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Hydrography and population genetic structure in brook charr (*Salvelinus fontinalis*, Mitchill) from eastern Canada

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Abstract

Despite the abundance of studies of genetic diversity in freshwater fishes, few have specifically addressed the role of habitat structure in partitioning genetic variance within and among populations. In this study, we analysed the variability of six microsatellite loci among 24 brook charr population samples in order to correlate hydrographic structure with genetic organization. These populations originated from three Canadian National parks (Kouchibouguac, Fundy and Forillon) that showed distinct hydrographic structure. Considering the general characteristics of these habitats, we formulated specific hypotheses in regard to genetic structure, which were principally based on the potential for gene flow and population size associated with each habitat. The hierarchical analysis of molecular variance and the genetic distances computed among populations revealed that habitat structure analyses constitute an important, but insufficient, predictor of genetic structure. We discuss the importance of habitat complexity on genetic structure in the context of management and conservation.

Keywords: brook charr, *fontinalis*, genetic structure, habitat, microsatellite, *Salvelinus*

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Introduction

Understanding the extent, causes and significance of genetic distribution in time and space is fundamental to many areas of evolutionary biology and ecology (reviewed in Carvalho 1993). Genetic variation is an important tool for defining management and conservation units, which interact in dynamic evolutionary and ecological processes (Waples 1991; Moritz 1994; Bernatchez 1995; Ryman *et al*. 1995). This helps to improve our understanding of environmental factors that may influence the intensity of evolutionary forces (Lewontin 1974; Soulé 1976; Lande 1988).

The structure and partitioning of habitat for life history stages of a given species may affect its population genetic diversity (Sugg *et al*. 1996; Dobson 1998; Piertney *et al*. 1998; Russel *et al*. 1999). For example, reduced levels of intrapopulation genetic diversity may result from restricted gene flow owing to physical barriers (Shaw *et al*. 1994; Hernandez & Smith 1997) and ecological barriers, such as the absence of suitable habitat, even for species with a high potential for dispersal (Piertney *et al*. 1998). A geographical structure favouring dispersal will, on the other hand, facilitate the spread of new allelic combinations (Slatkin 1987; Avise 1992). The extent of genetic divergence among subdivided populations may differ as genetic drift will be largely influenced by their effective population size, which itself may be controlled by the carrying capacity of habitats (Angers *et al*. 1999).

In fishes, trends of population genetic structure associated with habitat structure have been mainly documented by interspecific comparisons. For instance, a general pattern of pronounced, intermediate and weak population divergence has been documented in freshwater, anadromous and marine fishes, respectively (Gyllensten 1985; Ward *et al*. 1994). Such broad comparisons, however, are partly hampered by the difficulty of distinguishing the effects of habitat structure from the variable dispersal capabilities of divergent taxa with sometimes very different life histories. Indeed, the effect of habitat structure on patterns of genetic diversity may be better understood by comparing populations within the same species but found across widely contrasting habitats. Yet, this has been rarely achieved (Shaw *et al*. 1991, 1994; Congdon 1995).

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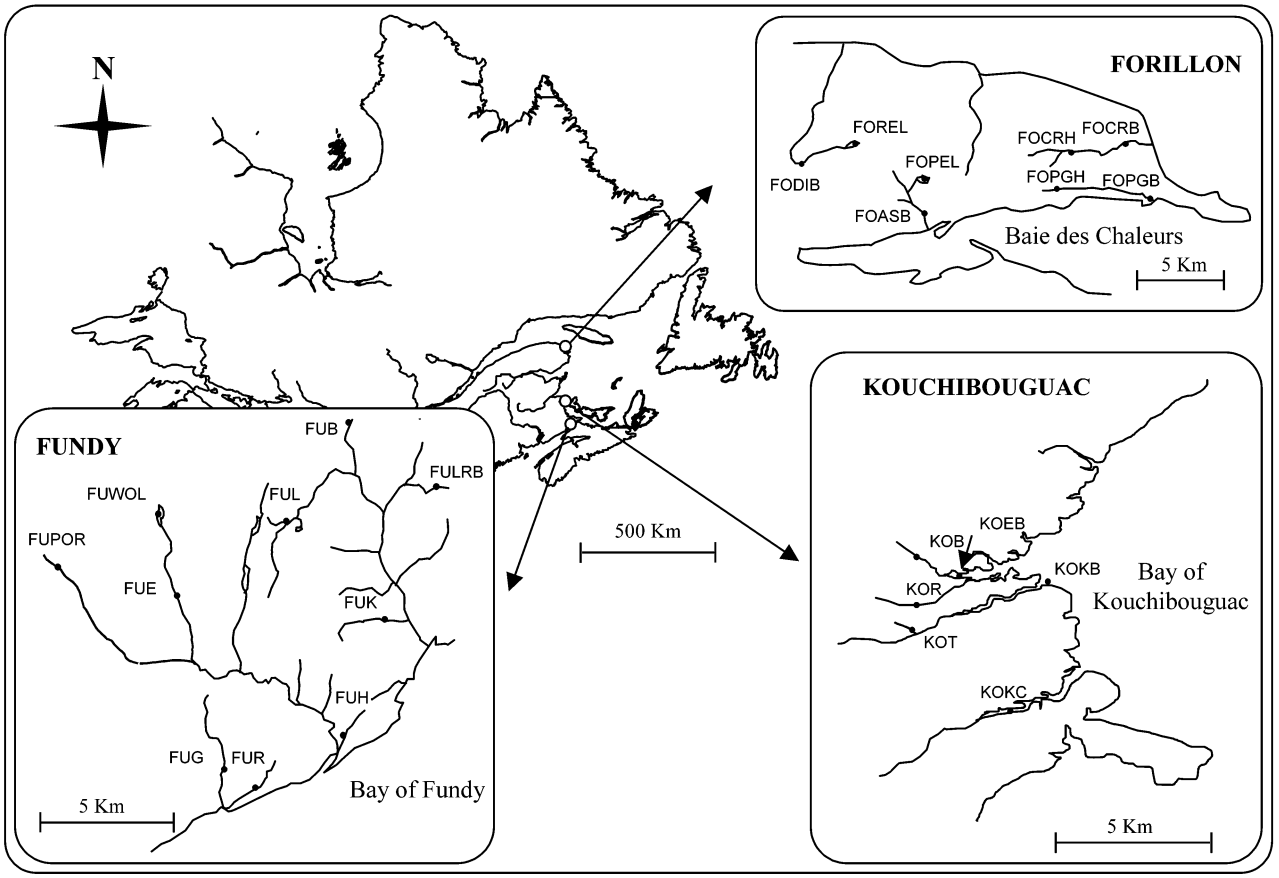

Fig. 1 Geographical locations of brook charr samples from Forillon, Fundy and Kouchibouguac National Parks. Abbreviations correspond to the locations described in Table 1.

The brook charr (*Salvelinus fontinalis*) is an endemic salmonid of eastern North America composed of anadromous and freshwater resident populations. It is found in a very broad range of habitats, from small, high-gradient creeks to large, low-gradient rivers and lakes (Bernatchez & Giroux 1991). Genetic analyses have revealed that this species ranks among the most highly structured animal species (Ward *et al*. 1994). Large-scale analyses based on allozymes and mitochondrial DNA (mtDNA) revealed that the majority of genetic variance in brook charr populations is partitioned among major drainages or regions associated with distinct refugial origins (Perkins & Krueger 1993; Danzmann *et al*. 1998). More recent studies, involving microsatellite loci, also revealed that strong population structure occurred on a geographical scale of a few kilometres, resolving the role contemporary drainage subdivision and patterns of historical recolonization in shaping the observed structure (Angers & Bernatchez 1998; Angers *et al*. 1999). Those studies did not examine, directly, patterns of genetic and habitat structure.

The objective of this study was to quantify the extent of genetic diversity among brook charr populations found in contrasting hydrographic systems, in order to test predictions concerning the effect of habitat on the extent of population genetic structure. Our general approach was to compare genetic structure (as measured by microsatellite DNA variation) among freshwater brook charr populations from three National Parks in eastern Canada, which are strikingly different in hydrographic structure.

Forillon National Park (48°50′N; 64°20′W) covers an area of 244 km^2 and is characterized by small, linear parallel streams draining into the St-Lawrence estuary and the Bay of Gaspé (Fig. 1). As no anadromy has been reported in sampled rivers of that park, and all streams drain directly into salt water, very restricted contemporary gene flow is expected to occur among drainages. Because streams are not subdivided into smaller branches and no obvious physical barriers isolate fish from upstream and downstream sections, little within-drainage structuring is expected. Finally, reduced intrapopulation diversity is

Table 1 Sampling location and mean values per population of number of alleles (A), observed heterozygosity (H_O) and gene diversity (H_E) in Forillon, Fundy and Kouchibouguac National Parks

	Code	N	Latitude	Longitude	A	H_O	H_E
Forillon							
Premier Lac de Penouille	FOPEL	32	48°53′30″	64°24′30″	7	0.69	0.71
Ruisseau Ascha	FOASB	30	48°52′00″	64°25′50″	8	0.70	0.67
Ruisseau de Petit-Gaspé (upstream)	FOPGH	30	48°50′20″	64°18′00″	8	0.55	0.76
Ruisseau de Petit-Gaspé (downstream)	FOPGB	30	48°48′30″	64°14′40″	8	0.76	0.75
Ruisseau Cap-des-Rosiers (upstream)	FOCRH	30	48°51′40″	64°16′00″	10	0.74	0.78
Ruisseau Cap-des-Rosiers (downstream)	FOCRB	30	48°51′00″	64°13′50″	10	0.69	0.75
Lac du Renard	FOREL	28	48°56′20″	64°26′10″	8	0.74	0.73
Rivière de la Division	FODIB	30	48°56′30″	64°30′00″	9	0.74	0.74
Fundy							
Goose River	FUG	32	45°32′00″	65°05′30″	7	0.65	0.74
Rossiter Brook	FUR	32	45°31′48″	65°05′30″	8	0.70	0.71
Hueston Brook	FUH	32	45°33′30″	65°00′30″	9	0.58	0.75
Kinney Brook	FUK	32	45°36′35″	64°57′38″	10	0.73	0.76
Long Reach Brook	FULRB	30	45°40′10″	64°56′10″	7	0.49	0.60
Broad River	FUB	31	45°41′55″	65°00′15″	10	0.68	0.71
Laverty Brook	FUL	32	45°39′40″	65°01′58″	8	0.63	0.66
East branch of Point Wolf River	FUE	32	45°37′55″	65°08′03″	9	0.62	0.69
Wolf Lake	FUWOL	32	45°39′30″	65°09′00″	8	0.51	0.54
Point Wolf River	FUPOR	32	45°38′50″	65°12′40″	10	0.71	0.76
Kouchibouguac							
Black River	KOB	30	46°51′00″	65°01′00″	9	0.72	0.77
Black River Mouth	KOEB	30	46°50′30″	65°58′00″	9	0.78	0.77
Rankin Brook	KOR	30	46°49′30″	65°00′20″	10	0.70	0.75
Kouchibouguac River Mouth	KOKB	24	46°50′00″	65°55′00″	11	0.66	0.78
Tweety Brook (Kouchibouguac River)	KOT	30	46°49′00″	65°07′20″	9	0.69	0.75
Kouchibouguacis River	KOKC	30	46°42′30″	65°05′00″	11	0.70	0.77

expected owing to the small size (estimated mean drainage area: 30 km²) and the physical isolation of the streams.

Fundy National Park (45°20′N; 65°00′W) covers an area of 207 km². It is essentially composed of two river systems with multiple ramifications draining into the Bay of Fundy (Fig. 1). Andromous populations are not known in this system, suggesting low migration among drainages, similar to Forillon. Owing to the multiple tributary ramifications in each river system, greater interpopulation (within-drainage) structure is expected relative to Forillon. Higher intrapopulation diversity is expected because of the overall larger size of drainages (estimated mean area: 135 km²) compared with Forillon.

Kouchibouguac National Park (46°40′N; 65°00′W) covers an area of 239 km² and is characterized by the occurrence of three large and low-gradient rivers that drain into Kouchibouguac and St-Louis Bays (Fig. 1). Anadromy occurs in all rivers and, consequently, higher gene flow and weak interdrainage divergence is expected. It is also expected that intrapopulation genetic diversity should be the highest in this National Park because of the larger (estimated mean drainage area: 275 km²) suitable brook charr habitat in Kouchibougouac than in the other two parks.

Materials and methods

Sampling

A total of 795 brook charr (*Salvelinus fontinalis*), representing 24 sampling sites, were collected in 1996 from the three Canadian National parks described above (Table 1). In Forillon National Park, one downstream and one upstream site were sampled for each of four tributaries. In Fundy National Park, four samples were obtained from the two major drainages and two additional samples were obtained from a smaller, adjacent drainage. Finally, in Kouchibouguac National Park, one downstream (anadromous fish) and one upstream sample were obtained from the Kouchibouguac and Black Rivers, and two additional upstream samples were collected in the Rankin and St-Louis Rivers.

Microsatellite analysis

DNA was extracted from tissues (adipose fins or white muscle), preserved in 95% ethanol, following the Chelex protocol of Estoup & Largiader (1996). Genetic diversity was screened at six microsatellite loci using primers

specifically developed for brook charr (*Sfo8*, *Sfo12*, *Sfo18* and *Sfo23*; Angers et al. 1995), *Salmo trutta* (*MST85*; Presa & Guyomard 1996) or *S. salar* (*SSA197*; O'Reilly *et al*. 1996). Microsatellite polymorphisms were assessed by radioactive (as described in Angers *et al*. 1995) and fluorescent detection. Fluorescent detection utilized two triplex polymerase chain reaction (PCR) amplifications (with primers *Sfo8*, *Sfo12*, *MST85* and *Sfo18*, *Sfo23*, *SSA197*). A denaturing step of 4 min at 95 °C was followed by 32 cycles of 1 min at 95 °C, 45 s at 58 °C and 45 s at 72 °C, and a final elongation step of 10 min at 72 °C. The primers *SSA197*, *Sfo12* and *Sfo18* were 5′ labelled with HEX (yellow); *Sfo8* and *MST85* with TET (green); and *Sfo23* with 6-FAM (blue) dyes. PCR amplifications were performed in a Perkin-Elmer 9600 thermocycler in 10-μL reaction volumes containing: 1 μL of reaction buffer (10 mm Tris-HCL, pH 9.0, 50 mm KCl), 1 U of *Taq* polymerase, 75 μm of each dNTP and ≈ 50 ng of DNA template. Primer concentration varied among loci: *Sfo8* and *SSA197*, 0.085 μm (each primer); *Sfo12*, 0.11 μm; *Sfo18*, 0.20 μm; *Sfo23*, 0.40 μm; and *MST85*, 0.08 μm. For each sample, 1 μL of each triplex PCR product was mixed with 2 μL of blue formamide containing 10% of GS350 internal size standard (red colour-TAMRA 350 bp) and electrophoresized in a 5% denaturing polyacrylamide gel for 2.25 h at 3000 V using an ABI 377 automated sequencer/genescanner. Scoring of allelic size was performed using Genescan (version 2.1) and Genotyper (version 2.0) ABI software, with reference to the internal standard and a reference brook charr sample, with known allelic sizes run on all gels.

Data analysis

Genetic polymorphism was estimated for each sample as the number of alleles per locus (*A*), observed heterozygosity (H_O) and gene diversity (H_E), computed using GENEPOP, version 3.1 (Raymond & Rousset 1995). Overall gene diversity (pooled samples) was also compared among parks. The probability test of Guo & Thompson (1992), available in GENEPOP, was used to test for locus conformity to Hardy–Weinberg equilibrium within samples under both alternative hypotheses of either heterozygote deficit or excess (Raymond & Rousset 1995).

The first step in defining population structure was to test the null hypothesis of homogeneity in allelic distribution, among all pairs of samples within parks, with Fisher's exact test through 1000 iterations using the Markov chain method (in GENEPOP), in order to identify population units. The hierarchical partitioning of genetic diversity among populations was quantified using an analysis of variance framework (Weir & Cockerham 1984) in the program ARLEQUIN, version 1.1 (Schneider *et al*. 1997). We computed estimates of population divergence using both variance in allelic frequency (*F*-statistics; Weir & Cockerham 1984) and allelic size (Φ–statistics; Michalakis & Excoffier 1996). Comparing the extent of population structure depicted from both approaches may allow assessment of the relative role of historical separation and more recent genetic drift processes in population divergence (e.g. Angers & Bernatchez 1998; Goodman 1998). By using this procedure, we first computed both F_{ST} and Φ_{ST} estimates within each park, without hierarchical structure, as an initial estimate of the overall extent of population differences. We then performed hierarchical analyses of gene diversity in order to assess the component of genetic variance corresponding to: (i) variance among individuals within a sample; (ii) among samples within drainage (F_{SC}); and (iii) among drainages (F_{CT}). The significance of *P*-values was adjusted following Bonferroni sequential corrections for multiple simultaneous statistical tests (Rice 1989).

We then assessed relationships among all populations analysed using chord distances (D_{CE}; Cavalli-Sforza & Edwards 1967), which assume pure genetic drift. Pairwise distances were computed using the GENEDIST program included in the PHYLIP computer package, version 3.57c (Felsenstein 1993). The matrix of pairwise distances obtained for D_{CE} was used to construct a population phenogram using the neighbour–joining algorithm available in PHYLIP. Confidence levels on tree topology were estimated by the percentage of 2000 bootstraps performed from resampling loci, and compiled using the CONSENSE program of PHYLIP.

Results

All microsatellite loci generally displayed high levels of polymorphism, with the total number of alleles per locus varying between nine and 61, and the overall gene diversity varying between 0.61 and 0.96 (Table 2). Intrasample diversity was also generally high, with the mean number of alleles per population varying between 3.9 and 15.4, and the mean gene diversity varying between 0.55 and 0.87. Significant departure from Hardy–Weinberg equilibrium (heterozygote deficiency) was detected by multilocus probability tests in five of the 24 samples. Generally speaking, intrasample diversity, expressed both in terms of number of alleles and gene diversity, was similar among parks (Table 1, Table 3). A *t*-test comparing *A* and H_E among each pair of parks for each locus revealed no significant differences for either parameter.

Population divergence

Homogeneity tests of allele frequency distributions revealed significant differences, using Bonferroni sequential corrections, in all pairwise sample comparisons within each park.

Table 2 Overall genetic diversity at six microsatellite loci over 24 populations of brook charr

Locus	Total number of alleles	Mean number of alleles per population (A)	Overall gene diversity (H_E)	Mean gene diversity per population	Mean observed heterozygosity per population	Allelic size range
SFO8	61	15.4	0.96	0.86	0.79	196–330
SFO12	13	4.8	0.74	0.65	0.63	197–275
SFO18	13	5.5	0.76	0.62	0.59	170–193
SFO23	38	13.4	0.94	0.87	0.82	139–231
MST85	30	8.2	0.88	0.75	0.67	142–238
SSA197	9	3.9	0.61	0.55	0.57	146–188
Mean	27.33	8.5	0.82	0.72	0.68	

Table 3 Gene diversity (H_E), observed heterozygosity (H_O) and number of alleles (A) for all populations at all loci

	SFO8			*SFO12*			*SFO18*			*SFO23*			*MST85*			*SSA197*		
	H_E	H_O	A	H_E	H_O	A	H_E	H_O	A	H_E	H_O	A	H_E	H_O	A	H_E	H_O	A
Forillon																		
FOPEL	0.83	0.56	8	0.71	0.68	5	0.56	0.56	5	0.91	1.00	12	0.71	0.69	8	0.53	0.66	4
FOASB	0.86	0.74	12	0.53	0.60	4	0.35	0.40	4	0.81	0.97	13	0.82	0.80	10	0.63	0.69	4
FOPGH	0.88	0.73	15	0.62	0.52	4	0.76	0.73	6	0.86	0.64	12	0.85	0.24	9	0.61	0.50	4
FOPGB	0.90	0.65	13	0.64	0.77	4	0.71	0.79	8	0.84	0.85	13	0.77	0.73	6	0.66	0.75	5
FOCRH	0.91	0.90	16	0.77	0.78	8	0.76	0.72	7	0.88	1.00	16	0.66	0.62	8	0.72	0.57	5
FOCRB	0.91	0.69	19	0.71	0.70	6	0.56	0.53	7	0.89	0.96	13	0.69	0.80	7	0.71	0.57	5
FOREL	0.87	0.93	14	0.67	0.71	8	0.68	0.71	4	0.78	0.82	10	0.79	0.85	7	0.59	0.57	3
FODIB	0.93	0.89	19	0.59	0.53	6	0.66	0.70	4	0.88	0.87	12	0.73	0.83	7	0.67	0.68	4
Mean	0.89	0.76	14.5	0.65	0.66	5.6	0.63	0.65	5.6	0.86	0.89	12.6	0.75	0.70	7.7	0.64	0.62	4.2
Fundy																		
FUG	0.84	0.67	10	0.68	0.66	4	0.70	0.50	6	0.85	0.75	11	0.76	0.70	7	0.57	0.63	4
FUR	0.92	0.87	18	0.55	0.39	3	0.77	0.80	6	0.84	0.96	9	0.56	0.59	5	0.63	0.66	6
FUH	0.94	0.96	17	0.56	0.33	4	0.78	0.70	6	0.90	0.73	13	0.81	0.45	12	0.48	0.45	4
FUK	0.95	0.95	22	0.65	0.61	3	0.75	0.68	7	0.92	0.84	15	0.75	0.55	10	0.55	0.74	3
FULRB	0.85	0.82	12	0.57	0.62	4	0.68	0.63	7	0.86	0.79	13	0.59	0.52	4	0.04	0.04	2
FUK	0.95	0.95	22	0.65	0.61	3	0.75	0.68	7	0.92	0.84	15	0.75	0.55	10	0.55	0.74	3
FULRB	0.85	0.82	12	0.57	0.62	4	0.68	0.63	7	0.86	0.79	13	0.59	0.52	4	0.04	0.04	2
FUB	0.91	0.92	17	0.62	0.71	6	0.59	0.53	5	0.91	0.83	16	0.77	0.75	10	0.49	0.52	3
FUL	0.83	0.89	15	0.54	0.48	4	0.49	0.48	3	0.90	0.96	14	0.76	0.68	7	0.46	0.45	3
FUE	0.90	0.77	15	0.66	0.71	6	0.49	0.42	4	0.89	0.84	18	0.78	0.76	8	0.40	0.41	3
FUWOL	0.57	0.53	12	0.57	0.69	3	0.52	0.53	5	0.71	0.57	13	0.54	0.53	8	0.34	0.34	4
FUPOR	0.92	0.97	22	0.45	0.56	3	0.86	0.81	8	0.91	0.79	13	0.78	0.46	6	0.62	0.70	5
Mean	0.81	0.79	15.1	0.59	0.58	3.8	0.61	0.55	5.4	0.86	0.79	13.0	0.71	0.62	7.6	0.46	0.48	3.5
Kouchibouguac																		
KOB	0.91	0.76	15	0.81	0.74	6	0.58	0.53	6	0.89	0.83	14	0.72	0.67	8	0.68	0.76	4
KOEB	0.90	0.89	15	0.74	0.87	5	0.64	0.63	5	0.92	0.93	14	0.80	0.83	10	0.65	0.66	4
KOR	0.89	0.72	15	0.75	0.76	6	0.68	0.70	6	0.74	0.61	13	0.85	0.73	13	0.59	0.69	4
KOKB	0.94	0.95	19	0.80	0.50	5	0.67	0.57	5	0.95	0.79	19	0.87	0.82	11	0.49	0.50	4
KOT	0.91	0.86	17	0.74	0.71	5	0.63	0.68	5	0.91	0.71	15	0.75	0.50	8	0.56	0.69	4
KOKC	0.94	0.80	21	0.77	0.67	7	0.47	0.47	5	0.95	0.80	17	0.85	0.80	9	0.66	0.67	5
Mean	0.91	0.83	17.0	0.77	0.71	5	0.61	0.60	5.3	0.89	0.78	15.3	0.81	0.72	9.8	0.60	0.66	4.1

The abbreviations correspond to the locations described in Table 1.

This suggests that each of the 24 locations analysed represented a genetically definable population. The overall level of population structure across all sampling site was moderate but very significant ($P < 0.00001$), and comparable values were observed using allelic frequency ($F_{ST} = 0.191$) and allelic size ($\Phi_{ST} = 0.216$) variance. The three parks, however, differed in the extent of overall population divergence. Lower values of both F_{ST} (0.033) and Φ_{ST} (0.089)

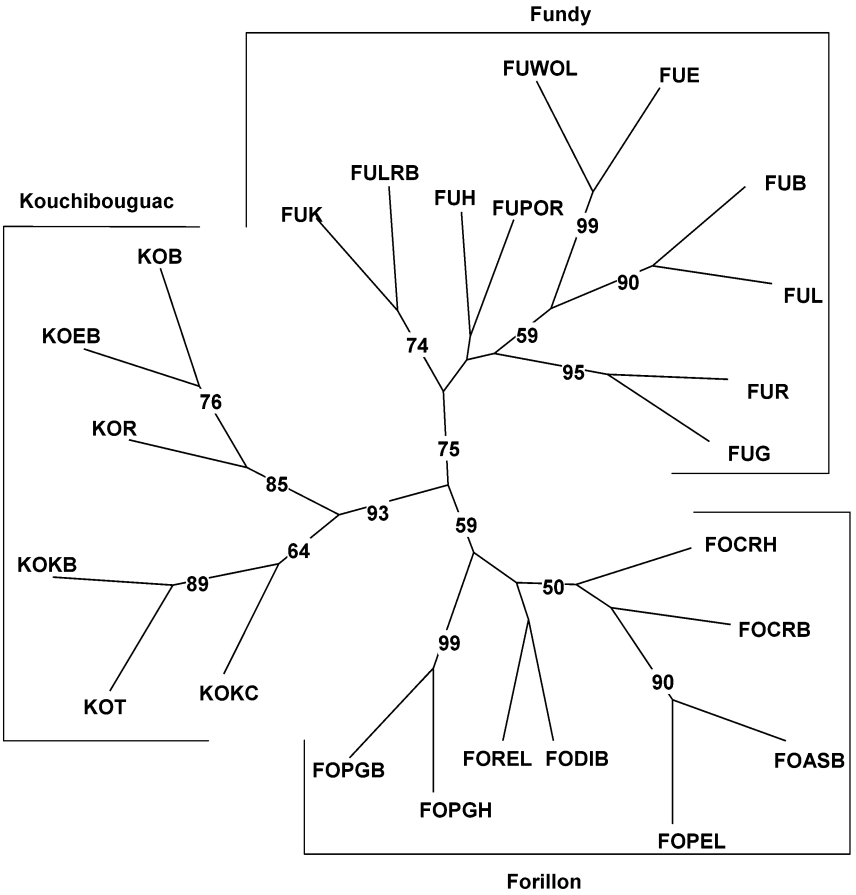

Fig. 2 Population phenogram based on the chord distance of Cavali-Sforza & Edwards (1967). Bootstrap values higher than 50% are indicated along branches.

were observed in Kouchibouguac vs. the other two parks. Both F_{ST} (0.145) and Φ_{ST} (0.149) estimates were similar in Fundy. In Forillon, the F_{ST} value (0.110) was slightly lower than that of Fundy. However, Φ_{ST} was much higher (0.280), suggesting that some populations in Forillon have been separated from others by a period of time sufficient for mutations to accumulate. Examination of allelic size distribution among populations (data not shown) suggested that the highest Φ_{ST} value relative to F_{ST} was driven by two populations from the same drainage (FOCRH and FOCRB). When that drainage was excluded from the analysis, the F_{ST} value remained essentially the same (0.092), whereas the Φ_{ST} was then comparable to that observed in Fundy (0.125).

Population relationships

The topology of the population phenogram constructed from the pairwise D_{CE} matrix revealed that populations of each of the three parks clustered separately (Fig. 2). Globally, populations of the three parks were characterized by qualitative differences in allelic composition (Fig. 3). For instance, alleles *Sfo8–218* to *Sfo8–224*, *Sfo12–251* and *MST85–200* were more frequent in Fundy than in the other two parks, whereas alleles *Sfo8–202*, *Sfo8–206* and *Sfo8–314* to *Sfo8–330* were predominant in Forillon. In Kouchibouguac, alleles *Sfo18–179* and *Sfo23–195* were predominant relative to the other two parks (Fig. 3).

Within parks, populations tended to group by drainage, in both Forillon and Kouchibouguac (Fig. 2). In Forillon, all pairs of populations from the same drainage clustered more closely together, except for FOCRH and FOCRB. Similarly, and despite differences in life history type (anadromous vs. resident), populations from Kouchibouguac also clustered by drainage. In contrast, populations from Fundy did not always group by drainage. Namely, populations FUL and FUB from the upper reaches of the eastern main drainage clustered more closely to upper populations from the other main drainage (FUWOL, FUE) than to more downstream populations of the same drainage (FUK and FULRB). This suggested that population subdivision in Fundy was partly determined by drainage subdivision, and also partly in an upstream/downstream manner, independent of actual drainages.

Hierarchical gene diversity analysis

Concomitant with the observation that populations tended to group by parks, the hierarchical gene diversity analysis

revealed that 7.28% and 15.04% of the total genetic variance was the result of interpark differences, based on variance in allelic frequency and size, respectively. The analysis of molecular variance (AMOVA) also revealed a contrasting pattern of hierarchical genetic structure among parks (Table 4). In Kouchibouguac, components of genetic variance imputable to differences among drainages were low, either based on allele frequency or size variance (with the ratio of interpopulation within drainage/interdrainage close to one). In contrast, Forillon was characterized by a greater component of interpopulation within-drainage relative to interdrainage difference (Table 4). This difference was more pronounced for allelic size variance (ratio interpopulation/interdrainage = 7.571) than for allelic frequency variance (ratio = 4.392). Examination of pairwise Φ_{ST} values (data not shown) revealed that this pattern was mainly driven by the divergence of population FOCRB from the other populations (mean $\Phi_{ST} = 0.563$), including population FOCRH from the same drainage, relative to the mean pairwise divergence among all others (mean $\Phi_{ST} = 0.103$). When the drainages containing FOCRB and FOCRH were excluded from the hierarchical analysis, both the among-drainages and among-populations within-drainage components of genetic variance were comparable based on allelic frequency variance ($F_{CT}/F_{SC} = 0.05656/0.04801$; ratio = 1.178) In contrast, the among-drainages component became more important based on allelic size variance ($\Phi_{CT}/\Phi_{SC} = 0.10217/0.04527$; ratio = 2.2569).

The hierarchical structure observed in Fundy also differed from the other two systems. Thus, the overall analysis

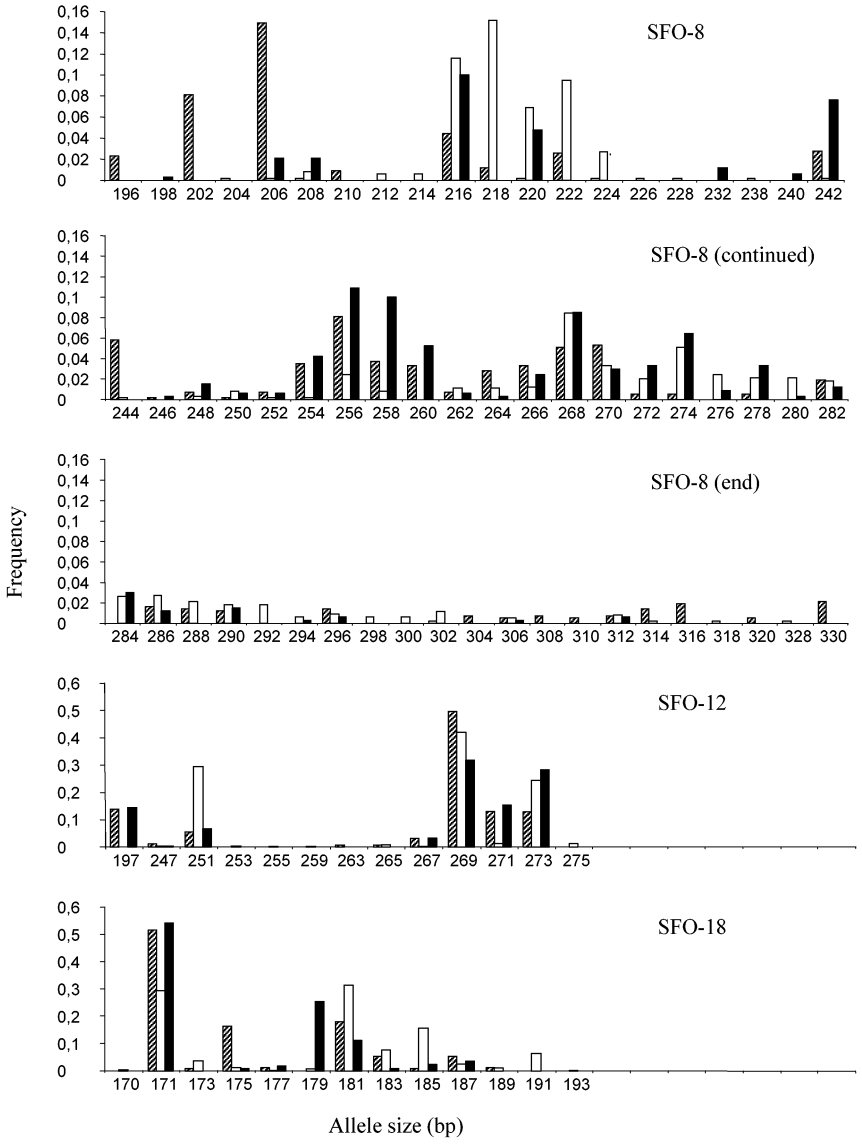

Fig. 3 Histogram illustrating allelic frequency differences among Forillon, Fundy and Kouchibouguac National Parks (hatched boxes, Forillon; open boxes, Fundy; black boxes, Kouchibouguac).

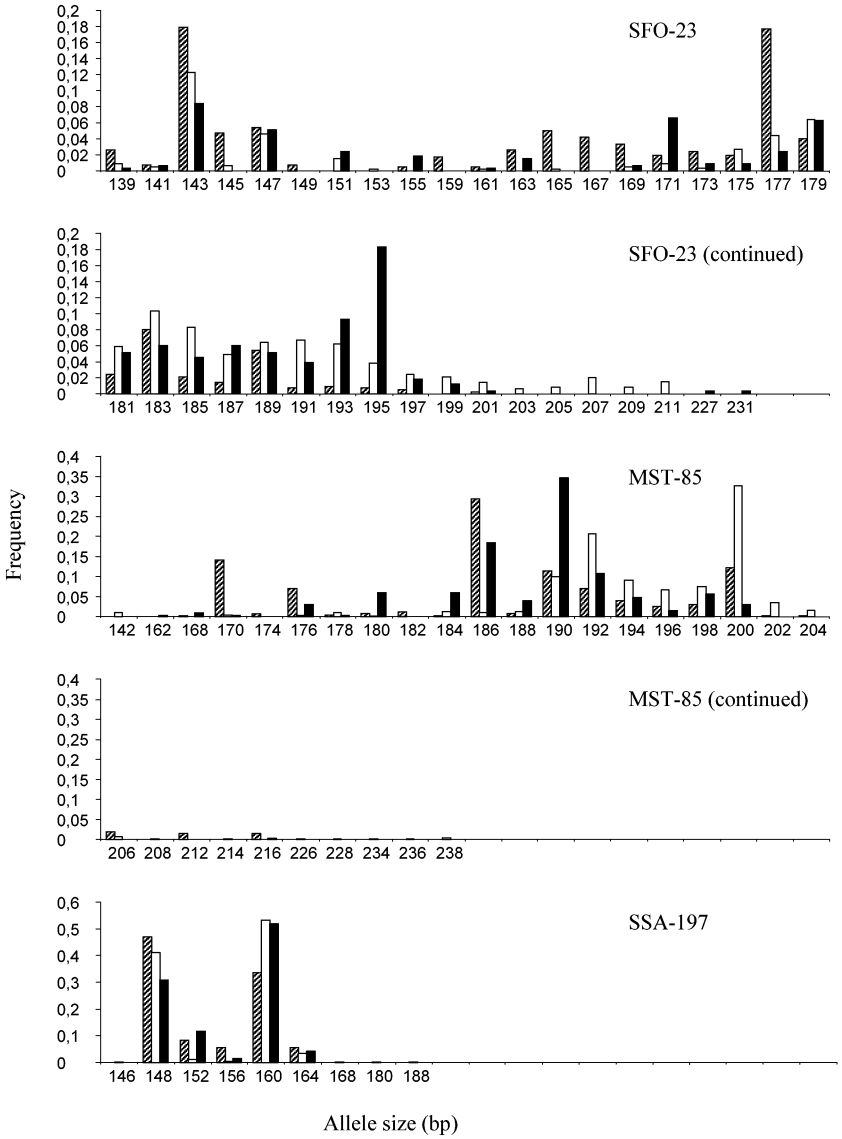

Fig. 3 *continued.*

showed no significant component of genetic variance attributable to interdrainage differences but relatively strong interpopulation differences within drainages, both for F_{SC} and Φ_{SC} (Table 4). The topology of the phenogram based on D_{CE} distance (Fig. 2) suggested the existence of a group of four populations located at the upstream section of the two major drainages (FUWOL, FUE, FUB, FUL). An AMOVA was then performed considering this group, relative to the all other populations, which revealed a more important intergroup component of genetic variance (F_{CT} = 0.159, P = 0.013) and Φ_{CT} = 0.117, P = 0.037) than observed considering groups based on drainages. For comparison, we performed the AMOVA by grouping populations as upstream vs. downstream for the two other parks and nonsignificant, almost null, values were obtained (data not shown).

Discussion

The main objective of this study was to quantify the extent of genetic diversity among brook charr populations found in contrasting hydrographic systems, in order to test predictions concerning the effect of habitat structure on the extent of population genetic structure. Our results both partly confirmed and refuted our predictions. A first prediction was that intrapopulation genetic diversity should have increased with size of habitat. Therefore, we predicted the following trend: Kouchibouguac > Fundy > Forillon. This prediction was refuted by the fact that very similar intrapopulation diversity (expressed by the number of alleles per locus) and gene diversity (H_E) was observed in all populations, independently of the hydrographic system. Similar conclusions were recently reached in a study of

Table 4 Hierarchical analysis of allelic and molecular variance, with intrapopulation/interpopulation and interpopulation/interdrainage ratio values

	Forillon		Fundy		Kouchibouguac	
	% variation	*F*-statistics or *Φ*-statistics	% variation	*F*-statistics or *Φ*-statistics	% variation	*F*-statistics or *Φ*-statistics
F-statistics						
Among drainages (F_{CT})	2.09	0.021	−6.15	−0.061	2.09	0.021*
Among populations within drainage (F_{SC})	9.18	0.094**	21.35	0.201**	1.81	0.018*
Within populations	88.73	0.113**	84.80	0.152**	96.10	0.039*
Ratios						
Intrapopulation/interpopulation (within drainage)	9.666	1.202	3.972	0.756	53.094	2.167
Among populations within drainage/interdrainages	4.392	4.476	—	—	0.866	0.857
Φ-statistics						
Among drainages (Φ_{CT})	3.31	0.033	−6.50	−0.065	5.53	0.055
Among populations within drainage (Φ_{SC})	25.06	0.259**	22.25	0.209**	4.77	0.051*
Within populations	71.63	0.284**	84.25	0.158**	89.70	0.103*
Ratios						
Intrapopulation/interpopulation (within drainage)	2.858	1.097	3.787	0.754	18.805	2.020
Among populations within drainage/interdrainages	7.571	7.848	—	—	0.863	0.927

Percentage of variation (%), allelic frquency (*F*-statistics) and allelic size (*Φ*-statistics) are presented for groups defined as drainage: *P-value < 0.05; **P-value < 0.00001.

The ratios of interpopulation/interdrainage are not presented for Fundy because of negative values for F_{CT}.

lacustrine populations of brook charr. Thus, Angers *et al*. (1999) found no correlation between lake size and genetic diversity measured by the same genetic markers used here. These authors interpreted their results according to the possibility that those populations did not reach mutation-drift equilibrium owing to periodical fluctuations in abundance. A similar explanation might also apply to riverine systems and, consequently, our results may generalize the view that for brook charr, habitat dimension is a poor predictor of the potential to maintain a given level of genetic diversity in contemporary populations.

In contrast, levels of intrapopulation gene diversity observed in riverine populations in this study were consistently higher than those reported for lacustrine populations of brook charr (Angers & Bernatchez 1998). For instance, the mean number of alleles per population per locus (average of 26 lakes), reported by Angers & Bernatchez (1998), varied from 2.42 to 6.85, depending on locus. In this study, it varied from 5.63 to 14.5 in Forillon, from 3.83–15.1 in Fundy, and from 5.33–17.0 in Kouchibouguac, for the same loci. Gene diversity estimates also followed the same pattern. Moreover, Angers *et al*. (1997) analysed populations from small streams within the same area as the lacustrine populations and found that genetic diversity was consistently higher for stream (mean $A = 28$; mean $H_E = 0.67$) than lacustrine (mean $A = 24$; mean $H_E = 0.48$) populations. Altogether, those observations suggest that while there is no association between intrapopulation genetic diversity and habitat size, there may be a distinction between habitat type. The potential explanation for this is currently only speculative. One possibility is that riverine populations are demographically more stable in time than lake populations, thus leading to the maintenance of an overall higher effective population size (N_e). More likely, however, is the possibility that genetically distinct populations within a stream system are not isolated, may exchange genes by migration and could be considered as a metapopulation (Harrison & Taylor 1997). It is theoretically demonstrated that such an association between several small populations may result in a higher level of genetic variation than a single panmictic population of the same census size (Kimura & Crow 1963). This could lead to a higher effective population size in riverine than lacustrine populations, thus explaining the higher intrapopulation genetic diversity. Whatever the definitive explanations, these observations

indicate that stream populations of brook charr should be considered as important as lacustrine populations for maintaining genetic diversity.

Our second prediction was that the extent of population structure should be correlated with the complexity of within-drainage habitat structure, and that the interdrainage component of genetic variance should be commensurate with the potential for gene flow among them. A salient result was that homogeneity tests of the distribution of the alleles indicated that each of the 24 samples analysed represented distinct, nonrandomly mating populations, even when found within the same drainage and geographically isolated by few kilometres (less than 5 km). This is congruent with the recent observation in brown trout (*Salmo trutta*), that resident stream salmonids can develop discrete reproductive units on a very small scale (Estoup *et al*. 1998).

The observed pattern of genetic structure was congruent with the predictions for Kouchibouguac populations. Globally, the extent of population structure both within and among drainages was low, indicating that anadromy maintains gene flow among populations from neighbouring rivers, reducing interdrainage divergence. These observations corroborate results of a previous study, based on allozymes and mtDNA, which revealed that anadromous and resident populations from a given drainage were more similar than anadromous populations of different drainages (Jones *et al*. 1997). It also fits the general pattern of differences in population divergences between freshwater and anadromous fishes (Ward *et al*. 1994).

The congruence between *a priori* predictions and patterns of population structuring observed in Forillon and Fundy National Parks was less clear. For both parks, we predicted a more important interdrainage divergence than interpopulation within-drainage divergence because we assumed the impossibility of contemporary gene flow among drainages. Surprisingly, no significant component of interdrainage divergence was observed in Fundy and Forillon in the first analysis. A more detailed examination revealed that the hierarchical pattern observed in Forillon was driven by one highly divergent population. However, even when this population was excluded from the analysis, interdrainage divergence remained relatively similar to interpopulation variation within drainage (ratio = 1.249) using allelic frequency information. This suggests that gene flow has been as limited within the drainages as among them. The relatively weak interdrainage divergence component (F_{CT} = 0.048, population FOCRB excluded) might indicate intermittent gene flow among drainages, even though anadromy has not been reported in those populations.

In Fundy, the extent of interpopulation variance within drainage/interdrainage was more important than in Forillon. This partly corroborates the prediction of a higher structure owing to multiple tributary ramifications in Fundy. Yet, this factor alone cannot explain the absence of significant interdrainage differentiation. Thus, the closer relationships among the four upstream populations from physically isolated drainages may indicate historical connections. Previous drainage rearrangement may be a plausible hypothesis explaining this unexpected grouping (Bishop 1995). In this way, populations from the two major drainages may have previously been in contact, exchanging genes. The pattern observed in Fundy may also have resulted from a different recolonizing population wave that then remained isolated. A similar scenario has been invoked to explain the latitudinal component of genetic structuring in brook charr from La Mauricie National Park, Canada (Angers & Bernatchez 1998; Angers *et al*. 1999).

Population origins

The observed pattern of population relationships and genetic divergence suggests that contemporary populations from these three regions may have distinct historical origins. Thus, the D_{CE} topology showed a significant grouping of population by region, which was confirmed by the AMOVA. Such a pattern would be unlikely under a scenario of a single origin for all populations, followed by differentiation by genetic drift, unless all populations within a single park were connected by gene flow. Although we cannot entirely refute this hypothesis, it appears unlikely in the light of our results. Alternatively, it is possible that the different parks were colonized by distinct waves of founding populations, as documented by Angers & Bernatchez (1998). These authors showed the potential of microsatellite analysis to identify historical events of recolonization by distinct ancestral populations issued from a single glacial race. A similar scenario could apply here, as Jones *et al*. (1996) and Danzman *et al*. (1998) showed from mtDNA analysis that Eastern Canada was probably colonized from a single glacial race of brook charr.

In conclusion, this study represents one of the few attempts to predict population genetic structure from habitat structure. Our results showed that such association is at best partial, and that many additional factors must be considered. This study indicates that historical demography may be as important as contemporary factors in explaining patterns of genetic diversity among populations of north temperate fishes (reviewed in Bernatchez & Wilson 1998). The lack of association between genetic and habitat structure may also partly reflect a lack of understanding of the species population biology. The apparent pattern of intrapopulation genetic diversity in stream vs. lake habitat is one case in point. Similarly, the divergences observed among populations within streams over short

distances in the apparent absence of physical barriers may be indicative of strong philopatry over small geographical scales. Altogether, these observations stress the need for further interactions of ecological and population genetic studies.

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