of evolutionary divergence in brook charr, making them of particular interest for understanding the processes involved in the early steps of ecological speciation.

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