

Combined Use of SMM and Non-SMM Methods to Infer Fine Structure and Evolutionary History of Closely Related Brook Charr (*Salvelinus fontinalis*, Salmonidae) Populations from Microsatellites

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In this paper, we characterized microsatellite and mtDNA variation among 779 individuals representing 26 brook charr (*Salvelinus fontinalis* Mitchell) populations from La Mauricie National Park (LMNP) to test the general hypothesis that microsatellite analysis may provide greater resolution than mtDNA to infer the evolutionary history of closely related and geographically proximate populations. We also assessed the relative efficiency of stepwise mutation model (SMM) and non-SMM genetic information, used separately and together, to depict population relationships and to quantify the divergence and the partitioning of genetic variance among populations. Based on restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism sequence data, LMNP displayed a disjunct mtDNA genetic composition compared to other northeastern North American populations. However, these populations formed a homogeneous group, suggesting that they originated from a different glacial refuge. The screening of five microsatellites revealed high levels of diversity, strongly partitioned among populations, based on both allelic and molecular variance ($F_{ST} = 37.04\%$; $\Phi_{ST} = 45.96\%$). The use of chord distance was more reliable than $(\delta\mu)^2$ distance in resolving population tree topology using the neighbor-joining (NJ) algorithm. However, the construction of a hybrid tree obtained using the NJ- D_{CE} topology and the least-squares method branch length estimation from $(\delta\mu)^2$ distances appeared to better reflect both the relationships and the divergence of populations. This tree was composed of seven population lineages characterized by different genetic compositions and the presence of unique alleles. These lineages coincided only partially with drainages, and, consequently, genetic variance was more strongly partitioned among them than among drainages. The magnitude of $(\delta\mu)^2$ distance suggested that LMNP has been recolonized by seven ancestral populations, already isolated prior to the beginning of the last Wisconsinian glacial period, and that differences among lineages could be as old as the refuge from which they originated. This supports the view that microsatellite polymorphism holds the potential for analysis of the evolutionary history of populations over short temporal and geographical scales. These results also suggest that the use of microsatellites in conservation genetics may change our views about the problems of temporal and geographical scales to be considered in order to optimize conservation strategies.

Introduction

Phylogeography, the fusion of phylogenetics and population genetics with biogeography, has revolutionized evolutionary biology and population genetics (Avice et al. 1987). Namely, phylogeographic studies have consistently revealed the dominant role of historical events in shaping the genetic compositions and structures of modern populations and have reinforced the view that attempts to interpret population differentiation in terms of ecological and evolutionary causes may lead to erroneous conclusions in the absence of historical considerations (Avice, Neigel, and Arnold 1984; Bermingham and Avice 1986; Avice et al. 1987; Bernatchez and Martin 1996; Bernatchez and Wilson 1998 and references therein). Because it allows delineation of patterns of evolutionarily distinct population segments within a species (or evolutionarily significant units [ESUs], *sensu* Ryder 1986; Waples 1991) and elucidates their evolutionary trajectories, the phylogeographic approach is also highly relevant to conservation issues (Moritz 1994; Bernatchez 1995).

Most phylogeographic studies of animals have relied on the analysis of mitochondrial DNA (mtDNA) sequence variation. Thanks to many of its unique attributes, such as the uniparental and nonrecombining mode of inheritance, simplicity of genomic organization, and relatively high point mutation rates compared to most nuclear genes (Moritz, Dowling and Brown 1987), the analysis of mtDNA has proven most useful in defining major phylogenetic assemblages within species that were often undetected by allozymes and other genetic methods and reconstructing their evolutionary history (Bermingham and Avice 1986; Cann, Stoneking, and Wilson 1987; Bernatchez and Dodson 1991; Avice 1992; Wayne et al. 1992; Shaffer and McKnight 1996; Weninck et al. 1996).

However, as for any other methods, mtDNA analysis is not without limitations. It provides phylogenetic information of a single gene tree, which may not accurately reflect a population tree in several demographic conditions, such as when there are major phylopatric discrepancies between sexes (Pamilo and Nei 1988; Degnan 1993; Palumbi and Baker 1994). More importantly, the usefulness of mtDNA in inferring population relationships may be hampered when the temporal scale of divergence has not been sufficient to lead to reciprocally monophyletic coalescence of mtDNA variants among populations, that, is when it is approximately

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<4Ne generations (Avise, Neigel, and Arnold 1984). In such cases, the number of mutational changes detected by current analytical tools may also not be sufficient for adequate phylogenetic reconstruction. For instance, assuming a mitochondrial mutation rate of 2×10^{-8} mutations/site/year and a genome size of 16,800 bp, an average of only one mutation will accumulate per lineage for every 500 bp over 100,000 years, on average. Many important evolutionary events, such as the establishment of modern human populations, the radiation of species flocks in freshwater lakes, and the recolonization of the northern hemisphere by plants and animals following the last glacier retreats, are believed or known to have occurred during such a short time frame. In all those cases, however, the analysis of mtDNA variation has apparently reached its limits of usefulness for resolving evolutionary histories, lacking resolution to delineate young species or define population assemblages even on large geographic scales, or resulting in clustering of individuals in nonconcordance with their geographic origins (Cann, Stoneking, and Wilson 1987; Meyer et al. 1990; Sturmbauer and Meyer 1992; Horai et al. 1995; Wilson et al. 1996; Pigeon, Chouinard, and Bernatchez 1997). This may also have consequences for conservation issues, for instance, in cases where founding populations separated by thousands of generations and representing evolutionary distinct lineages are confounded because they go undetected. These observations illustrate that the study of evolutionary history on small temporal scales may benefit from the investigation of more rapidly evolving genetic markers, which may hold the potential for finer resolution of phylogenetic signals among recently diverged groups of organisms.

Microsatellite loci are a special class of tandemly repeated DNA which are increasingly replacing or complementing other markers for numerous applications in evolutionary and conservation genetics. High mutational rates of microsatellites lead to extensive polymorphism and increase the probability that isolated populations diverge rapidly at these loci. Such characteristics make them particularly useful in the study of population genetics of species showing limited variability at other markers (Bruford and Wayne 1993; Hugues and Queller 1993; Paetkau and Strobeck 1994; Taylor, Sherwin, and Wayne 1994; Angers et al. 1995; Pope, Sharp, and Moritz 1996; Tessier, Bernatchez, and Wright 1997). To a lesser extent, microsatellites are beginning to be used for phylogenetic reconstruction among populations (Bowcock et al. 1994; Estoup et al. 1995a; Forbes et al. 1995; Goldstein et al. 1995b; Shriver et al. 1995; Nei and Takezaki 1996), although little attempt has been made to infer microsatellite patterns of diversity in a phylogeographic context. Intraspecific phylogenetic relationships inferred from microsatellites have often been congruent with those obtained from other approaches in depicting deeper branching (e.g., Estoup et al. 1995a; Nei and Takezaki 1996) and have sometimes provided more accurate geographic clustering of more closely related populations (e.g., Bowcock et al. 1994).

Direct estimates of microsatellites' mutation mechanisms have shown that most (but not all) mutations

involve the addition or subtraction of a small number of repeat units (e.g., Henderson and Petes 1992; Weber and Wong 1993), which apparently violates the assumptions of the infinite-alleles mutation model (IAM; Kimura and Crow 1964). To account for these observations, various estimators of genetic differentiation based on the stepwise mutation model (SMM; Ohta and Kimura 1973) assumptions have been proposed for microsatellites (Goldstein et al. 1995a, 1995b; Shriver et al. 1995; Slatkin 1995; Michalakis and Excoffier 1996; Rousset 1996; Feldman et al. 1997). Applied to microsatellites, SMM assumes that mutations add or subtract a single or a small number of repeat units from a given allele with equal probability. Consequently, such a mutational process retains a memory of allelic relationships which can be used to develop an estimator of separation time among groups of organisms (e.g., Goldstein 1995b).

Despite these theoretical developments, there is much debate concerning alternative approaches to estimate the extent of genetic divergence among populations and infer their phylogenetic relationships. Contrary to the fact that they should better fit linearity with time and, consequently, provide better estimates of divergence, the use of SMM-based distances to infer population relationships from phylogenetic reconstruction has not consistently performed better than IAM-based approaches (e.g., Goldstein et al. 1995b; Bentzen et al. 1996; Valsecchi et al. 1997). Several factors may account for these observations. Namely, there is increasing evidence that mutational rates and processes may vary substantially among and even within loci (e.g., Di Rienzo et al. 1994; Estoup et al. 1995a; Jin et al. 1996; Angers and Bernatchez 1997), which cannot easily be taken into account by current distance measures (Feldman et al. 1997). More importantly, most microsatellite studies have involved the use of low numbers of loci, which generates much larger variance values around estimates of distance based on SMM assumptions than around those based on others (e.g., Nei 1995; Takezaki and Nei 1996). Based on results obtained from simulation studies, Takezaki and Nei (1996) (reiterating the view of Nei 1995) recently proposed that in the face of such constraints, a combined use of both SMM and non-SMM information, following the approach of Nei, Tajima, and Tateno (1983), could be optimal in inferring population relationships. More precisely, they proposed that the use of distances that are independent of mutation models and that have lower sampling error than mutation-based methods (e.g., chord distance) should be more efficient in obtaining correct tree topology, whereas SMM-based distances, which better fit linearity with time (e.g., $(\delta\mu)^2$), should better approximate real branch lengths. To our knowledge, however, the efficiency of this approach has not been evaluated empirically.

In this paper, microsatellite and mtDNA variation of brook charr (*Salvelinus fontinalis* Mitchell) populations from La Mauricie National Park (LMNP) were compared to test the general hypothesis that microsatellite analysis provides more reliable information regarding relationships and divergence among closely related and geographically proximate populations, from which

their evolutionary history may be inferred. This system was also used to assess the relative efficiency of a combination of SMM and non-SMM genetic information in depicting relationships and inferring the divergence of populations compared to the sole use of one or the other. We argue that microsatellite analysis provided evidence for the recolonization of a small area that was deglaciated 10,000 years ago by several evolutionary lineages whose divergence predated postglacial times and that were undifferentiated based on mtDNA analysis. We also empirically support the view that a combined use of both SMM and non-SMM information represents the best approach to inferring relationships among recently diverged populations when a relatively low number of variable microsatellites are available and their mutational process is uncertain. Finally, the relevance of the results obtained for conservation issues is discussed.

Materials and Methods

Biological Material and Study Area

The brook charr (*Salvelinus fontinalis* Mitchell) is an endemic salmonid of eastern North America (Scott and Crossman 1973) and ranks among the most highly structured animal species (Gyllensten 1985; Ward, Woodward, and Skibinski 1994), with most of its genetic variance partitioned among major drainages (Ferguson, Danzmann, and Hutchings 1991; McCracken, Parker, and Guffey 1993; Perkins, Krueger, and May 1993; Angers et al. 1995; Danzmann and Ihssen 1995; Jones, Clay, and Danzmann 1996). It is generally assumed that the species' genetic structure largely reflects the differential recolonization of various parts of the species range by different evolutionary groups that evolved in allopatry during the Pleistocene glaciations and among which gene flow has remained limited over time (Perkins, Krueger, and May 1993; Danzmann and Ihssen 1995). Thus, the analysis of mtDNA variation suggested that two glacial races of brook charr identified by distinct mtDNA clades (named "A" and "B"; fig. 1) were involved in the northeastern North American recolonization process (Ferguson, Danzmann, and Hutchings 1991; Jones, Clay, and Danzmann 1996; R. G. Danzmann et al., unpublished data). The vast majority of charr from this region (>90%), however, are characterized by a single haplotype (haplotype 1) from clade B, resulting in the almost complete absence of mtDNA differentiation over hundreds of thousands of square kilometers. These observations, along with allozyme surveys (McGlade 1981; Perkins, Krueger, and May 1993), are highly suggestive that brook charr identified by mtDNA clade B have been the most important in the postglacial recolonization of northeastern North America.

The LMNP is located at the margin of the Canadian shield in south-central Quebec, Canada (46°46'N; 73°00'W). The park covers an area of 544 km², and its waters drain into the St. Maurice River watershed (fig. 2). The Wisconsinian ice mass covered LMNP until approximately 11,000 years ago (Dyke and Prest 1987), and the Champlain postglacial sea that developed fol-

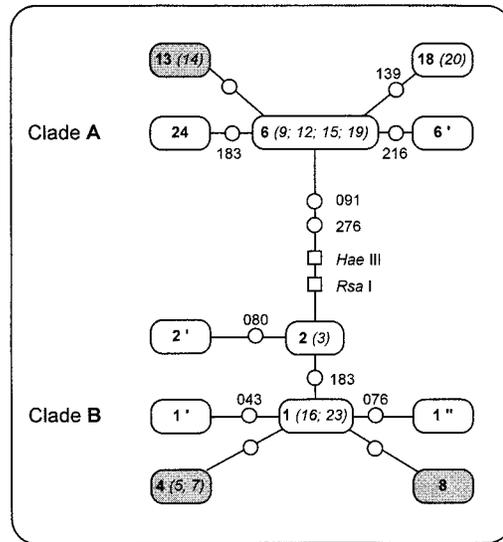


FIG. 1.—Parsimonious network among mtDNA haplotypes for brook charr, adapted from Bernatchez and Danzmann (1993), illustrating the relationships of new haplotypes depicted in this study (table 2) to those previously identified. Italic numbers refer to mtDNA variants detected by RFLP analysis over the entire mtDNA but not discriminated by sequence. New haplotypes are designated by numbers followed by ' or ". Numbers along the branches refer to positions of mutations as described in table 3. Shaded haplotypes were described in Bernatchez and Danzmann (1993) but not observed in this study.

lowing the last deglaciation between 12,000 and 9,800 years ago have surrounded the low (<210 m) altitudinal area at the margin of the park.

A total of 779 brook charr were collected from 26 lakes (table 1), located 3–42 km apart, and representative of seven different drainages of LMNP (fig. 2). Samples were obtained either from anglers for lakes where fishing is allowed, or by angling, netting, and electrofishing performed by the personnel of Canada Park Services. Whenever possible, adipose fins were clipped from subsequently released fish and preserved in 95% ethanol until total DNA extraction was performed according to Taggart et al. (1992).

Mitochondrial Analysis

mtDNA characterization of LMNP populations was performed in three complementary steps. The presence of mtDNA clades A and B was first screened for all individuals by a diagnostic restriction fragment length polymorphism (RFLP) analysis performed on a PCR-amplified segment. As most fish were unexpectedly characterized by the mtDNA clade A profile (see *Results*), polymorphism within clades was screened further on a subset of 305 individuals (table 1) by the analysis of single-strand conformation polymorphism (SSCP; Orita et al. 1989; Sheffield et al. 1993). Finally, sequence analysis was performed on each of the different SSCP conformers for the final identification of mtDNA haplotypes.

The segment analyzed by PCR-RFLP encompassed the ND/5–6 subunits and was amplified using the C-leu and C-glu primers (Park et al. 1993) prior to digestion with restriction endonucleases *Hae* III and *Rsa* I, which

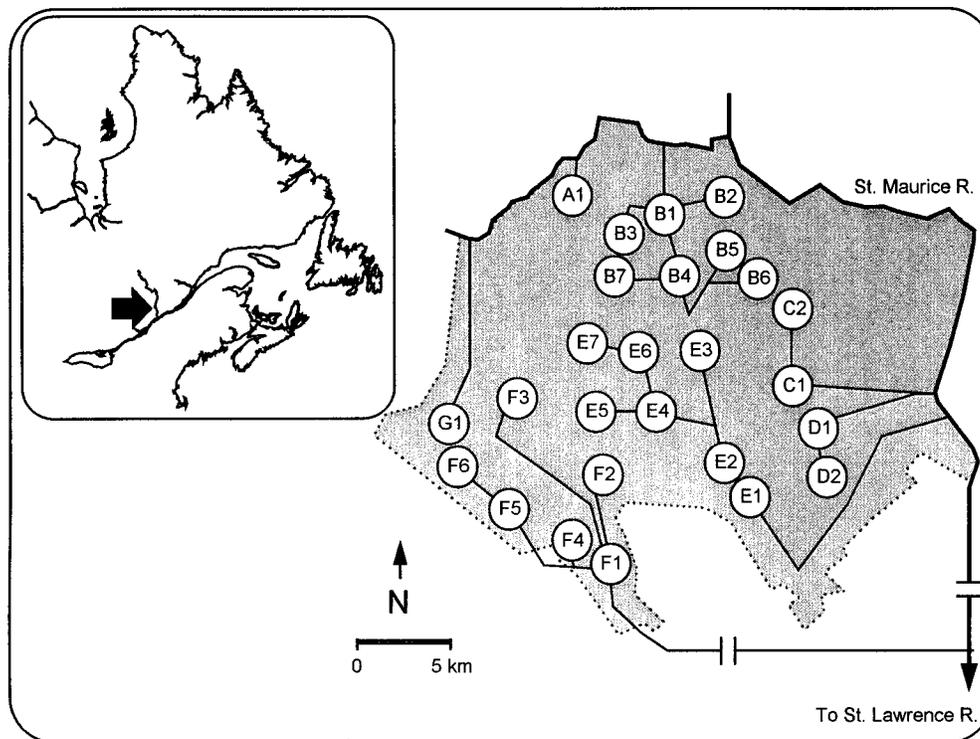


FIG. 2.—Map of the La Mauricie National Park indicating the relative locations of sampled brook charr populations among the seven drainages (table 1). The inset represents the geographical situation of the study. Codes correspond to names of lakes given in table 1.

Table 1
La Mauricie National Park Brook Charr Populations
Sampled for Genetic Analyses

CODE	DRAINAGE	POPULATION	SAMPLE SIZES ^a			
			A	B	C	D
A1	Théode R.	Théode	30	30	5	2
B1	Des Cinq R.	Des Cinq	30	30	5	2
B2		William	30	30	5	
B3		Eclair	30	30	5	
B4		Dauphinais	30	30	5	
B5		Archange	30	30	5	
B6		Petit Archange	30	30	5	
B7		Messieurs	30	30	5	
C1	Du Fou R.	Du Fou	30	30	20	2
C2		Alice	29	29	20	2
D1	Bouchard R.	Pimbina	30	30	20	4
D2		Benoit	30	30	5	
E1	A la Pêche R.	Marie	30	30	30	2
E2		Edouard	30	30	30	4
E3		Giron	30	30	20	2
E4		Ecarté	30	30	5	
E5		Alphonse	30	30	5	
E6		Formont	30	30	5	
E7		Arthur	30	30	5	
F1	Shawinigan R.	Wapizagonke	30	30	30	5
F2		Guilliette	30	30	30	2
F3		Avalon	30	30	20	2
F4		Modène	30	30	5	
F5		Caribou	30	30	5	2
F6		Maréchal	30	30	5	
G1	Anitagamak R.	Waber	30	30	5	2

^a A = microsatellite; B = mtDNA RFLP; C = mtDNA SSCP; D = mtDNA sequence.

generated diagnostic fragment patterns between mtDNA clades A and B (fig. 1). The digested fragments were electrophoretically separated on 2% agarose gels run for 1 h at 98 V.

Radioactive PCR for SSCP analysis was carried out in 10 μ l reaction volume using [α -³⁵S] dATP and primers LN20 and H2 of Bernatchez and Danzmann (1993), which amplify a segment encompassing 40 bp of the 3' end of the proline tRNA and the adjacent 300 bp of the 5' end of the control region. Migration conditions were modified from Sheffield et al. (1993) as followed; after 2 min at 95°C, heat-denatured fragments were loaded onto a non-denaturing gel consisting of 5.5% acrylamide (49–1 acrylamide-bis), 7.0% (v/v) glycerol, and 0.5 \times TBE. Samples were electrophoresed in 0.5 \times TBE for 14 h at 15 W, where constant temperature (approximately 25°C) was maintained using an air fan. Two individuals from each drainage (table 1) for each SSCP conformer were then amplified in 50 μ l PCR and sequencing was performed on double-stranded DNA products using the Sequenase 2.0 kit (USB) following fragment isolation in 1% agarose gel and purification with the Qiagen kit. Sequences were visually aligned and compared with those reported in Bernatchez and Danzmann (1993).

Microsatellite Analyses

Genetic analysis of the nuclear genome was performed with five microsatellite loci specifically designed for brook charr (SFO-8, SFO-12, SFO-18, and SFO-23; Angers et al. 1995) or brown trout, *Salmo trutta* (MST-85; Presa and Guyomard 1996), known a priori to be

moderately to highly polymorphic for the species (Angers et al. 1995). Microsatellite polymorphism was assessed using either radioactive or fluorescent detection methods. Radioactive PCR amplifications were carried out as described in Angers et al. (1995). Briefly, PCR was performed in 15 μ l reaction volume using 100 ng DNA template, 0.2 pmol primer end-labeled with [γ - 32 P] dATP, 15 pmol of the other primer, 75 μ M of each dNTP, 1.5 mM MgCl₂, 1 \times *Taq* buffer, and 0.25 U *Taq* polymerase. The following PCR profile was used: an initial denaturing step of 3 min at 95°C; 5 cycles of 60 s at 94°C, 35 s at annealing temperature (SFO = 60°C, MST-85 = 55°C), and 1 s at 72°C; followed by 25 cycles of 35 s at 94°C with all other parameters unchanged. Multiplexed PCR was used for SFO-12 and SFO-18 loci. Microsatellite polymorphism was then screened in polyacrylamide sequencing gels followed by autoradiography. PCR amplifications using fluorescent dyes were performed using labeled primer as specified in Angers et al. (1995)—SFO-8: HEX; SFO-12: FAM; SFO-18: FAM; SFO-23: TET. Locus MST-85 was not analyzed by the fluorescent method. The same PCR conditions described above were used, and the detection of polymorphism was then screened by DNA sequencer (ABI 373A) as detailed in Ziegler et al. (1992). Congruence of allelic identification between radioactive and fluorescent detection methods was ensured by screening a subset of individuals over the whole size range of each locus by both methods.

Statistical Analyses

Genetic diversity was quantified by the number of alleles per locus (A), observed heterozygosity (H_O) and the unbiased estimates of heterozygosity (H_E , Nei 1987). Genotypic phase disequilibrium was estimated to test for locus independence prior to other analyses using version 1.2 of GENEPOP (Raymond and Rousset 1995). Within-sample deviation from Hardy-Weinberg equilibrium was tested using null hypotheses of both excess and deficit in heterozygotes at individual loci and over all loci. GENEPOP was also used to assess heterogeneity in allelic frequencies among pairwise comparisons of samples and to compute values of significance for all parameters by unbiased estimates of Fisher's exact test through 1,000 iterations using the Markov chain method (Guo and Thompson 1992). Critical significance levels for simultaneous statistical tests were computed using sequential Bonferroni adjustments (Rice 1989).

The hierarchical partitioning of the genetic variance was quantified using an analysis of variance framework (Weir and Cockerham 1984; Excoffier, Smouse, and Quattro 1992; Michalakis and Excoffier 1996). We computed estimates using allele frequencies only (Weir and Cockerham 1984), and then using an analysis of molecular variance framework, which accounts for variance in size between pairs of alleles (Michalakis and Excoffier 1996). The information of mutational differences among alleles was entered as a matrix of Euclidian squared distances. By this procedure, we first computed both F_{ST} and Φ_{ST} estimates over all populations (without hierarchical structure) at individual loci using the program

winAMOVA version 1.5 (Excoffier, Smouse, and Quattro 1992). Estimates over all loci were computed by dividing the sums of variance by the sums of the total variance. We then performed the same analyses including a hierarchical structure in order to assess the component of genetic variance imputable to two different population groupings; by drainage systems (as illustrated in fig. 2), and according to significant populations assemblages defined a posteriori by phylogenetic reconstructions (see below). This allowed us to assess the relative role of historical versus contemporary population subdivisions.

The efficiencies of different approaches to assessing population relationships were then compared: tree on individuals; population trees reconstructed under a single method (without inference to mutational process or SMM-based) to estimate both topology and branch length; and population trees reconstructed under a combined method, where topology and branch length were estimated from non-SMM and SMM assumptions, respectively.

Chakraborty and Jin's (1993) distance based on the proportion of shared alleles (D_{AS}) was used to assess relationships among individuals. D_{AS} is the simplest estimation of genetic distance, and its application has proven useful in identifying populations issued from reticulate processes or assigning particular individuals to their original populations (Bowcock et al. 1994; Estoup et al. 1995a). D_{AS} on individuals and the index of classification, which measures how well individuals from a given group cluster together in a tree (Estoup et al. 1995a), were computed using a program written by J.-M. Cornuet (INRA, Laboratoire de Neurobiologie Comparée des Invertébrés, Bures-sur-Yvette, France). Because phylogenetic reconstruction methods are based on the assumption of radiative evolution, which is violated by reticulate processes such as hybridization and introgression events, three populations, which obviously had much lower classification scores than the other 23 populations (suggestive of their hybrid origins; see *Results*), were discarded from further analyses.

Genetic divergence among populations was first quantified by Cavalli-Sforza and Edwards' (1967) chord distance (D_{CE}), which makes no assumption regarding constant population size or mutation rates among loci. The magnitude of this distance is not proportional to evolutionary time, but its use generally leads to a higher probability of depicting the correct tree topology among closely related populations under either IAM or SMM assumptions (Nei and Takezaki 1996; Takezaki and Nei 1996). D_{CE} distances were computed using the GENE-DIST program included in the PHYLIP computer package, version 3.5c (Felsenstein 1993).

We then computed Goldstein et al.'s (1995b) $(\delta\mu)^2$ pairwise population distances using the Microsat program, version 1.4 (Minch 1996). $(\delta\mu)^2$ takes into account deviations of allele size variance from theoretical expectations under SMM reported in several studies (Shriver et al. 1993; Di Rienzo et al. 1994; Estoup et al. 1995a; Garza, Slatkin, and Freimer 1995). Contrary to other SMM-based estimators, $(\delta\mu)^2$ has the advantage

Table 2
Distribution of mtDNA Variants Among La Mauricie National Park Populations as Detected with RFLP Among Clades A and B, and SSCP/Sequence for the Nine Haplotypes

	CLADE (RFLP)		HAPLOTYPE (SSCP and sequence)								
			B				A				
	A	B	1	1'	1''	2	2'	6	6'	18	24
*a	1	0	0	0	0	0	0	1	0	0	0
C1-C2	1	0	0	0	0	0	0	0	1	0	0
D1	0.60	0.40	0	0	0	0	0.40	0.60	0	0	0
E1	0.32	0.68	0.60	0	0	0.08	0	0.32	0	0	0
E2	0.35	0.65	0.65	0	0	0	0	0.35	0	0	0
E3	0.89	0.11	0	0	0.11	0	0	0.89	0	0	0
F1	0.76	0.24	0.20	0.04	0	0	0	0.73	0	0	0.04
F2	0.76	0.24	0.20	0	0	0.04	0	0.68	0	0	0.08
F3	1	0	0	0	0	0	0	0.85	0	0.05	0.10

*a * Included A1, B1 to B7, D2, E4 to E7, F4 to F6, and G1. Haplotype numbers refer to Bernatchez and Danzmann (1993). Numbers followed by ' or '' refer to new haplotypes identified in the present study.

es of being independent of population size (under mutation–drift equilibrium), and of allowing direct estimation of time since population divergence when mutation rates can be approximated (Goldstein et al. 1995b). $(\delta\mu)^2$ has also been shown to be more appropriate than other methods for approximating real branch lengths (Takezaki and Nei 1996).

Pairwise distances obtained both from D_{CE} and $(\delta\mu)^2$ estimates were independently used to construct population trees using the neighbor-joining (NJ) algorithm (Saitou and Nei 1987) available in PHYLIP 3.5c. Confidence estimates on tree topologies obtained from observed distance values were estimated by the percentage of 1,000 bootstraps performed resampling loci, and compiled using the CONSENSE program of PHYLIP.

Finally, a population tree was reconstructed using a combined approach, according to Nei (1995). In this tree, D_{CE} topology was imposed by the user tree option available in PHYLIP (in the FITCH program), and the

Cavalli-Sforza and Edwards (1967) least-squares method was used to estimate branch lengths from the $(\delta\mu)^2$ distance matrix.

Results

Mitochondrial DNA Diversity

RFLP of all 779 individuals revealed that both clades A and B were present among LMNP brook charr populations (table 2). Unlike in previous mtDNA studies of the species, however, mtDNA clade A largely predominated in most of LMNP, being fixed in 20 out of 26 populations, whereas mtDNA clade B was restricted to six populations and exceeded the relative frequency of clade A in populations only two (table 2).

SSCP analysis of a subset of 305 individuals revealed nine variants, all corresponding to different sequences, differing by one to five point mutations (tables 2 and 3). Thus, under the conditions used in this study, SSCP was apparently able to detect single-nucleotide mutations despite the relatively large size of the fragment analyzed (377 bp). Sequences of the same conformers found in different drainages were always completely identical. Five of these nine sequence variants were previously reported by Bernatchez and Danzmann (1993), four of which corresponded to groups of distinct haplotypes resolved by RFLP analysis over the entire mtDNA using 46 endonucleases (Bernatchez and Danzmann 1993; table 3). The other four haplotypes differed by a single nucleotide replacement from these haplotypes (table 3 and fig. 1).

Geographic partitioning, partially coinciding with drainage subdivisions, was observed in the distributions of several haplotypes (table 2). For instance, populations C1 and C2 were fixed for haplotype 6' which was observed nowhere else, haplotype 24 was found only in populations F1, F2, and F3 (although at very low frequency), and haplotype 1 was detected at high frequencies in E1 and E2. All other haplotypes (except haplotype 2) were restricted to single populations. Altogether, this pattern resulted in a significant genetic structure among populations ($F_{ST} = 68.3\%$; $\Phi_{ST} = 50.9\%$). However, given the fixation of a single haplotype in 17 sam-

Table 3
Variable Nucleotide Positions in the Amplified D-loop Region Sequence of the Nine mtDNA Haplotypes Observed in the La Mauricie National Park Brook Charr Populations

	*	2	*	3	4	6	*	9	
	043	076	080	091	139	183	216	276	<i>N</i>
Clade A									
6	G	T	A	G	A	C	A	T	14
6'	G	T	A	G	A	C	C	T	4
18	G	T	A	G	del	C	A	T	1
24	G	T	A	G	A	T	A	T	2
Clade B									
2	G	T	A	A	A	C	A	C	3
2'	G	T	del	A	A	C	A	C	2
1	G	T	A	A	A	T	A	C	4
1'	A	T	A	A	A	T	A	C	1
1''	G	C	A	A	A	T	A	C	2

NOTE.—Upper numbers refer to the positions of the mutational site corresponding to Bernatchez and Danzmann (1993). Asterisks indicate mutations not detected in the latter study. Lower numbers refer to the sequence positions; del refers to indels. *N* = number of individuals sequenced for each variant.

Table 4
Genetic Diversity at Five Microsatellite Loci Over All La Mauricie National Park Brook Charr Populations

	A_T	A_S	H_T	H_S	H_I	S_T	S_S
SFO-8	37	6.85 (3.76)	0.86	0.61 (0.20)	0.64 (0.22)	149.55	68.84
SFO-12	9	2.42 (1.36)	0.67	0.21 (0.22)	0.22 (0.23)	55.19	37.99
SFO-18	10	3.54 (1.17)	0.77	0.52 (0.18)	0.55 (0.21)	6.69	5.06
SFO-23	24	6.96 (2.65)	0.89	0.70 (0.14)	0.74 (0.18)	29.23	22.87
MST-85	15	4.23 (1.88)	0.62	0.41 (0.21)	0.44 (0.24)	22.80	6.58

NOTE.— A_T = total number of alleles; A_S = mean number of alleles per population; H_T = overall expected heterozygosity; H_S = mean expected heterozygosity per population; H_I = mean observed heterozygosity per population; S_T = overall allelic size variance; S_S = mean allelic size variance per population; numbers in parentheses are standard deviations.

ples distributed throughout all but one drainage, resulting in $F_{ST} = 0$ among 65% of all populations surveyed (table 2), more detailed analysis of population structure based on mtDNA variation was uninformative and not considered further.

Microsatellite Overall Diversity

In contrast to mtDNA, microsatellites generally displayed high levels of polymorphism, with the observed number of alleles per locus varying from 9 (SFO-12) to 36 (SFO-8) and the overall expected heterozygosity ranging from 0.62 (MST-85) to 0.89 (SFO-23) (table 4). Allelic size distributions varied among loci, resulting in major differences in their overall variances, with that of SFO-8 being 22 times higher than that of SFO-18 (table 4). Most loci showed disjunct allelic size distributions in which size classes were often separated by several base pairs (fig. 3). This was particularly obvious at SFO-8, for which allelic size ranged between 202 and 316 bp with modes separated by size gaps (e.g., no alleles found in the 212–220, 226–240, 286–294, or 298–310 size ranges). A major disjunction in allelic size distribution was also observed at SFO-12, for which 50 bp separated allele 197 from the second smallest allele. As recently reported, this locus is an imperfect microsatellite in which gaps among allelic size classes are due to large indels of repeat and nonrepeat motifs (Angers and Bernatchez 1997).

Intrasample diversity was also generally high, with the mean number of alleles per locus ranging from 2.42 (SFO-12) to 6.96 (SFO-23) and the mean heterozygosity ranging from 0.21 (SFO-12) to 0.70 (SFO-23) (table 4). However, genetic diversity varied considerably among samples (data not shown), with the number of alleles detected over all loci combined ranging from 10 to 43, and gene diversity averaged across loci varying from 0.17 to 0.79. Disjunct allelic size distribution (e.g., SFO-8 and SFO-23 in A1, SFO-8 in F6; fig. 3A and D) was also frequently observed within samples, resulting in large mean size variance per population at several loci relative to the overall size variance (table 4).

Genotypic Linkage and Hardy-Weinberg Disequilibriums

Exact tests for genotypic linkage disequilibrium depicted four significant adjusted P values out of 260 (1.5%) comparisons, a proportion lower than that expected by chance alone (13 expected from type I error at $\alpha = 0.05$). No more than one significant value was

observed for a given pairwise comparison across populations, suggesting the absence of physical linkage of those loci and of population substructuring within samples. Significant departure from Hardy-Weinberg equilibrium was detected by multilocus probability tests (two-tailed) in only one sample. When heterozygote deficit or excess was tested for each locus separately, significant departure from Hardy-Weinberg equilibrium was detected in two additional samples at SFO-23 (6.5 significant deviations expected from type I error at $\alpha = 0.05$). Altogether, these results indicated genotypic equilibrium (or nearly so) at all microsatellites within each sample.

Population Differentiation

Highly significant differences ($P < 0.001$; data not shown) in allele frequencies between populations were observed at one to five loci for all but one pairwise comparison, which corroborates expectations of genetic drift processes acting on physically isolated, nonrandomly mating populations. Altogether, 1,454 of the 1,625 pairwise comparisons (89.5%) revealed significant heterogeneity in allele frequencies among populations.

Gene diversity analysis revealed extensive genetic variance among populations, based on either allelic ($F = 37.04\%$) or molecular ($\Phi = 45.96\%$) variance (table 5). The relative similarity of these two measures averaged across loci was apparently purely stochastic, as exemplified by the major discrepancies observed at individual loci. At SFO-12, genetic variance among populations estimated from allelic variance was more than twice (70.00% vs. 30.36%) that calculated from molecular variance, whereas the opposite was observed at SFO-8 and MST-85, for which the estimates of interpopulation genetic variance were increased by 77% and 104%, respectively, when using molecular variance. Both estimates were more similar for SFO-18 and SFO-23. Such differences may most likely be attributed to differential mutational rates or processes among loci, which, in turn, translates into major differences in their numbers of alleles and size variances (table 4). This is particularly obvious for SFO-12 already known to strongly deviate from SMM expectations (Angers and Bernatchez 1997).

Population Relationships

The analysis of relationships based on D_{AS} among the 779 individual brook charr (phenogram not shown) and estimates of classification indices revealed that in-

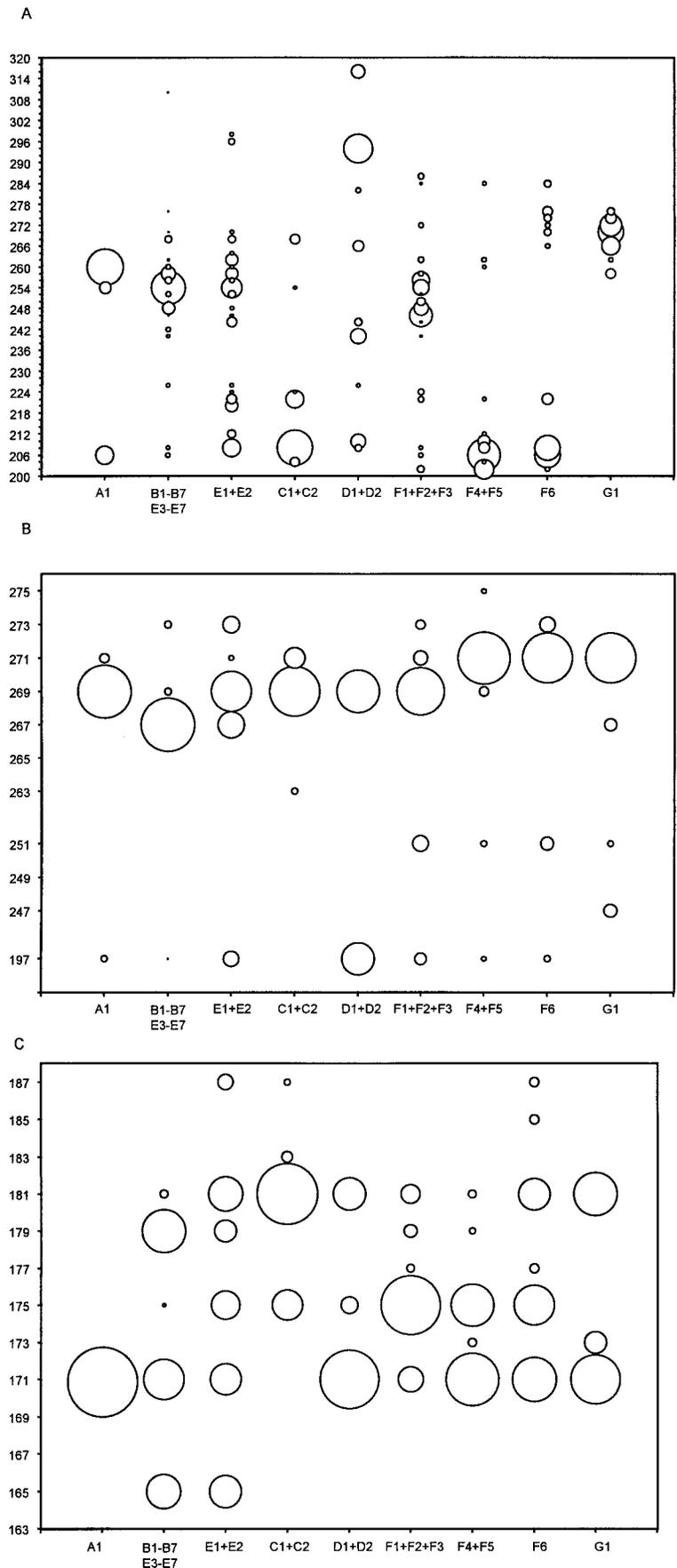


FIG. 3.—Schematic illustration of relative allelic frequencies at five microsatellite loci for seven population groups in La Mauricie National Park defined by $D_{CE}(\delta\mu)^2$ tree. A, SFO-8. B, SFO-12. C, SFO-18. D, SFO-23. E, MST-85. Circles represent distinct alleles, and their surfaces are directly proportional to their relative frequencies.

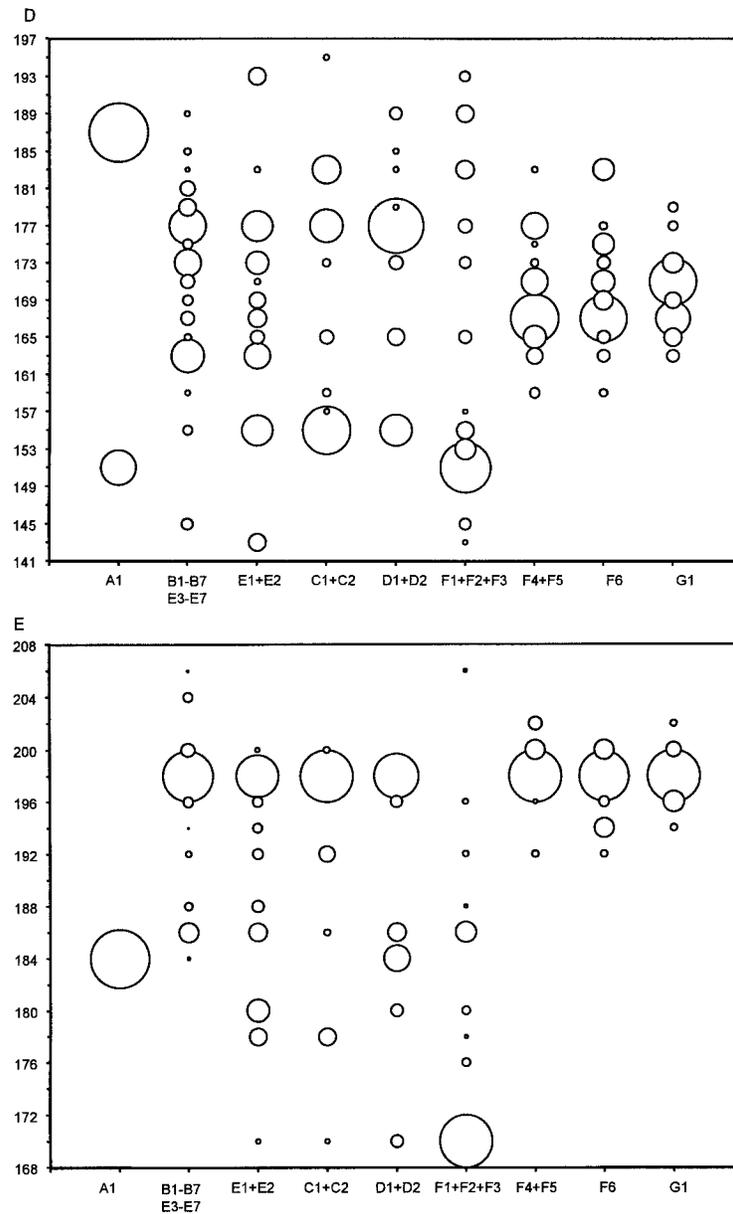


FIG. 3—Continued

dividuals of each population generally clustered as monophyletic units, with three notable exceptions. Individuals of populations E1 and E2 formed several clusters distributed throughout the tree topology, whereas most brook charr of population F6 were divided between populations G1, F4, and F5. This resulted in very low classification indices for these three populations (E1: 0.21; E2: 0.02; F6: 0.29) compared to all others, for which this measure varied between 0.51 and 1.00. These three populations thus appeared to be the result of admixture of two or more distinct evolutionary population groups (see below) and, consequently, were excluded from further phylogenetic analyses.

The topology of the population tree reconstructed from the pairwise D_{CE} matrix revealed six population assemblages that were all (except one) supported by high bootstrap values (86%–100%) (fig. 4A). These as-

semblages corresponded to drainage subdivisions, with two notable exceptions. All populations from the physically isolated Des Cinq (B populations) and à la Pêche (E populations) drainages composed a unique cluster supported by a bootstrap value of 96%. In contrast, populations from the Shawinigan drainage (group F) formed two distinct clusters corresponding to the two major sub-drainages within this watershed. These were as divergent from each other as from any of the other groups. These observations are indicative that either mutation/drift (in the case of the B/F group) or gene flow (in the case of the two F groups) has been insufficient to override the historical signals of their genetic composition.

Tree reconstruction from the $(\delta\mu)^2$ distance matrix did not improve the resolution of population relationships (fig. 4B). (SFO-12 was excluded from calculations of $(\delta\mu)^2$ since it was known a priori not to conform to

Table 5
Partitioning of the Genetic Variance at Microsatellite Loci, Using Allelic (F) or Molecular Variance (Φ) Over All Populations (Global) and Using Hierarchical Analysis Where Groups Were Defined as Either Drainage (fig. 2) or Population Lineages by D_{CE} - $(\delta\mu)^2$ Topology (fig. 4C)

	SFO-8	SFO-12	SFO-18	SFO-23	MST-85	Overall
Global						
Among populations						
F ...	30.76	70.00	33.65	22.74	34.75	37.04
Φ ...	54.40	30.36	25.48	20.69	70.97	45.96
Drainages						
Among groups						
F ...	21.38	49.75	23.56	11.70	14.68	23.82
Φ ...	39.06	21.79	13.84	6.09	11.83	28.65
Among populations within group						
F ...	12.04	22.94	12.97	12.67	21.78	15.91
Φ ...	18.54	11.30	13.59	15.46	59.76	20.12
Lineages						
Among groups						
F ...	27.55	71.62	29.03	17.36	33.45	35.20
Φ ...	59.64	23.93	13.73	15.30	73.20	48.17
Among populations within group						
F ...	7.41	3.12	8.91	8.31	6.07	6.84
Φ ...	0.75	10.11	14.09	8.04	2.42	3.63

SMM expectations.) In contrast to the D_{CE} tree, this tree revealed only four population groupings that were supported by much lower bootstrap values (ranging from 37% to 68%). With the exceptions of population group F1–F3, clustering had little correspondence with drainage subdivisions or geographic proximity (fig. 1; e.g., population G1 clustering with populations D1 and D2). These observations indicated that the D_{CE} tree topology was more reliable than that of the $(\delta\mu)^2$ tree for inferring population relationships.

The combined use of the D_{CE} tree topology and branch length estimation from $(\delta\mu)^2$ distances is presented in figure 4C. In comparison to the D_{CE} tree, this resulted in a substantial reduction in branch lengths among populations within groups relative to those among groups. The relative lengths of second-order branches relating population assemblages were also dramatically reduced, such that they became insignificant in comparison to the lengths of those delineating the different population groupings. This created a polytomy involving all groups and the dissociation of population G1 from populations F4 and F5, which resulted in the definition of seven distinct population assemblages of brook charr in LMNP: A1; B1 to B7 and E3 to E7; C1 and C2; D1 and D2; F1 to F3; F4 and F5; and G1 (fig. 4C).

Each of these were characterized by strong differences in allele frequencies (and in mtDNA haplotypes in some cases), sometimes involving the presence of unique alleles (fig. 3). For instance, both population groups G and F4/F5 were characterized by an almost complete fixation of allele SFO-12*271 which was not found or was observed only at low frequency elsewhere. However, these two groups were very distinct in allelic composition at SFO-8 and SFO-18. All 12 populations

from the B/E grouping were characterized by the unique (or nearly so) fixation of SFO-12*267 and the presence of allele SFO-18*165. The population assemblage F1–F3 was characterized by allele MST-85*170, which was found only in very low proportions in a few other populations, and the unique presence of mtDNA haplotype 24. Population A1 was characterized by the unique allele SFO-23*187. As stated previously, the population assemblage C1/C2 was fixed for mtDNA haplotype 6', which was observed nowhere else, and these populations were also characterized by the absence of allele SFO-18*171, which was found at relatively high frequency in all other populations. Populations D1 and D2 were characterized by relatively high frequencies of alleles SFO-8*294 and SFO-8*312, which was not observed elsewhere. Many additional, although less obvious, differences in allele frequencies can be found among all population groupings (fig. 3).

The presumption based on individual clustering analysis and classification index that populations E1, E2, and F6 appeared to be the result of historical introgressive hybridization between evolutionarily distinct population groups was further supported by their allelic composition. Thus, the admixture of groupings G and F4/F5 in population F6 appeared particularly evident based on its allelic composition at SFO-8, for which two very disjunct allelic classes that were characteristic of the two “pure” groups were observed (fig. 3A). Most of the alleles detected in the population grouping B/E were also detected in populations E1 and E2 as well, including unique alleles such as SFO-18*165 and SFO-12*267 (figs. 3B and C), which indicated their close affinity with this group. However, the high heterozygosity values of these two populations (0.73 and 0.78) compared to any others (0.18–0.64), the high frequency of mtDNA clade B (absent in all other B/E populations but E3), and the sharing of several alleles with nearby groups (namely C and D) (fig. 3) that were generally absent from other populations of the B/E group all indicated the reticulate origin of populations E1 and E2.

Hierarchical Gene Diversity Analysis

The hierarchical analysis of gene diversity revealed that the overall proportion of genetic variance imputable to interpopulation differences was partitioned among groups and among populations within groups (table 5). Both components were relatively similar when dividing populations by drainages, based either on allelic (ratio of among groups/among populations within group = 1.50) or molecular (ratio = 1.42) variance. However, the among-groups component of genetic variance relative to among-populations-within-group component increased dramatically when population groups corresponded to the seven population assemblages that were phylogenetically defined (table 5). This increase was also more pronounced when considering mutational processes (ratio = 13.27) than when considering drift alone (ratio = 5.15).

Discussion

Mitochondrial DNA

The salient feature of mtDNA results was the unexpected predominant abundance of clade A haplotypes

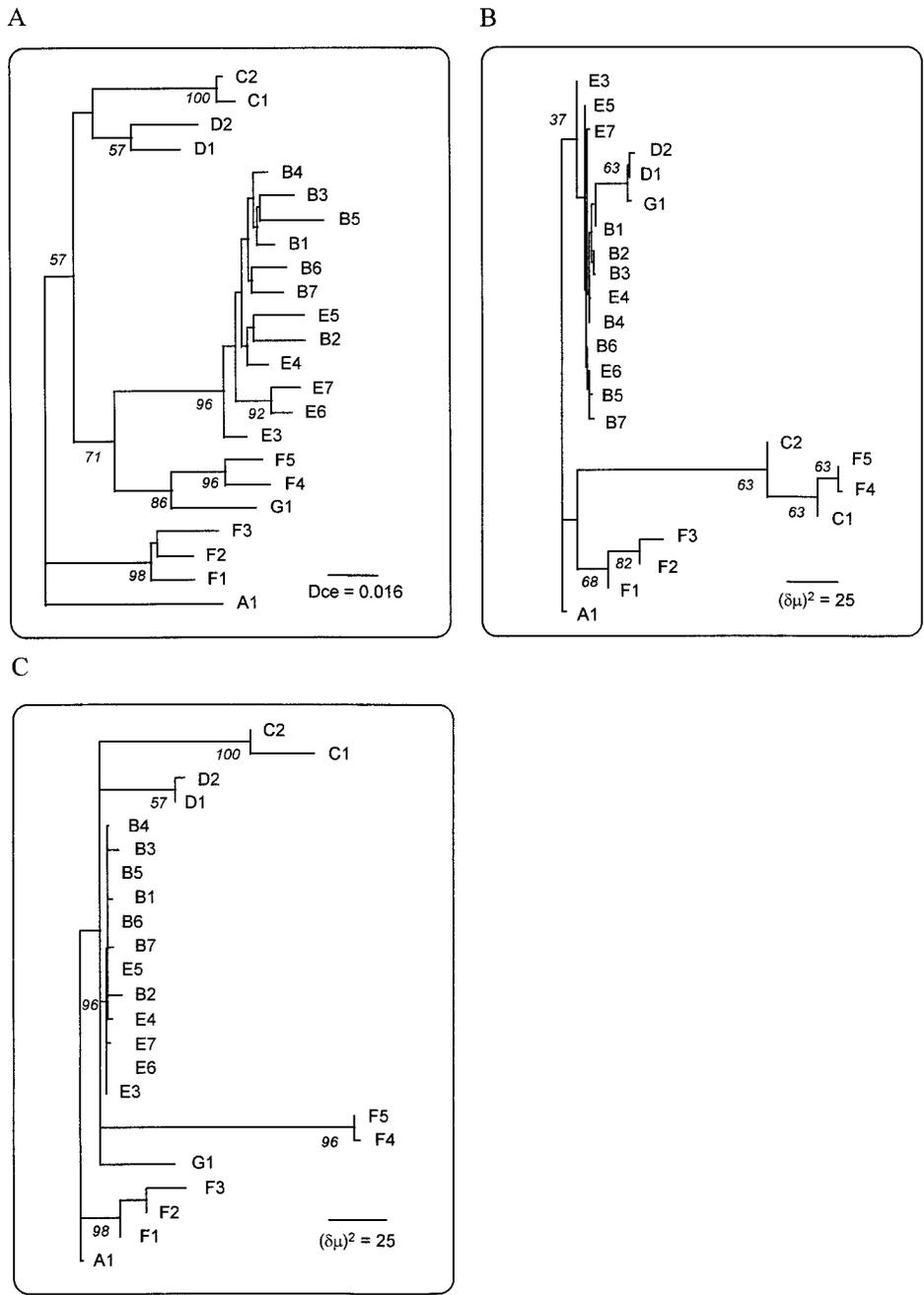


FIG. 4.—Population phenograms illustrating relationships among 23 La Mauricie National Park brook charr populations. Codes refer to populations identified in table 1. Percentages of replication of the observed topology based on 1,000 bootstraps on loci are given along branches. A, Neighbor-joining tree from D_{CE} distances. B, Neighbor-joining tree from $(\delta\mu)^2$ distances. C, Hybrid tree from D_{CE} (topology) and $(\delta\mu)^2$ (branch length).

(namely haplotype 6) in all but two of the LMNP populations. Indeed, previous studies reported that all brook charr populations from northeastern North America were fixed (or nearly so) for haplotype 1 of clade B (Ferguson, Danzmann, and Hutchings 1991; Jones, Clay, and Danzmann 1996; Danzmann et al., unpublished data). Those studies, however, included a single sample from south central Quebec. These observations are likely indicative that this part of the species range has been recolonized mainly by brook charr that sur-

vived the last glaciation events in a refuge separate from others involved in the recolonization of more northern and eastern regions, corroborating the view that brook charr that reinvaded northeastern North America have multiple origins (e.g., Perkins, Krueger, and May 1993).

Elsewhere, mtDNA clade A predominates only in the coastal Atlantic and Great Lakes regions, where it is found in sympatry with less abundant clade B haplotypes, namely haplotype 1 (Quattro, Morgan, and Chapman 1990; Danzmann and Ihssen 1995; Danzmann

et al., unpublished data). Since the Great Lakes region was covered by glaciers during the Wisconsinian glaciation events, coastal Atlantic plains represent the most likely source of origins of populations that recolonized LMNP. Suitable brook charr habitats, such as streams, rivers, and lakes, were available in this vast region that extended from 36° to 41° latitude north (discussed in Bernatchez 1997). There is also evidence that this region served as a glacial refuge for other north temperate fishes, including rainbow smelt, *Osmerus mordax* (Bernatchez 1997); whitefish, *Coregonus clupeaformis* (Bernatchez and Dodson 1991); lake trout, *Salvelinus namaycush* (Wilson and Hebert 1995); Arctic charr, *Salvelinus alpinus* (Wilson et al. 1996); and white sucker, *Catostomus commersoni* (Lafontaine and Dodson 1996). Inland dispersal northward from this refugial region was possible via several rivers, namely the Hudson and the Connecticut, that connected with proglacial Lake Vermont, which developed 12,900 years ago in south-central Quebec, and the brackish Champlain Sea, which replaced it following marine waters intrusion that persisted between 12,000 and 9,800 years ago (detailed in Bernatchez 1997). The environmental conditions prevailing in these systems were apparently favorable for brook charr dispersal, and the end of Champlain sea phase (approximately 10,000 years ago) coincided with the deglaciation of LMNP, suggesting that the establishment of brook charr in this region occurred at that time (Lacasse and Magnan 1993).

Other than providing evidence for the large geographic origin of LMNP brook charr populations, mtDNA analysis provided limited information on their genetic structuring due to the reduced polymorphism observed. Geographic partitioning of several mtDNA haplotypes was partly congruent with drainage separation and population phylogenetic groupings defined by microsatellites, resulting in a significant genetic structure as indicated by either F_{ST} and Φ_{ST} values. The low abundance of these haplotypes, the almost complete fixation of the same haplotype in most populations independent of drainages ($F_{ST} = 0$ among 65% of all populations), and the small number of mutations that differentiated them precluded a finer characterization of population phylogeographic structure and the interpretation of the evolutionary histories of populations. Specifically, it remained impossible to assess whether the geographic distribution of mtDNA haplotypes reflected recent postglacial differential lineage extinction among populations, or the retention of more ancient preglacial separation signals.

Microsatellite DNA

In contrast, the analysis of microsatellite variation revealed extensive polymorphism which resolved a finer population structuring. These results supported our working hypothesis that microsatellites may be more appropriate than mtDNA for inferring relationships among closely related populations. The salient feature of this analysis was the definition of seven population assemblages, all characterized by different genetic composition and the presence of many unique alleles. These as-

semblages were generally highly divergent relative to the extent of genetic differentiation observed among the populations that composed each of them.

Even when classified as parts of a same drainage, many of the lakes surveyed are like islands with no possible physical connections, the effects of such isolation being reflected by differential allelic frequencies among all brook charr populations from a given drainage. The phylogenetic definitions of several population assemblages did not coincide with their drainages of origin. Namely, all populations from Des Cinq (B) and À la Pêche (E) drainages composed a single phylogenetic group of populations among which the low level of divergence was comparable to that observed among populations within other drainages. Similarities among populations from these two physically isolated systems clearly indicated that these adjacent drainages were initially combined before they became divided following isostatic rebound or other environmental changes. In contrast, two phylogenetically distinct population groups were defined within the single drainage F, among which the extent of divergence was comparable with and even more important than that observed among other separate drainages (fig. 4). Consequently, gene diversity analysis revealed a much higher among-groups component of genetic variance when the analysis was based on evolutionary lineages rather than on drainage subdivisions. Finally, the poor classification indices, higher heterozygosity values, and obvious admixture of distinct allelic size classes observed in three populations (E1, E2, and F6) all suggested that these were issued from introgressive hybridization between well-defined population groupings.

Two hypotheses may conceivably account for such a pattern of genetic partitioning. First, LMNP may have been recolonized mainly by a single panmictic population. Once established, genetic drift and local mutations may have led to population divergence, while extensive gene flow within drainage may have hampered differentiation among these populations, resulting in a more important differentiation among than within drainages. An alternative hypothesis to the single-invasion hypothesis is that LMNP was recolonized by several founding populations that were already differentiated prior to their postglacial dispersal and among which intermixing has remained limited.

Under SMM and mutation–drift assumptions, $(\delta\mu)^2$ distance is theoretically linear with time and, consequently, allows direct estimation of divergence times independent of population sizes (Goldstein et al. 1995b). Assuming that divergence times of populations cannot exceed that of the deglaciation of LMNP, the isolation of phylogenetic groups under the first hypothesis occurred approximately 10,000 years ago (Dyke and Prest 1987), while this time frame corresponds to isolation of populations within groups under the second scenario. The average $(\delta\mu)^2$ value estimated over all pairwise comparisons of populations belonging to the seven different population groups defined in the $D_{CE}-(\delta\mu)^2$ tree (fig. 4C) was 74.45 (SD = 59.16, $n = 181$) whereas the same estimate calculated among populations within

groups was 2.85 (SD = 2.03, $n = 72$). Using 10,000 years as divergence time and distance values of either comparison, along with a mean generation time of 3 years (Scott and Crossman 1973), we then calculated the mutation rate averaged over all loci using equation A3 of Goldstein et al. (1995b). This resulted in an estimates of 1.12×10^{-2} mutations/locus/generation for the first hypothesis and 4.28×10^{-4} for the second one. The latest estimate is comparable to that of 5.6×10^{-4} estimated for humans based on 15 dinucleotide microsatellites (Weber and Wong 1993) and the calibrated estimate of 7.96×10^{-4} obtained by Goldstein et al. (1995b). This value is also similar to the average mutation rate of 4.75×10^{-4} estimated for honey bee, *Apis mellifera* (Estoup et al. 1995a). Mutation rate on the order of 10^{-4} is also frequently used to infer demographic parameters for fishes (Garcia de Leon, Chikhi, and Bonhomme 1997; O'Connell et al. 1997).

On the other hand, the literature reported only a few individual microsatellite loci that exhibit the mutation rate implied by the first hypothesis, which resulted in much higher polymorphism (e.g., Primmer et al. 1996) than that observed here. Because bottlenecks and founder events associated with the recolonization of the different drainages by a few individuals may have influenced genetic distance, the estimated mutation rate could have been overestimated. However, the high levels of heterozygosity observed in most populations are not compatible with such demographic events.

Without firmly rejecting the first hypothesis, the multiple-invasions hypothesis therefore appeared more congruent with the actual knowledge of microsatellite mutation rates. Assuming linearity of $(\delta\mu)^2$ estimates with time, our results suggest that the mean divergence time among phylogenetically distinct population groups is approximately 26 times larger than that involving populations belonging to the same group. Under the multiple-invasions hypothesis (mutation rate = 4.28×10^{-4}), this ratio suggests that the different population groups diverged approximately 260,000 years ago, on average. The hypothesis that the different population groups defined in this study diverged well before the last postglacial recolonization events is further supported by the fact that their mean divergence times fall within the range of that estimated among major brook charr refugial groups. Tentatively applying the molecular clock of 1% sequence divergence per million years estimated for salmonids (Smith 1992) to the sequence differences observed in this study, the mtDNA clades A and B that distinguished major refugial groups in brook charr diverged approximately 600,000 years ago, a value similar to that estimated from a more extensive study based on RFLP analysis over the entire mitochondrial genome (Danzmann et al., unpublished data).

Without denying the obvious value of more traditional markers such as mtDNA, these results indicate that microsatellites may reach a finer scale of resolution to infer population relationships and phylogeographic structure over relatively short (yet important from an evolutionary perspective) temporal frames or small geographic scales. Thus, the analysis of microsatellites of-

fers the potential to gain further insight into, and possibly a more realistic view of, the evolutionary history of recently diverged populations. This is well illustrated by the case of north temperate freshwater fishes. Typically, mtDNA phylogeographic studies of such species have revealed that they are composed of major population groups diagnosed by phylogenetically distinct lineages that most likely survived in distinct refugial regions (e.g., Bernatchez and Dodson 1991, 1994; Bernatchez, Guyomard, and Bonhomme 1992; Wilson et al. 1996). Logically, fish that survived within such large refugial regions were most likely subdivided into populations that were geographically isolated into separate drainages or other physical barriers, just as in contemporary populations. Following deglaciation and the opening of new dispersal routes, these populations may then have independently recolonized new areas, with or without admixing with each other. Most likely because of the relatively short time frame involved in the separation of such populations, however, mtDNA generally failed to reveal phylogenetic signals of the existence of such subdivisions, rather, projecting the picture of a given refugial population as a single panmictic unit. Besides potentially providing a finer understanding of historical events in shaping phylogeographic structure, the resolution of historical population structure at the sub-refugial level could be of importance for the understanding of evolutionary processes, for instance, in assessing the hypotheses of single- versus multiple-invasion events as related to the origin of sympatric species pairs (e.g., Bernatchez and Dodson 1990; McPhail 1994).

Distance Measures and Phylogenetic Reconstruction

While there is little doubt that microsatellite analysis offers great potential for inferring small temporal and geographic scale issues in population genetics, there are still major controversies about which genetic distance measures are more appropriate for assessing population relationships.

Given the constraints imposed by the low number of loci analyzed in the present study, three criteria indicated that tree topology based on D_{CE} distances was more effective than that based on $(\delta\mu)^2$ in depicting the most reliable population relationships, providing higher confidence values on tree topologies, better geographic concordance of population clustering, and higher components of genetic variance among population groups. Very limited success has been obtained thus far with genetic distances developed for microsatellites in phylogenetic reconstruction of populations or closely related species compared to the use of IAM-based measures or analyses making no inference about mutational processes (Estoup et al. 1995a; Goldstein et al. 1995b; Boyce et al. 1997; Valsecchi et al. 1997; but see Bentzen et al. 1996).

Despite limitations imposed by sampling errors for tree reconstruction, SMM-based measures are nevertheless those that better approach linearity with time for microsatellites and, consequently, are most likely to depict correct branch lengths (Takezaki and Nei 1996). Consequently, $(\delta\mu)^2$ distances better approximated real

branch lengths than did D_{CE} based on the higher congruence in the ratio of branch lengths among phylogenetically distinct population groups versus those among populations within the same group with the intragroup versus intergroup partitioning of genetic variance based on either allelic or molecular variance. These results therefore indicate that the use of a genetic distance that makes no inference of mutational process (such as D_{CE}) to construct tree topology, combined with that of a distance that incorporates information of mutational differences among alleles (such as $(\delta\mu)^2$) to adjust branch lengths of the resulting tree, as theoretically proposed by Takezaki and Nei (1996) (reiterating the view of Nei 1995), may represent the optimal approach for assessing the evolutionary history of closely related populations until a high number of loci of known mutational processes can be routinely used without compromising the number of populations and individuals that must be analyzed in such studies. To our knowledge, however, the efficiency of this approach had not previously been evaluated empirically for microsatellites.

Conservation Issues

An important issue in the elaboration of conservation strategies is the identification of conservation units. There is now a consensus that priority recognition for protection of such units should be commensurate with their evolutionary distinctiveness. The concept of ESUs (*sensu* Ryder 1986) has recently been reconsidered in an effort to better define subunits of species for conservation purposes (Waples 1991). According to this principle, such groups must represent an important component of the species' evolutionary legacy. While few conservation biologists argue the legitimacy of this definition, there is no general agreement about the criteria to be used in defining ESUs. For instance, Moritz (1994) suggested that ESUs should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci. However, because several population groups may show local adaptations in the absence of any detectable mtDNA variation, likely due to insufficient evolutionary times to develop reciprocal monophyly (e.g., Magnusson and Ferguson 1987; Meyer et al. 1990; Sturmbauer and Meyer 1992; Legge et al. 1995), less stringent criteria have been proposed. For instance, Bernatchez (1995) suggested that any differentiable populations could be recognized as ESUs in situations in which it can be shown that these populations follow a distinct evolutionary trajectory. In such cases, populations that exhibit adaptive uniqueness receive priority for conservation. Clearly, the interpretation of ESUs will depend of the tools used to define them. Globally, the mtDNA homogeneity observed in this study indicated a common monophyletic origin of population groups found in LMNP, which sharply contrasts with those observed in other northeastern North American lakes. Thus, the entire LMNP could be considered as a single ESU relative to neighboring populations. However, microsatellites indicated the presence of seven phylogenetically distinct units, associated with the differential recolonization of the park by distinct

population groups that have evolved independently during a time period possibly as long (at least on the order of magnitude) as that involved in the differentiation of distinct glacial races diagnosed by mtDNA lineages. Such a temporal frame of isolation leading to pronounced genetic differentiation may have been sufficient to develop heritable, adaptive differences among the different groups. Consequently, if one objectively applies recognized criteria for the definition of ESU, each population group of LMNP may represent an ESU, potentially as important for conservation issues as the different glacial races themselves. The results of this study therefore suggest that the use of microsatellites in conservation genetics may change our views about the problems of temporal and geographical scales to be considered in order to optimize conservation strategies.

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