Microsatellite gene diversity analysis in anadromous arctic char, *Salvelinus alpinus*, from Labrador, Canada

Louis Bernatchez, J. Brian Dempson, and Sylvain Martin

Abstract: We analysed six loci among 257 Arctic char (*Salvelinus alpinus*) representing seven locations in Labrador and Newfoundland to provide a first assessment of microsatellites gene diversity in anadromous char and to determine the geographic scale of population structuring within the species. The number of alleles per locus varied between 9 and 48, and gene diversity ranged from 0.190 to 0.968. Significant F_{ST} and differences in allele frequencies were observed among most samples, as well as heterozygous deficiency, which was indicative of a Wahlund's effect. These results implied the existence of genetically distinct populations on a microgeographic scale (less than 10 km) and that our samples represented an admixture of char from those populations that interchange among rivers for owerwintering, in congruence with tagging investigations. These results indicate that microsatellites potentially offer more sensitivity than allozymes and mitochondrial DNA to infer fine-scale population structure in anadromous arctic char.

Résumé : Nous avons analysé six loci parmi 257 ombles chevalier *(Salvelinus alpinus*) provenant de sept sites au Labrador et à Terre-Neuve dans le but d'établir l'utilité des microsatellites pour la quantification du partage de variance génétique parmi les populations anadromes et pour documenter l'échelle de structuration génétique chez cette espèce. Les microsatellites se sont révélés modérément à hautement variables, avec un nombre d'allèles par locus variant entre 9 et 48 et une hétérozygotie attendue variant entre 0.190 et 0.968. Des valeurs de F_{ST} significatives, de même qu'une hétérogénéité des fréquences alléliques ont été observées entre la plupart des échantillons. Nous avons observé un déficit en hétérozygote associé à un effet Wahlund dans tous les échantillons, sauf un. Ces résultats traduisent l'existence de populations génétiquement différenciées sur une échelle microgéographique (inférieure à 10 km), de même qu'un mélange de ces populations dans les tributaires d'hivernage, corroborant ainsi les études de marquage. Nous concluons que les microsatellites offre une sensibilité supérieure aux marqueurs traditionnels pour l'étude de structures populationelles fines.

Introduction

Explaining the extent, causes, and consequences of biotic distribution in space is fundamental to our understanding of how species evolve to cope in particular environments, as well as for their conservation and management. In the past decade, the member–vagrant hypothesis has provided an important conceptual framework for studying the role of ecological processes in determining spatial patterns of abundance in aquatic species (Sinclair and Iles 1988). One prediction of this hypothesis is that the number of populations composing a given species will primarily be determined by the number of environmental settings (either geographic, physical, or oceanographic) allowing the closure of a species life cycle. Thus, the member–vagrant hypothesis predicts that anadromous fish species, typically homing to a given river for spawning, and into which juvenile fish grow until moving down to sea where they live until sexual maturation, should be composed of numerous populations determined by the number of rivers available to the species. This has been corroborated by both ecological and genetic studies in species of the genus *Salmo* and *Oncorhynchus* (e.g., Davidson et al. 1989; Sinclair and Iles 1988). Predicting population structure from such theoretical framework may, however, be more complicated in species exhibiting complex migratory behavior, for instance, involving sporadic coastal movements at both juvenile and adult stages and (or) overwintering migration in different rivers, and for which homing behavior has not been documented.

Anadromous arctic char (*Salvelinus alpinus*) represents an extreme in complexity of migratory behavior among anadromous fishes. Periodic migrations of adult and juvenile fish to the sea enable anadromous populations to take advantage of marine food resources (reviewed in Dempson 1995). The seaward movement coincides with spring runoff and ice breakup in coastal rivers and consists of both first-time and repeat migrants. First-time migrants have spent 1–8 years in fresh water. Repeat migrants include adults (maturing and nonmaturing) and juveniles. Arctic char are not known to

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Table 1. Sample locations with their abbrevaiations, date of sampling, and life-history stage sampled (A, adult; J, juvenile).

Region and river	Latitude (N)	Longitude (W)	Date	Stage	*N*
Labrador					
Pangertok Inlet River (PI)	58°21′	63°10′	08/1991	A	30
Sachem Bay Pond (SB)	56°41′	61°48′	08/1988	A	41
Fraser River (FR)	56°39′	63°10′	07/1988	A	39
Reid Brook (RB)	58°18′	62°05′	08/1996	A, J	46
Ikadlivik Brook (IB)	58°18′	62°10′	08/1996	A	30
Kogluktokluk Brook (KOB)	58°18′	62°07′	08/1996	J	30
Newfoundland					
Gander Lake (GL)	48°55′	54°35′	07/1995	A, J	40

overwinter at sea and reenter freshwater yearly at the end of the summer and early fall.

Such complexity in migratory behaviour and the lack of clear information about the extent of homing behaviour makes it difficult to predict the population structure in Arctic char. Namely, it is not clear if the fact that the species use more than one river to complete its life cycle should result in populations structured by region rather than by river. Tagging studies revealed that summer coastal movements are geographically localized and indicated that char from different geographic areas may be considered different population units (Dempson 1995). On a smaller scale, tagging studies revealed the propensity of char to move among different rivers emptying within a same bay from year to year (Dempson and Kristofferson 1987). These observations are compatible with the hypothesis that char populations may be structured by region rather than by river. On the other hand, it is not known whether straying fish only comprised nonspawning, overwintering components of the populations or also included mature fish, therefore precluding to refute the hypothesis that the species may be genetically structured by river.

In theory, allele frequency data from neutral loci may provide the best approach to assess genetic population structure. Thus far, the usefulness of allozymes and mitochondrial DNA (mtDNA) to characterize genetic structure in arctic char has been hampered bythe extremely reduced polymorphism generally observed in the species (e.g., Wilson et al. 1996). In addition, there are increasing concerns that the non-neutrality of enzymatic loci may, in some cases, mask the identity of existing population differentiation (e.g., Verspoor and Jordan 1989).

Microsatellites are a class of highly polymorphic and presumably neutral nuclear loci that are receiving increasing attention (Jarne and Lagoda 1996). The usefulness of microsatellites for addressing fine-scale population structure has been demonstrated in *Salvelinus* species (Angers and Bernatchez 1998). In the only use of microsatellites in arctic char published thus far, a recent study on European populations from central alpine lakes clarified the species population structure on a mesogeographic scale where other markers failed to detect significant genetic diversity partitioning (Brunner et al. 1998).

In this study we performed a gene diversity analysis among arctic char from Labrador and Newfoundland to assess the scale of population structuring in anadromous species using more than one river to complete their life cycle. This study also provides an assessment of the potential usefulness of microsatellites to infer fine-scale population structure in species exhibiting extremely reduced genetic diversity at both allozymes and mtDNA, such as observed in arctic char. We also document the usefulness of comparing data analyses based on the consideration, or not, of mutational differences among alleles to infer genetic structure among closely related populations. Finally, we discuss the importance of the observed population structure in the context of environmental impacts.

Materials and methods

Samples and microsatellite loci

A total of 257 arctic char were collected to cover different geographic scales from six sampling sites along the Labrador coast and from an inland lake in Newfoundland, as a reference population to ensure that statistically significant genetic divergence can indeed be quantified among populations known a priori not to be currently connected by gene flow (Table 1). Three of the most important tributaries of Voisey's Bay that support arctic char were surveyed (Fig. 1). Ikadlivik Brook is a tributary of Kogluktokoluk Brook, which empties into the head of Voisey's Bay. Reid Brook's mouth essentially empties at the same location as the Kogluktokoluk River (Fig. 1) and is normally considered as an extended tributary of the Kogluktokoluk–Ikadlivik river system. The other samples represent char originally assigned to several different management stock complexes distinct from Voisey's Bay (Dempson and Kristofferson 1987). Reid Brook samples were captured either in Reid Brook itself or in Reid Pond located about 15 km upstream of Reid Brook's mouth. Tissues used for DNA extraction consisted of either adipose fins, white muscle, or liver first kept at –20°C and then fixed in 95% ethanol for shipment from Newfoundland to Québec City for genetic analysis. Genetic diversity at six microsatellite loci was screened using primers originally developed for other salmonids to amplify homologous microsatellite loci in *S. alpinus* (Table 2). The methods used for DNA extraction, PCR reactions, electrophoresis, autoradiography and allele scoring were as outlined in Brunner et al. (1998).

Genetic diversity

Genetic polymorphism for each sample (=location throughout the text) was estimated as the mean number of alleles per locus (*A*),

Fig. 1. Location map showing sampling sites for Arctic char from northern Labrador and Newfoundland.

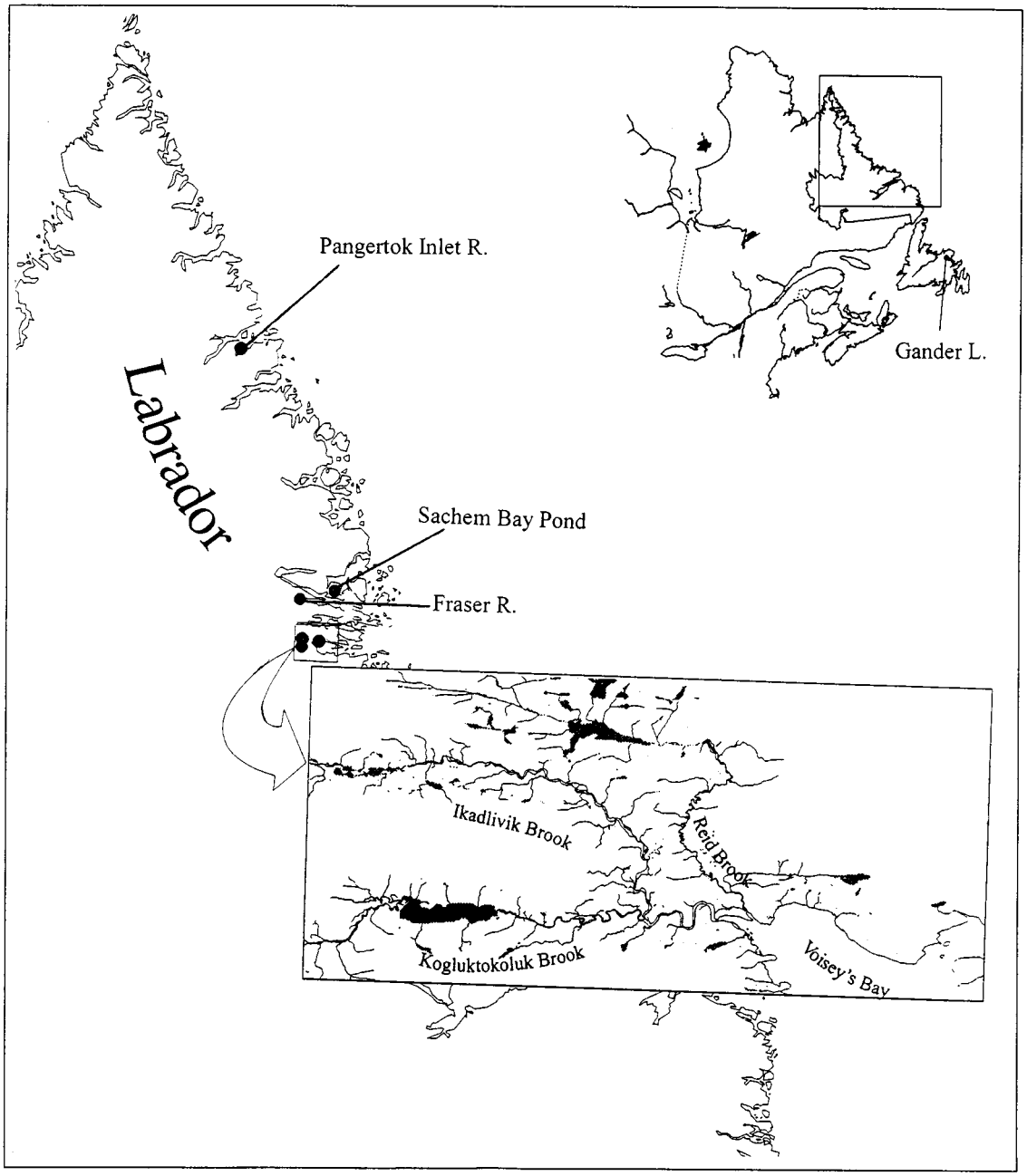


Table 2. Source species, reference, annealing temperature, allelic size range, number of fish successfully analyzed per locus (*N*), number of alleles per locus (*A*), and overall gene diversity (H_e) for six microsatellite loci used in this study.

Locus	Species	Reference	Temperature (°C)	Size range (bp)	*N*	*A*	H_e
MST85	*Salmo trutta*	Presa and Guyomard 1996	55	170–220	248	16	0.521
Pupupy	*Oncorhynchus mykiss*	Morris et al. 1996	60	387–492	247	9	0.411
Cocl-3	*Coregonus clupeaformis*	Bernatchez 1996	50	219–289	240	33	0.760
Ssa-85	*Salmo salar*	O'Reilly et al. 1996	60	115–255	257	46	0.911
Sfo-8	*Salvelinus fontinalis*	Angers et al. 1995	60	216–316	234	33	0.916
Sfo-23	*Salvelinus fontinalis*	Angers et al. 1995	60	159–289	237	48	0.907

observed heterozygosity (H_o), and gene diversity (H_e), using the version 3.0 of GENEPOP (Raymond and Rousset 1995). GENEPOP was also used to assess heterogeneity in allele frequencies among pairwise comparisons of samples and to test for deviations from Hardy–Weinberg (HW) equilibrium within samples for every single locus and over all loci. Values of significance were estimated by Fisher's exact test in cases of five alleles per locus or less, and a Markov chain method to obtain unbiased estimates of the exact test through 1000 iterations when the number of alleles was higher. As discussed by Rousset and Raymond (1997), exact tests share several properties that make them one of the most powerful method to circumvent potential problems imposed to the use of more traditional statistical procedures by high number alleles relative to the sample size, a typical situation with microsatellites. Briefly, exact tests do not require the use of an asymptotic distribution (exactness) and rely on probability distribution independent of unknown parameters under the null hypothesis. Together, this leads to the use of particular conditional distributions that is independant of unknown frequencies. It has been shown that the power of exact tests remains high at elevated heterozygosities (>0.70) and that power increases and asymptotic variance around genetic parameters estimates decreases with the number of alleles (Rousset and Raymond 1997, and references therein).

The population genetic structure was quantified using an analysis of variance framework, which involves computing correlation of a pair of alleles drawn from the same subpopulation relative to that randomly drawn from a group of subpopulations (Weir and Cockerham 1984). Fixation indices were also computed in an analysis of molecular variance framework (AMOVA), using information on the mutational differences among alleles, which was entered as a matrix of Euclidian squared distances (Michalakis and Excoffier 1996). Comparing the extent of population structure depicted from allele frequency and mutational information may allow to assess the relative role of long-term separation and contemporary genetic drift in population differentiation (Bernatchez and Martin 1996). By this procedure, genetic structure indices were computed at three hierarchical levels: within individuals, among individuals within samples (F_{IS}), and among samples (F_{ST}) using the program ARLEQUIN (Schneider et al. 1997). The significance of the variance components associated with the different levels of genetic structure was tested using nonparametric permutation procedures (1000 permutations in the present case). Pairwise F_{ST} estimates among all population combinations were also computed in the same manner.

Population relationships

Because of the uncertainty regarding what constitutes the most appropriate method for quantifying population differences based on microsatellite polymorphism, two measures of genetic distance were performed; chord distance (D_{CE}; Cavalli-Sforza and Edwards 1967), assuming pure genetic drift, and the $(\delta\mu)^2$ genetic distance, which takes into account size differences between alleles and theoretically fits linearity with time better than non-SMM based distance measures when microsatellites follow a strict stepwise mutation model (SMM) (Goldstein et al. 1995). Simulation and empirical studies recently demonstrated that the use of chord distance generally leads to a higher probability of obtaining the correct tree topology under either the infinite allele model (IAM) or SMM assumptions, whereas branch lengths may be better approximated from SMM-based methods with microsatellites (Takezaki and Nei 1996; Angers and Bernatchez 1998). Therefore, only the D_{CE} distance matrix was used to obtain the topology of neighbour-joining phenograms. Branch lengths obtained from both D_{CE} and $(\delta\mu)^2$ were then compared by imposing the D_{CE} topology when using the $(\delta\mu)^2$ distances with the user tree option in PHYLIP, version 3.5 (Felsenstein 1993). Comparisons of both phenograms may then provide insight into the relative role of genetic drift and mutations on population divergence. Bootstrap values on branching patterns were obtained by 1000 resamplings of loci and individuals within samples using a program developed by J.M. Cornuet (Institut national de la recherche agronomique, Laboratoire de neurobiologie comparée des invertébrés, Bures-sur-Yvette, France).

Results

Overall and intrapopulation gene diversity

All six microsatellite loci used were moderately (*MST-85**, *Pupupy**, *Cocl-3**) or highly (*Sfo-8**, *Ssa-85**, *Sfo-23**) polymorphic, with the total number of alleles per locus varying between 9 and 48 and overall heterozygosity at each locus ranging between 0.411 and 0.916 (Table 2). The intrapopulation diversity was also generally high for all populations with gene diversity (H_e) ranging between 0.190 and 0.968 depending on locus and population (Table 3). *F* statistics revealed highly significant positive F_{IS} estimates based either on allelic or molecular variance analysis with a higher component observed in the latter case (Table 4), which indicates an overall departure from HW genotypic frequency expectations due to heterozygous deficiency in one or more samples. Indeed, global F_{IS} estimates were positive for each sample except for FR (abbreviations are given in Table 1), although only two of these values (GL and PI) were statistically significant (Table 1). However, when RB was split into Reid Brook and Reid Pond, a highly significant F_{IS} value was observed in Reid Brook ($F_{IS} = 0.108$, $P = 0.005$), whereas char from Reid Pond appeared to be in HW equilibrium ($F_{IS} = -0.014$, $P = 0.9079$). Overall tests revealed that three loci (*MST-85**, *Pupupy**, and *Cocl-3**) were globally responsible for the significant F_{IS} values observed ($0.00001 < P < 0.003$). Significant values were not consistently observed for the same loci in all populations, and a significant value was also observed at *Sfo-8** in one population (Table 3). Significant genotypic phase disequilibrium was also detected for one different locus pair in each sample except for GL and PI: RB (*MST-85*/Cocl-3**, $P < 0.00001$), IB (*Cocl-3*/Sfo-8**, $P = 0.01$), KOB (*MST85*/Pupupy**, $P = 0.035$), SB (*Pupupy*/Ssa-85**, $P < 0.00001$), which suggested the absence of physical linkage. Globally, highly significant F_{IS} and (or) genotypic phase disequilibrium estimates are indicative of either small population sizes, the presence of null alleles, or Wahlund's effect (see Discussion).

Population structure

Population structure analysis led to rejection of the null hypothesis of no differentiation among samples, indicating the existence of two or more genetically distinct populations among the samples analysed. *F* statistics revealed an overall moderate but highly significant interpopulation component based either on allelic or molecular variance analysis, with a higher value obtained in the latter case (Table 4). Pairwise population analysis based on allelic variance revealed significant F_{ST} values in all comparisons, except between FR and PI ($P = 0.120$) (Table 5). A different scenario was observed in the analysis based on molecular variance where only comparisons involving GL and KOB populations remained highly significant. In those cases, F values were largely increased, whereas they were reduced for the others. Both analyses, as expected, were congruent in showing a high differentiation of GL from other populations, and interestingly enough, a higher differentiation between KOB and its

Table 3. Total number of alleles scored for each sample and locus (*N*), number of different alleles (*A*), observed heterozygosity (H_o), gene diversity (H_e), and significant ($P < 0.05$) exact probability or unbiased estimate of type-I error for Hardy-Weinberg (HW) departure proportions per locus and population.

	GL	IB	KOB	RB	FR	SB	PI
MST-85							
N	80	60	60	92	60	78	60
A	7	8	7	9	9	6	7
H_o	0.400	0.400	0.733	0.608	0.467	0.436	0.467
H_e	0.696	0.475	0.622	0.706	0.408	0.415	0.508
HW	$<10^{-5}$ns	ns	0.014	ns	ns	0.043	
Pupupy							
N	80	60	60	92	54	82	60
A	4	5	5	7	4	4	3
H_o	0.400	0.200	0.133	0.478	0.519	0.561	0.500
H_e	0.443	0.533	0.190	0.659	0.545	0.646	0.538
HW	ns	0.002	0.042	ns	ns	0.007	ns
Cocl-3							
N	80	60	60	88	48	80	58
A	10	12	16	18	13	15	13
H_o	0.875	0.700	0.833	0.750	0.750	0.725	0.690
H_e	0.873	0.835	0.877	0.884	0.890	0.844	0.835
HW	ns	0.023	ns	ns	ns	ns	ns
Ssa-85							
N	80	60	60	92	74	82	60
A	26	25	18	22	34	25	20
H_o	0.925	1.000	0.900	0.870	1.000	0.854	0.833
H_e	0.950	0.957	0.913	0.929	0.965	0.929	0.893
HW	ns	ns	ns	ns	ns	ns	ns
Sfo-8							
N	78	60	60	90	34	82	68
A	14	15	14	19	15	11	21
H_o	0.821	1.000	0.966	0.956	0.880	0.829	0.862
H_e	0.871	0.898	0.896	0.902	0.919	0.831	0.940
HW	$<10^{-5}$ns	ns	ns	ns	ns	ns	
Sfo-23							
N	72	60	56	90	60	70	60
A	34	20	20	23	23	22	18
H_o	0.833	0.933	0.964	0.978	0.967	0.800	0.867
H_e	0.968	0.930	0.935	0.922	0.902	0.837	0.924
HW	0.043	ns	ns	ns	ns	ns	ns
F_{IS} (overall)	0.118***	0.084	0.078	0.081	–0.084	0.086	0.091***

Note: Abbreviations for populations are given in Table 1. ns, not significant. *** Significant ($P < 0.00001$) overall F_{IS} estimates, using the Fisher's method and following sequential Bonferroni corrections ($k = 7$).

Table 4. Fixation indices based on allelic (F_{ST}) and molecular (Φ) variance analysis.

	F_{ST}	*P**	Φ	*P**
F_{IS}	0.0574	$<10^{-5}$	0.1412	$<10^{-5}$
F_{ST}	0.0586	$<10^{-5}$	0.1082	$<10^{-5}$
F_{IT}	0.1126	$<10^{-5}$	0.2341	$<10^{-5}$

**P*, Probability of difference from zero.

tributary, IB, than between the latter one and the neighbouring RB population.

Concomitant with *F*-statistic results, the existence of more than one population in the system was also supported by significant differences in allele frequency between all pairwise sample comparisons. The number of significant comparisons (out of 21 per locus) varied between 14 and 20 depending on locus. Most samples were characterized by the presence of specific alleles or striking differences in frequencies. For instance, GL population was characterized by high

Table 5. Pairwise differentiation estimates based on allelic (F_{ST}; below diagonal) and molecular (Φ; above diagonal) variance analysis.

	GL	IB	KOB	RB	FR	SB	PI
GL		0.2525*	0.2609*	0.1640*	0.1417*	0.2324*	0.2758*
IB	0.0927*		0.1409*	0.0020	0.0235	0.0000	0.0000
KOB	0.0845*	0.0329*		0.1300*	0.1500*	0.1280*	0.1002*
RB	0.0765*	0.0161*	0.0471*		0.0000	0.0117	0.0156
FR	0.1155*	0.0225*	0.0725*	0.0255*		0.0258	0.0439
SB	0.1244*	0.0397*	0.0790*	0.0370*	0.0266*		0.0000
PI	0.1111*	0.0339*	0.0711*	0.0359*	0.0091	0.0300*	

Note: Abbreviations refer to populations in Table 1.

*Statistically significant ($\alpha = 0.05$) following adjustment for multiple tests with the sequential Bonferroni method ($k = 21$).

frequencies of alleles *MST-85*196* and *MST-85*204* (relative frequency (RF) > 0.325), which were found at low frequencies elsewhere (RF < 0.083). Within Voisey's Bay, KOB char were characterized by a relatively high frequency of allele *MST-85*208* (RF = 0.217) compared with IB and RB (RF < 0.05). Similarly, allele *Pupupy*459* was frequent in those two populations (RF > 0.239) compared with KOB (RF < 0.033). Allele *Cocl-3*225*, which was relatively abundant elsewhere (RF > 0.136), was not observed in IB population. Northern populations (PI and SB) differed by a relatively high frequency of allele *SSa-85*189*, which was absent or observed at very low frequency among more southern populations. Allele *Sfo-23*183* was abundant in the FR population (RF = 0.283) but relatively rare elsewhere (RF < 0.10). Many additional differences were observed at all loci (data not shown but available upon request).

Such differences in allele frequencies resulted in relatively large genetic distances among samples (Fig. 2). Gander Lake population was the most distant, as expected from its physical isolation. All Voisey's Bay samples clustered together, although supported by a relatively low bootstrap value (either based on locus or individual bootstraping) and FR clustered closer to PI than SB samples, thus not correlating with the geographic distance separating them. The most striking difference observed between the D_{CE} and $(\delta\mu)^2$ phenograms was the much longer branch length differentiating the KOB char from all other anadromous samples in the latter one. The $(\delta\mu)^2$ phenogram also increased the divergence of the GL population relative to anadromous ones.

Discussion

Microsatellite diversity in anadromous arctic char

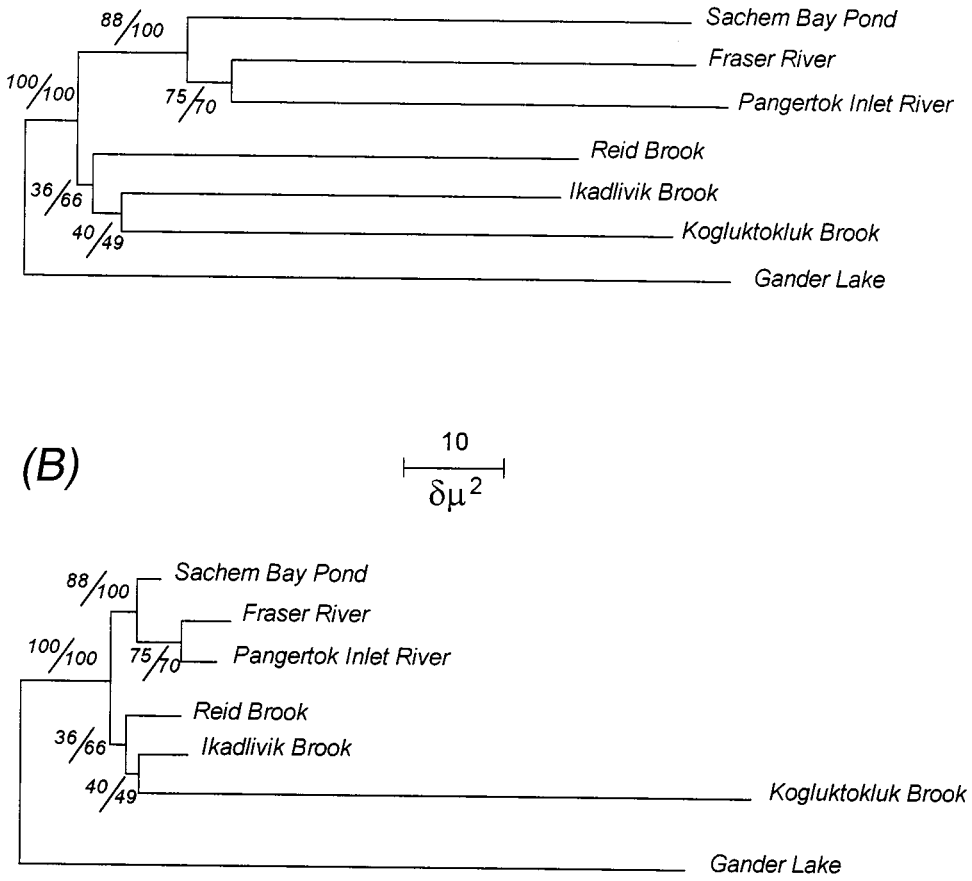
High level of polymorphism of microsatellite loci used in this study is in sharp contrast with the extremely low levels of variation detected in arctic char with other genetic markers. For instance, average heterozygosity ($H_e = 0.737$) and number of alleles per locus ($A = 30.8$) observed in microsatellites largely exceeds values generally reported in allozyme studies in which average heterozygosity and number of alleles for polymorphic loci do not exceed 0.05 and 2.0 (e.g., Osinov et al. 1996). Levels of mtDNA diversity detected by either RFLP or sequencing in arctic char are also much lower than for microsatellites, with overall haplotypic diversity and the total number of haplotypes reported never exceeding 0.40 and 10, respectively (e.g., Wilson et al. 1996). The extent of microsatellite variation among arctic char from Labrador and Newfoundland is, however, comparable with that recently reported in a study on European arctic char populations from central alpine lakes (Brunner et al. 1998), indicating that microsatellites potentially offer more sensitivity than traditional markers to infer fine-scale population structure in arctic char.

The scale of population structure in anadromous arctic char

All genetic measures employed in this study led to the rejection of the null hypothesis of absence of genetic differentiation among arctic char samples, implying the existence of genetically differentiated populations among which gene flow has been restricted and on which mutation and drift may independently act to enhance genetic divergence. The overall F_{ST} (0.059) and F (0.108) values observed were, however, relatively low, suggesting only mild genetic differentiation among char from different locations, as generally reported in anadromous fishes (Ward et al. 1994). Such levels of interpopulation genetic divergence in anadromous species most likely reflect the long-term combined effect of their general propensity for homing to natal sites and the potential of straying in absence of firm physical barriers to gene flow.

Results of this study indicated various levels of genetic divergence among samples. As expected, landlocked arctic char from Gander Lake, represented the most distinct population. While this can certainly be attributed to its physical isolation from anadromous populations, the magnitude of its genetic differentiation could also be potentially due to historical factors, namely to a distinct postglacial origin (e.g., Wilson et al. 1996). The significant genetic differentiation observed among anadromous char from the four different geographic areas surveyed in is congruent with limited movements observed in tagging studies (Dempson and Kristofferson 1987). These involved the tagging of more than 7500 char, of which 1886 (nearly 25%) were subsequently recaptured. Results indicated that dispersal at sea was very limited by comparison with either Atlantic (*Salmo salar*) or various Pacific salmon (*Oncorhynchus* sp.) species**.** Thus, 95% of all recaptures of fish tagged in Nain Bay were within 70 km from tagging sites and only 2.3% were recaptured 100 km away or more. Consequently, results of these tagging experiments also

Fig. 2. Neighbour-joining phenogram showing relationships among char populations from Labrador and Newfoundland. Values along branches indicate bootstrap values in percent either based on locus (above) or individual. (A) Phenogram based on chord distance; (B) $(\delta\mu)^2$ phenogram built by imposing tree topology obtained from chord distance.



revealed little straying over a time frame of 7 years among widely distributed areas along 300 km of the northern Labrador coast. For instance, only 1.3% (including juvenile, sexually immature, and mature fish) of char tagged in Nain Bay and Voisey's Bay were subsequently recaptured in more northern sites such as Saglek Fjord. In the present study, gene flow estimates based on the theoretical relationships between fixation index and gene flow ($Nm = [(1/F_{ST}) - 1]/ 4$) varied between 4.6 and 27.5 effective number of migrants per generation among the four major regions surveyed. Although the veracity of these absolute values may be biased by not satisfying theoretical assumptions, namely that populations are in equilibrium with respect to genetic drift and migration, and that their population structure fit an island model, they nevertheless corroborate tagging results by indicating limited intermixing among char populations from different areas at each generation.

The exact definition of population genetic composition and their association to specific tributaries was partly hampered in this study by the fact that fish could not be sampled on spawning grounds and evidence that our samples represented a mixture of nonrandomly mating fish (see below). Nevertheless, the results obtained were sufficient to refute the hypothesis of a single panmictic population within a same area, such as Voisey's Bay. The magnitude of genetic divergence (F_{ST} and genetic distance) among the three char samples from Voisey's Bay, captured less than 10 km apart, was within the range of that observed among wide geographic areas. This, and the observation of tributary specific alleles, is not compatible with the hypothesis of a single panmictic population, therefore being indicative of the existence of more than one reproductive unit among tributaries draining in proximity one of each other. These results, therefore, reinforce the view that anadromous Arctic char may possess strong homing capability (Johnson 1980), perhaps comparable with that of Atlantic salmon as suggested by F_{ST} values similar to those quantified in that species using similar markers (e.g., Fontaine et al. 1997).

The salient feature of this study, however, was the demonstration of a more pronounced genetic differentiation between char from Kogluktokluk Brook and those from its tributary, Ikadlivik Brook, than that observed between Ikadlivik Brook and the neighbouring Reid Brook. In fact, the magnitude of differentiation of Kogluktokluk Brook char exceeded that observed among other char from wide geographic areas. At present, the possible causes of such differentiation are only hypothetical. If the potential for straying was the primary cause of the extent of their genetic differentiation, one would intuitively conclude that gene flow should be more important among tributaries draining into the same river, which was

clearly not the case for Kogluktokluk and Ikadlivik brooks. Instead, the observed population structure could be the result of differential ecological selection acting to maintain local adaptation (discussed in Tessier et al. 1997). Alternatively, the higher differentiation of the Kogluktokluk Brook compared with other anadromous populations could partly be explained by historical rather than ecological factors. For instance, the development of reproductive isolation between populations that evolved in separate glacial refugia and subsequently came into secondary contact have been documented in other fishes (e.g., Bernatchez 1997). At present, the hypothesis that a similar scenario could be involved in Voisey's Bay arctic char is indirectly supported by the striking frequency differences of several alleles between Kogluktokluk Brook and all other anadromous populations, and by the fact that, as for Gander Lake, the extent of differentiation of the Kogluktokluk Brook population increased substantially when molecular rather than allelic variance was considered. This suggests an important mutational component, likely implying long-term separation, whereas pure genetic drift may mainly be responsible for differentiation among other populations.

Evidence for straying among rivers

Heterozygous deficiency in all but one sample was indicative of a Wahlund's effect, implying that our samples were often composed of a mixture of char from different populations. Alternative explanations could conceivably result in the same pattern, but those appear less parsimonious in the present context. Stochastic departures from HW equilibrium are expected to occur in small populations. Such processes, however, are considered negligible when effective population size (N_e) is greater than 500 (Hartl and Clark 1989). Although we cannot estimate effective sizes for char populations, it is obvious from commercial landings data that their current census (N) must be relatively large. For instance, more than 10 000 char per year have been harvested from the Voisey stock complex over the period 1977–1994 (Dempson and Shears 1995). Although there may be important discrepancies between a population census and its effective population size, anadromous char populations appear sufficient to consider stochastic processes negligible among cohorts of a same generation, given the fact the ratio of N:N_e reported in other salmonids are usually smaller than 20:1 (e.g., Hedrick et al. 1995). In addition, stochastic departures from HW equilibrium would be expected to equally imply both excess and deficiency of heterozygotes, which was not the case here.

The occurrence of null alleles has also been regularly reported as a major explanation for observed heterozygous deficiency (discussed in Brookfield 1996). Several lines of evidence suggest that the occurrence of null alleles would not affect HW equilibrium importantly in this study. Heterozygous deficiency was due to different loci in different populations, which would imply both the occurrence of null alleles at many loci and the differential absence of those alleles depending on populations. In addition, heterozygous deficiency has not been observed in other studies where the same loci were used, including arctic char (e.g., Angers and Bernatchez 1998; Tessier et al. 1997; Brunner et al. 1998), suggesting that the occurrence of null alleles would be confined to char populations from Labrador and Newfoundland. While those factors could still partly explain the observed heterozygous deficiencies, it appears more plausible that these mainly reflect the effect of population admixture. Further evidence of population admixture was also provided by significant genotypic phase disequilibrium, which cannot easily be explained by the effects of null alleles unless those were linked with alleles at other loci. Since char captured at most sites comprised both spawning and nonspawning fish, it is likely that our samples represented a mixture of both local spawners returning to their natal site and fish reentering rivers at the end of the summer for overwintering. The hypothesis of genetically mixed samples is also compatible with knowledge on movements obtained from tagging studies. For instance, multiple recaptures within the same river revealed that some char did not reenter the river for 2 years before the second recovery, and char tagged in a given river were also subsequently recaptured in a different tributary (Dempson and Kristofferson 1987). Gyselman (1994) has also reported that some char returning to Nauyuk Lake, Northwest Territories, do so following an absence of 1 or 2 years, and that part of the Nauyuk Lake population is likely composed of immigrants from other river systems**.** Altogether, the observed pattern of gene diversity partitioning in anadromous populations from Labrador lends support to the hypothesis that arctic char have the capacity to home to their natal rivers but that the behavioural pattern does not appear to be rigid (e.g., Johnson 1980). Straying has been proposed as an evolutionary alternative strategy to homing (Quinn 1984). Arctic char may utilize this tactic in seeking alternative overwintering and perhaps spawning localities. There may be several advantages to this strategy for a species living in areas where environmental conditions can be unpredictable and extreme. For instance, homing may ensure distribution of reproductive effort in "successful areas," whereas straying and overwintering in alternate systems may allow for the colonization of new systems (Johnson 1980).

Implications for conservation and management

These results has several implications for conservation and management. They first indicate that more than one management units may occur in anadromous char on a very fine geographic scale. Proposed development following the discovery of rich mineral deposits in the watershed of Reid Brook within Voisey's Bay raised concerns about the potential for detrimental impacts due to habitat disturbance on char populations. In this context, our results imply that severe habitat perturbation, such as destruction of spawning grounds, a toxic contaminant spill, etc., could lead to a net loss of genetic diversity within the species. The fact that Reid Brook may also be used as an overwintering site by char from other Voisey's Bay rivers implies that any habitat disturbance that would lead to loss of overwintering grounds could also have effects on the demography of other populations within Voisey's Bay.

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