Transactions of the American Fisheries Society 131:1106–1118, 2002
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Microsatellite Gene Diversity Analysis in Landlocked Arctic Char from Maine

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Abstract.—Using six microsatellite loci, we characterized the 12 remaining populations of Arctic char *Salvelinus alpinus* naturally occurring in Maine. More specifically, we challenged the hypotheses based on previous analyses with other markers that (1) Arctic char from Floods Pond (known locally as silver char) represent a distinct evolutionary lineage and (2) all other Arctic char populations from Maine belong to the same evolutionary lineage and therefore do not require individual consideration for conservation. The high level of polymorphism observed at microsatellite loci in this study contrasted sharply with the extremely low levels of variation previously reported at other markers. Analyses confirmed that all lakes possess genetically distinct populations among which gene flow is restricted and on which other evolutionary forces may act independently, enhancing their genetic divergence. However, hierarchical gene diversity, population clustering, and population assignment analyses all indicated that the populations from different drainages did not originate from genetically distinct ancestral population assemblages. Our results thus contradict previous conclusions, as we found that the Arctic char from Floods Pond likely did not originate from a distinct evolutionary lineage. Secondly, although all Maine Arctic char appear to belong to a single evolutionary lineage, sufficient divergence was found to reject the hypothesis that all other populations should be considered as genetically equivalent for conservation. We discuss the implications of these findings for the management and protection of these unique Arctic char populations.

The Arctic char *Salvelinus alpinus* has a holarctic distribution and exhibits a complex pattern of variability in morphology, coloration, ecology, and life history traits (reviewed in Behnke 1972; Johnson 1980). The contemporary distribution, diversity, and population structure of Arctic char were molded by repeated glacial advances and retreats during the Pleistocene Epoch, as has been the case with other northern temperate freshwater fishes (Bernatchez and Wilson 1998). Recent phylogeographic surveys of mitochondrial DNA (mtDNA) variation revealed that in North America these historic events have led to the evolution of four major lineages of Arctic char that are largely allopatric in distribution (Wilson et al. 1996; Brunner et al. 2001). One of these, the Laurentian lineage, likely dispersed from a now submerged Atlantic coastal refuge and is mainly composed of landlocked populations that became isolated from the sea during the last glacial retreat. The present distribution of the Laurentian lineage includes populations from southeastern Quebec, New Brunswick, and the northeastern United States (Wilson et al. 1996; Brunner et al. 2001). Based on unique phenotypes and morphological differences from Arctic char in Labrador and Newfoundland, these landlocked populations have been recognized as a distinct subspecies, *S. alpinus oquassa* (Qadri 1974).

Landlocked Arctic char are intolerant of environmental disturbance, and consequently many populations have become extinct due to either competition from human-introduced fish or human-induced environmental changes (reviewed in Kircheis 1989). Maine is now the only region in the United States that still has relict populations of the Laurentian lineage of Arctic char, which are found in 12 lakes scattered in three major watersheds. Based on its unique breeding color and oth-

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Received November 1, 2001; accepted April 14, 2002

TABLE 1.—Sample locations with their abbreviations, drainages of origin, areas, and sample sizes. Asterisks indicate populations not included in gene diversity analysis due to small sample size.

Water body	Abbreviation	Drainage	Latitude (N)	Longitude (W)	Area (ha)	Sample size
Gardner Lake	GAL	St. John	46°58′	68°53′	117	15
Pushineer Pond	PU	St. John	46°58′	68°58′	22	3*
Big Black Pond	BB	St. John	46°59′	68°50′	59	10
Big Reed Pond	BR	St. John	46°21′	69°03′	36	5*
Debouillie Pond	DP	St. John	46°58′	68°51′	106	23
Floods Pond	FP	Union	44°45′	68°30′	260	39
Green Lake	GL	Union	44°40′	68°32′	1,196	6*
Bald Mountain Pond	BMP	Penobscot	45°15′	69°44′	461	28
Penobscot Lake	PL	Penobscot	45°56′	70°13′	408	24
Rainbow Lake	RA	Penobscot	45°49′	69°07′	666	43
Wadleigh Pond	WA	Penobscot	46°21′	69°43′	62	30
Wassataquoik Lake	WAS	Penobscot	46°01′	68°57′	71	30

er external phenotypic traits, the Arctic char population of Floods Pond has been recognized as a distinct entity referred to as silver char or Sunapee (Kircheis 1989). Other populations, known locally as blueback char, are all located in remote unpopulated areas of the state. Floods Pond, however, is the main source for domestic water in an area of very rapid human population growth, and consequently the long-term survival of the population in this lake may be threatened by the periodic lowering of water levels (Kircheis 1989). Recent successes with artificial spawning habitats in Floods Pond have mitigated some of these concerns (F. W. Kircheis, unpublished data).

Conservationists regularly use the amount of genetic divergence between populations as a major criterion for determining population uniqueness and thus protection regimes (e.g., Moritz 1994; Bernatchez 1995; Waples 1995; Petit et al. 1998; Crandall et al. 2000). However, there is limited information on the genetic relationships among Maine Arctic char populations. A previous allozyme survey reported seven slightly polymorphic enzyme-coding loci but it failed to differentiate between four Maine populations, including Floods Pond (Kornfield et al. 1981). An analysis of restriction fragment length polymorphisms (RFLPs) in mtDNA showed predominantly similar results, with the exception of a unique, slightly divergent haplotype found only in Floods Pond (Kornfield and Kircheis 1994). This led the authors to conclude that the preservation of the Floods Pond silver char was warranted on genetic grounds and that all other populations should be viewed as a genetically cohesive group. Highly reduced allozyme and mtDNA polymorphism within evolutionary lineages is, however, a common feature of Arctic char throughout its range (Brunner et al. 1998; Osinov and Pavlov 1998) and hampers the use of those markers in characterizing the genetic structure of populations beyond the scale of glacial races. Therefore, it may be hazardous to equate the apparent homogeneity observed at these markers with the lack of genetic distinction among populations that are physically isolated.

Microsatellites are a class of highly polymorphic nuclear loci that are receiving increasing attention (Estoup and Angers 1998). The usefulness of these markers for addressing fine-scale population structure has recently been demonstrated in members of the genus *Salvelinus*, such as the brook char *S. fontinalis* (Angers and Bernatchez 1998; Castric et al. 2001), the Dolly Varden *S. malma*, and the bull trout *S. confluentus* (Taylor et al. 2001). The use of microsatellites in Arctic char has also resolved population structure on small geographic scales where other markers had failed to detect significant partitioning of genetic diversity (Bernatchez et al. 1998; Brunner et al. 1998; Primmer et al. 1999). In this study, we performed a microsatellite gene diversity analysis among all known relict populations of lacustrine Arctic char from Maine to elucidate whether they are composed of two evolutionary lineages, one unique to Floods Pond and the other represented by all other populations. Secondly, we tested the conclusion of Kornfield and Kircheis (1994) that with the exception of the Arctic char in Floods Pond, all of the populations in Maine are genetically indistinguishable.

Methods

Sample collection and microsatellite analysis.— A total of 256 specimens were sampled from the 12 lakes known to contain Arctic char populations in Maine (Table 1; Figure 1). The average sample

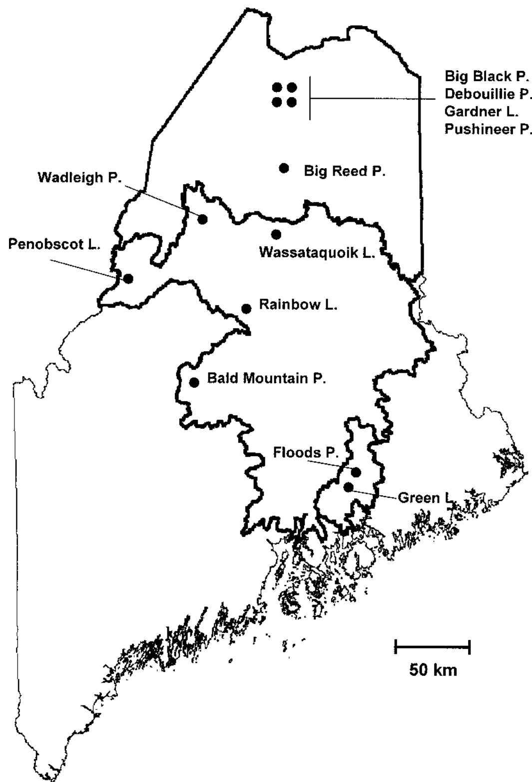


FIGURE 1.—Map showing the locations from which Arctic char samples were taken (P stands for pond, L for lake).

size for 9 of the lakes was 27. However, sample size was very limited for three populations (Big Reed Pond, Green Lake, and Pushineer Pond; $n \leq 6$), and consequently estimates of genetic diversity were not attempted for these populations. Samples from Gardner Lake and Big Black Pond were also smaller than average, so the results obtained for these populations should be interpreted cautiously. DNA was extracted from either adipose fins or white muscle fixed in 95% ethanol as detailed in Bernatchez et al. (1992).

Genotyping of individuals was performed by screening microsatellite loci using primers originally developed for other salmonids to amplify homologous microsatellite loci in Arctic char. Primers for *Sfo-8* and *Sfo-23* were developed from brook char (Angers et al. 1995), *Cocl-3* from lake whitefish *Coregonus clupeaformis* (Bernatchez 1996), *MST-85* from brown trout *Salmo trutta* (Presa and Guyomard 1996), *Sco-19* from bull trout (Taylor et al. 2001), and *Ots-1* from chinook salmon *Oncorhynchus tschawytscha* (Banks et al. 1999). One primer for each locus was labeled with one of three colored dyes: green (TET) for *Cocl-3, MST-85,* and *Sfo-8,* blue (6-FAM) for *Sco-19* and *Sfo-23,* and yellow (HEX) for *Ots-1.* Two polymerase chain reaction (PCR) multiplex reactions were used to amplify three loci at a time. Multiplex A consisted of a 15-μL reaction containing 1 unit of *Taq* polymerase, 3.0 μL 10× PCR buffer (Boehringer Mannheim), 2 mM $MgCl_2$, 0.28 mM deoxynucleotide triphosphate (dNTP), 50 ng DNA template, 133 pmol of each *Sco-19* primer, 133 pmol of each *Ots-1* primer, and 166 pmol of each *Cocl-3* primer. Multiplex B consisted of a 10-μL reaction with 1 unit of *Taq* polymerase, 1.0 μL 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 50 ng DNA template, 80 pmol of each *MST-85* primer, 100 pmol of each *Sfo-8* primer, and 700 pmol of each *Sfo-23* primer.

The PCR was conducted in a Perkin-Elmer 9600 thermocycler with the following profile: initial denaturation at 95°C for 3 min; 32 cycles of 94°C for 1 min, the annealing temperature (50°C for multiplex A and 60°C for multiplex B) for 45 s, and 72°C for 45 s; and final extension at 72°C for 10 min. Prior to electrophoresis, 1.5 μL of PCR product was mixed with 0.2 μL of internal size standard (TAMRA; 350 base pairs) and 1.8 μL of deionized formamide. The loading product was then heat-denatured and immediately placed on ice. Electrophoresis was conducted on a 5% polyacrylamide gel (19:1 acrylamide:bisacrylamide) and migrated for 2.25 h at 3,000 V using an ABI 377 (Perkin-Elmer) automated sequencer. Allelic size was determined using Genescan. Final scoring and data tabulation was conducted using Genotyper.

Gene diversity.—The software GENEPOP (Raymond and Rousset 1995a) was used to examine conformity to Hardy−Weinberg (HW) equilibrium and to catalog population statistics and do primary analysis. The genetic polymorphism for each population was thus estimated as the mean number of alleles per locus (A), observed heterozygosity (H_o), and gene diversity (H_e). A homogeneity test among all pairs of samples was performed to test for genic differentiation under the null hypothesis of homogeneous allelic distribution among samples (Raymond and Rousset 1995b). Global values of significance over all loci were estimated for both tests of HW equilibrium and genic differentiation using Fisher's method of combining probabilities from tests of significance (Sokal and Rohlf 1995).

The population genetic structure was quantified using an analysis of variance (ANOVA) framework that involves computing the correlation be-

tween a pair of alleles drawn from the same subpopulation relative to that between pairs randomly drawn from a group of subpopulations (Weir and Cockerham 1984). Fixation indices were also computed in an analysis of molecular variance (AMOVA) framework, using information on the allelic size variance that was entered as a matrix of Euclidian squared distances (known as Φ-statistics; Michalakis and Excoffier 1996). Both analyses were performed with the program ARLEQUIN (Schneider et al. 1997). Comparing the extent of population structure depicted from allele frequency and mutational information aids in the understanding of the relative roles of long-term separation and contemporary genetic drift in population differentiation (Slatkin 1995; Goodman 1998). We first computed both F_{ST} (the genetic variance statistic) and Φ_{ST} estimates for all pairwise comparisons of populations by this procedure. We then performed a hierarchical analysis of gene diversity to assess the component of genetic variance imputable to variance among individuals within each sample, among populations within a given drainage, and among drainages. Significance values were obtained by a permutation procedure (1,000 permutations). When multiple tests were conducted for a single hypothesis, the significance of the *P*-values was adjusted by means of Bonferroni sequential corrections for multiple simultaneous statistical tests (Rice 1989) with an initial α value of $0.05/k$, k being the number of tests.

Population relationships.—Because of uncertainty regarding what constitutes the most appropriate method for quantifying genetic distances among populations based on microsatellite polymorphism, two measures of genetic distance were computed: (1) the Nei et al. (1983) D_A distance and (2) the $(\delta\mu)^2$ genetic distance, which takes into account the size differences between alleles and theoretically represents linearity with time better than distance measures based on non-stepwise-mutation-models when microsatellites follow a strict stepwise mutation model (Goldstein et al. 1995). The matrices of pairwise distances were used to construct population phenograms using the neighbor-joining algorithm. Confidence levels on tree topology were estimated from the percentage of 1,000 bootstraps, which performed resampling of loci within samples. All procedures were performed using the program NJBPOP provided by J.-M. Cornuet (Laboratoire de Modélisation et de Biologie Évolutive, URBL-INRA, Montpellier, France).

Individual-based analyses.—We also performed a population allocation test that estimated the probability of correctly reassigning individuals to their known population of origin (Paetkau et al. 1995; Waser and Strobeck 1998). This method calculates the probability of occurrence of the multilocus genotype of a given individual in each population and then assigns the individual to the population with the largest probability of occurrence. Used in this context, the population allocation test represents a useful measure of genetic differentiation among populations (Paetkau et al. 1995). This test was performed using a program written with the algebric computer system MapleV (Bernatchez and Duchesne 2000).

Results

Intrapopulation Gene Diversity

All six microsatellite loci used were highly polymorphic, with the total number of alleles per locus varying between 12 for *MST-85* and 48 for *Sfo-23.* This translated into generally high intrapopulation diversity, with the number of alleles per locus between 3 and 28, and gene diversity between 0.298 and 1.000, depending on the locus and population (Table 2). Following Bonferroni corrections for simultaneous multiple tests, conformation to Hardy−Weinberg equilibrium was rejected in 9 out of 54 tests (8 involved deficits in heterozygotes), more than the 3 expected by chance alone with $\alpha = 0.05$. Three of the significant departures from HW equilibrium occurred in Floods Pond, which was in disequilibrium globally across loci (Fisher's method). Other departures from equilibrium were dispersed among loci and populations.

Genetic Differentiation among Lakes

Highly significant differences in allelic frequency distribution (global *P*-values across loci by Fisher's method) were observed among most pairwise comparisons of samples, generally indicating that each lake harbors a genetically distinct Arctic char population (Table 3). The average number of loci showing significant differences among samples was 4.7; the number ranged from 0 to 6.

The comparisons with the fewest number of significant differences mainly involved geographically proximate populations (e.g., Big Black and Debouillie ponds and Gardner Lake). Most samples were characterized by the presence of specific alleles or striking differences in frequencies (Figure 2). For instance, at locus *Cocl-3,* allele 225 was predominant in Wadleigh Pond but observed at very low frequencies elsewhere (Figure 2A).

TABLE 2.—Number of different alleles (A), observed heterozygosity (H_o), gene diversity (H_e), and significant (following Bonferroni corrections; $\alpha = 0.05$, $k = 6$) unbiased estimates of type 1 error for Hardy–Weinberg (HW) departure proportions per locus and population. Abbreviations for populations are given in Table 1; ns = not significant; NA = not applicable.

Locus	BB	BMP	BR	DP	FP	GAL	GL	PL	PU	RA	WA	WAS
Cocl-3												
A	0	10	4	11	6	8	7	7	4	12	7	9
H_o	0.800	0.857	0.400	0.783	0.923	0.600	0.833	0.750	1.000	0.953	0.539	0.967
H_e	0.874	0.828	0.711	0.797	0.741	0.635	0.773	0.665	0.867	0.889	0.594	0.841
HW	ns	ns	NA	ns	0.002	ns	NA	ns	NA	ns	ns	ns
Ots-1												
A	10	12	6	15	9	12	4	9	4	28	6	8
H_o	0.800	0.821	0.800	0.763	0.590	0.933	0.800	0.913	0.667	0.810	0.762	0.931
H_e	0.889	0.886	0.844	0.891	0.745	0.892	0.844	0.862	0.800	0.927	0.757	0.927
HW	ns	ns	NA	ns	ns	ns	NA	ns	NA	ns	ns	ns
Sco-19												
A	9	7	4	16	9	8	6	10	4	21	7	16
H_o	0.900	0.857	0.400	0.913	0.769	0.800	0.667	0.833	0.667	0.954	0.533	0.500
H_e	0.826	0.803	0.800	0.897	0.823	0.823	0.561	0.801	0.800	0.952	0.452	0.520
HW	ns	ns	NA	ns	ns	ns	NA	ns	NA	ns	ns	ns
MST-85												
A	5	6	3	6	5	7	3	6	4	5	3	7
H_o	0.500	0.714	0.600	0.783	0.205	0.867	0.167	0.583	0.667	0.279	0.000	0.733
H_e	0.711	0.707	0.778	0.772	0.543	0.832	0.530	0.653	0.867	0.417	0.298	0.601
HW	ns	ns	NA	ns	<0.0001	ns	NA	0.006	NA	<0.0001	<0.0001	ns
Sfo-8												
A	11	17	9	15	13	13	5	13	4	17	12	13
H_o	0.889	0.852	1.000	0.826	0.872	0.667	0.833	0.792	0.667	0.810	1.000	0.862
H_e	0.902	0.935	0.978	0.892	0.906	0.915	0.833	0.903	0.800	0.918	0.921	0.906
HW	ns	ns	NA	ns	<0.0001	<0.0001	NA	ns	NA	ns	ns	0.006
Sfo-23												
A	11	28	8	25	14	22	9	15	6	26	11	27
H_o	1.000	1.000	1.000	1.000	0.897	0.933	1.000	0.826	1.000	0.758	0.750	1.000
H_e	0.958	0.948	0.956	0.972	0.868	0.982	0.955	0.915	1.000	0.954	0.958	0.961
HW	ns	ns	NA	ns	ns	ns	NA	ns	NA	<0.0001	ns	ns

The same was true for allele 232 at *Ots-1*. A pattern of population-specific alleles was particularly apparent at *Sco-19* (Figure 2C), for which most populations were characterized by groups of alleles that were absent or observed at very frequency low elsewhere. Additional differences were observed at all loci.

The strong heterogeneity in allele frequency distribution also translated into significant estimates of population divergence based on allelic variance (F_{ST}; Table 4, below diagonal). Excluding the three samples with small sizes, F_{ST} values varied between 0.05 and 0.20, approximately. The values of Φ_{ST} exceeded those of F_{ST} in virtually all cases (Table 4, above diagonal). The general pattern of population genetic uniqueness was also illustrated

TABLE 3.—Pairwise comparison of the number of loci with significantly different allele frequencies (Fisher's exact test; $\alpha = 0.05$). The asterisk indicates an insignificant difference of the pooled P-values (Fisher's method) following adjustment of the multiple tests by mean of the sequential Bonferroni method ($k = 36$).

	Population							
Population	BB	BMP	DP	FP	GAL	PL	RA	WA
BMP	4							
DP	3	5						
FP	6	6	6					
GAL	3	5	0*	6				
PL	5	5	5	6	5			
RA	4	5	4	6	4	5		
WA	4	4	4	6	4	2	5	
WAS	5	5	5	6	5	5	6	5

by the assignment tests (Table 5). In most cases, a majority of individual multilocus genotypes could be reallocated to their population of origin (average = 79%, range = 40−97%). In accordance with the above analysis, the lowest assignment success was observed in geographically proximate populations from Big Black and Debouillie ponds and Gardner Lake. In these cases, incorrectly assigned fish were reallocated to geographically proximate populations. This was not necessarily the case elsewhere. For instance, several misassigned individuals from Floods Pond, Bald Mountain Pond, Rainbow Lake, and Wassataquoik Lake were reallocated to populations from separate and remote river drainages.

Population Relationships

While all lakes contain genetically distinct populations, no clear pattern of relationships emerged among them (Figure 3). Except for the strong grouping observed for the geographically proximate Debouillie Pond and Gardner Lake, the overall topology of the D_A population phenogram was poorly supported. Confidence estimates on the branching pattern of the $(\delta\mu)^2$ tree (not shown) were even worse, with bootstrap values ranging between 1% and 38%. Generally, there was no evidence of population grouping by drainage. (For example, Big Black Pond aggregated with Wadleigh Pond and Bald Mountain Pond clustered with Floods Pond and Penobscot Lake.) It is noteworthy that the silver char population (Floods Pond) did not cluster distinctively away from the other Arctic char populations.

The lack of correspondence between population relationships and drainage of origin was also reflected in the hierarchical analysis of molecular variance (Table 6). Thus, the amount of genetic variance explained by the interdrainage component was 2.2 times less (3.57/7.71) than that explained by variations among populations within a drainage. The small interdrainage component was, however, statistically significant, most likely because of the close relationships between fish in Debouillie Pond and Gardner Lake, as detailed above.

Discussion

High Levels of Intrapopulation Gene Diversity in Maine Arctic Char

The overall high level of polymorphism observed at microsatellite loci in this study contrasted sharply with the extremely reduced levels of variation previously reported for Maine Arctic char at other markers. The average heterozygosity (H_e = 0.819) and number of alleles per locus (11.9, excluding the three samples with $n \leq 6$) observed within each population greatly exceeded the variation previously reported for enzyme-coding loci, for which only 7 out of 26 screened loci were polymorphic and heterozygosity averaged 0.026 (Kornfield et al. 1981). The level of mtDNA diversity detected by RFLP analysis in Arctic char from Maine was also much lower than with microsatellites (Kornfield and Kircheis 1994). The extent of polymorphism found in microsatellites in Arctic char from Maine is, however, very similar to that recently reported in a microsatellite study of landlocked Arctic char populations from lakes in the European Alps. Brunner et al. (1998) reported average heterozygosity and allelic diversity values of 0.851 and 11.6 across 15 populations. These studies indicate that microsatellite loci are more sensitive than traditional markers with respect to gene diversity and population structure in Arctic char. It is also noteworthy that globally the level of intrapopulation genetic diversity in Arctic char from Maine generally exceeded that reported in lacustrine populations of the closely related brook trout (Angers and Bernatchez 1998; Hébert et al. 2000), including Maine populations (Castric et al. 2001).

The high level of intrapopulation diversity that we documented appears somewhat paradoxical because most of the populations surveyed in this study are physically isolated from the others, which would prevent gene flow, and population sizes are believed to be low for several of them (Kircheis, unpublished data). This diversity may be partially related to the higher mutation rate of microsatellite loci compared with that of either mtDNA or enzymatic loci (Estoup and Angers 1998); a more rapid rate of mutation is expected to promote the establishment of a higher effective number of alleles at mutation-drift equilibrium for a given effective population size (Ewens 1972). Also, the effective population size may have remained sufficiently large that not enough generations have occurred since the founding of the population (approximately 10,000 years or 2,000 generations) to enable populations to reach mutation-drift equilibrium (Castric et al. 2001). As such, the general high level of intrapopulation genetic diversity in landlocked populations from Maine could still partly reflect the original diversity of the founding anadromous ancestral population. Whatever the correct explanation, the high level of microsatellite variation is not compatible with the view that these populations are genetically de-

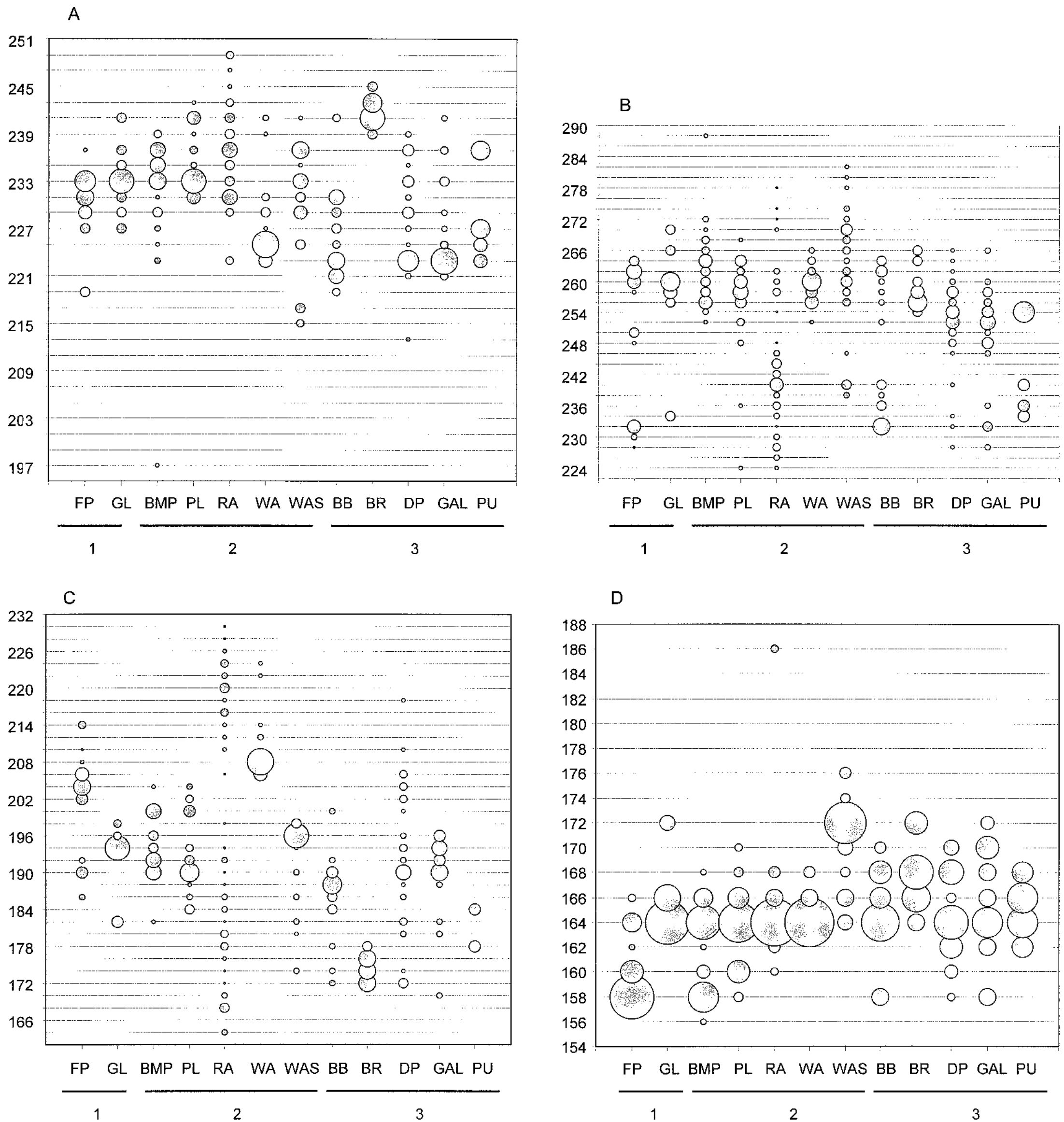
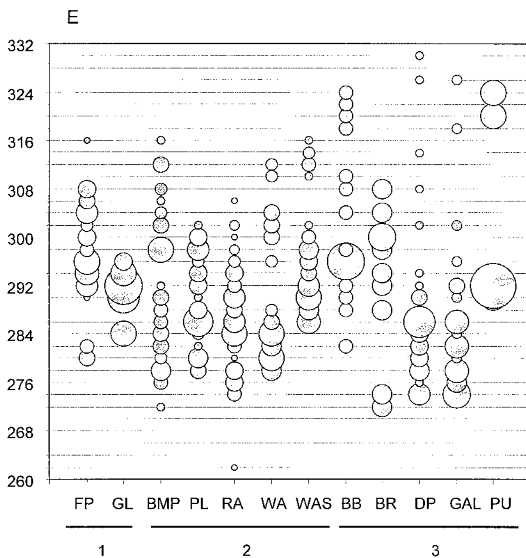


FIGURE 2.—Allele frequency histogram of the six microsatellite loci studied: **(A)** *Cocl-3,* **(B)** *Ots-1,* **(C)** *Sco-19,* **(D)** *MST-85,* **(E)** *Sfo-8,* and *(F) Sfo-23.* Populations are clustered by drainage (1 = Union, 2 = Penobscot, and 3 = St. John); see Table 1 for location abbreviations. (The raw data can be downloaded from L. Bernatchez's personal Web page at http://www.bio.ulaval.ca/ (click on the department's logo and then on ''Professeurs'').

pauperate or that they have suffered severe reductions in recent evolutionary times, explanations that are commonly invoked for the reduced levels of diversity observed with other markers in Arctic char (reviewed in Brunner et al. 1998).

Genetic Structure and Population Uniqueness

All of the statistical methods used in this study reject the null hypothesis of no genetic differentiation among Maine Arctic char from different lakes. This confirms that all of these lakes contain genetically distinct populations among which gene flow has been restricted and on which other evolutionary forces may be acting independently to enhance divergence. Genetic differentiation also occurs among the populations from the St. John River drainage (more precisely, the Fish River, which drains into the St. John) that are geograph-



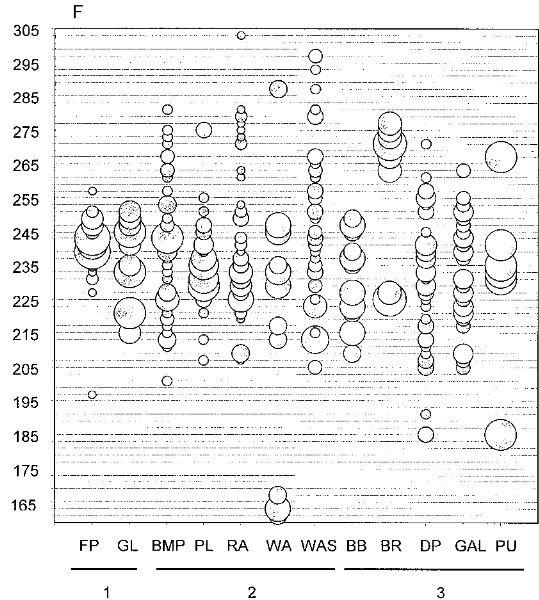


FIGURE 2.—Continued.

ically very close to each other and not physically isolated, namely, those from Gardner Lake and Debouillie Pond. Significant genetic differentiation among physically isolated populations is certainly what would be predicted intuitively. Yet our results contrast with those of previous analyses of both allozymes and mtDNA of Maine Arctic char, which generally failed to reject the hypothesis of no differentiation (Kornfield et al. 1981; Kornfield and Kircheis 1994). A similar contrast, in which strong genetic divergence was observed at microsatellite loci but was absent at other markers, was reported by Brunner et al. (1998) for populations in the European Alps. As discussed by these authors, the discrepancies in results can be attributed to the limited resolution of allozyme and mtDNA RFLP analyses, which reveal insufficient polymorphism to infer population structure in Arctic char. As pointed out by Waples (1998), it may be hazardous to infer genetic homogeneity from the absence of statistically significant genetic differences without considering alternative explanations, such as insufficient resolution. This, however, has been a common mistake in previous studies, including those on Arctic char from Maine (Kornfield et al. 1981; Kornfield and Kircheis 1994).

Although we found that the lakes we surveyed have genetically distinct populations, the amount of divergence appears to be relatively small for physically isolated populations, with a maximum observed F_{ST} value of 0.17. However, as discussed

TABLE 4.—Pairwise differentiation estimates based on allelic (F_{ST}; below diagonal) and molecular (ϕ_{ST}; above diagonal) variance analysis. Asterisks indicate insignificant difference of *P*-values following adjustment for multiple tests by means of the sequential Bonferroni method ($k = 36$).

Population	Population								
	BB	BMP	DP	FP	GAL	PL	RA	WA	WAS
BB		0.28073	0.11619	0.36005	0.18189	0.34717	0.19843	0.61077	0.18030
BMP	0.07038		0.13314	0.18091	0.19889	0.02183*	0.18775	0.30184	0.05418
DP	0.04591	0.06016		0.26994	−0.01384*	0.06737	0.09744	0.22324	0.15399
FP	0.10522	0.08352	0.12299		0.33057	0.26916	0.18724	0.47123	0.18504
GAL	0.05123	0.06784	0.00251*	0.13999		0.15807	0.09983	0.42051	0.18157
PL	0.08312	0.04519	0.06252	0.10935	0.09049		0.14753	0.54989	0.08156
RA	0.06064	0.06704	0.05749	0.14301	0.08715	0.06930		0.24464	0.17171
WA	0.11827	0.05794	0.03209	0.12807	0.07411	0.08553	0.06590		0.12796*
WAS	0.14235	0.11711	0.12712	0.17136	0.13061	0.14623	0.14085	0.14623	

TABLE 5.—Population reassignment analysis. Numbers indicate the proportions of individuals reclassified to the specified populations. Populations are arranged by drainage: FP (Union), BB-GAL (St. John), and BMP-WAS (Penobscot).

	Population								
Population	FP	BB	DP	GAL	BMP	PL	RA	WA	WAS
FP	0.897	0.077	0.000	0.000	0.000	0.026	0.000	0.000	0.000
BB	0.000	0.900	0.000	0.100	0.000	0.000	0.000	0.000	0.000
DP	0.000	0.043	0.652	0.304	0.000	0.000	0.000	0.000	0.000
GAL	0.000	0.267	0.267	0.400	0.067	0.000	0.000	0.000	0.000
BMP	0.000	0.071	0.000	0.020	0.679	0.179	0.000	0.000	0.000
PL	0.000	0.083	0.000	0.000	0.000	0.917	0.000	0.000	0.000
RA	0.000	0.488	0.047	0.000	0.000	0.116	0.349	0.000	0.000
WA	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.967	0.000
WAS	0.000	0.067	0.000	0.033	0.000	0.000	0.000	0.000	0.900

by Jin and Chakraborty (1995) and Hedrick (1999), the interpretation of the extent of differentiation among populations has been dominated by data from bi-allelic loci. In such a case, the F_{ST} value may range from 0 (when the populations all share the same allele frequencies) to 1 (when the populations are fixed for different alleles). Yet when there are multiple alleles, as for microsatellite data, the range of F_{ST} will have a maximum value lower than unity. From the average value of gene diversity for each population observed in Maine Arctic char (0.81), the equation (1b) of Hedrick (1999) predicts an approximate maximum F_{ST} value of 0.19. We observed no F_{ST} values higher that this predicted maximum. If one interprets the extent of population differentiation on the scale 0–0.19 rather than 0–1, then the average of F_{ST} values observed among all pairwise comparisons (0.10) is high, representing 52% of the possible maximum. Several populations approached the maximum value, such as Penobscot Lake versus Debouillie Pond and Gardner Lake (90% and 93%; Table 4). The lowest value (15%) was observed between Deboullie Pond and Gardner Lake, where

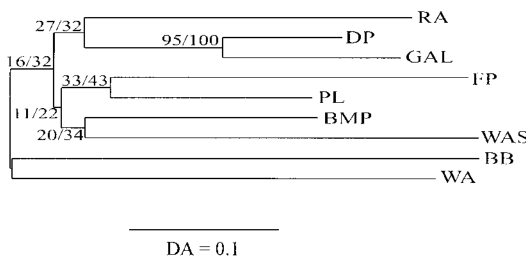


FIGURE 3.—Neighbor-joining phenogram (Nei's D_A distance) showing relationships among Arctic char populations in Maine. Values along branches are bootstrap values in percent by locus and individual. See Table 1 for location abbreviations.

gene flow may occur. Based on these estimates and the observation of numerous population-specific alleles (Figure 2), we conclude that Arctic char populations from Maine are highly differentiated overall and that each represents a unique gene pool.

Absence of Hierarchical Genetic Structure

Hierarchical gene diversity, population clustering, and population assignment analyses all indicated that populations within a given river drainage were generally not more closely related to each other than to others from separate river systems. These results show that populations from different drainages did not originate from genetically distinct ancestral population assemblages (for similar analyses, see Angers and Bernatchez 1998; Brunner et al. 1998; Castric et al. 2001; Taylor et al. 2001). They also indicate that the populations within a given drainage have generally been isolated for a considerable time, as the expected pattern for gene flow—producing more genetic similarity within than between drainages—was absent. The only evidence for gene flow occurred in the geographically proximate populations of the Fish River system. Although comparisons of microsatellite diversity with other populations on a broader geographic scale would be required to make firm conclusions, these results indicate that the Arctic char populations of Maine originated from the same ancestral group, which recolonized all of the study lakes as they became accessible following the retreat of the Wisconsin glaciation. These populations would then have become physically isolated from one another due to geological and environmental changes and evolved distinct allelic compositions through genetic drift and mutation.

The view that all Arctic char populations in Maine descended from a single Laurentian lineage

TABLE 6.—Hierarchical analysis of molecular variance (equal distances among alleles) among populations from the Union, Penobscot, and St. John River drainages.

Source of variation	df	Percentage of variation	Fixation indices	P
Among drainages	2	3.57	0.03574	0.02118
Among populations within drainage	6	7.71	0.07994	<0.00001
Within populations	475	88.72	0.11283	<0.00001

is compatible with Wilson et al. (1996) and Brunner et al. (2001), but it contradicts the conclusion of Kornfield and Kircheis (1994) that the silver char from Floods Pond has a distinct evolutionary lineage. Kornfield and Kircheis' (1994) interpretation was based on the observation that Floods Pond possessed a unique mtDNA banding pattern for one restriction enzyme not observed in fish from any other sampling locality. However, these authors surveyed a limited number of lakes, including only three from Maine. In a more extensive survey of mtDNA variation based on sequence analysis, Brunner et al. (2001) recently observed the apparently unique haplotype of Floods Pond in Penobscot Lake, Maine. Hence, the fixation of a single mtDNA haplotype in silver char reported by Kornfield and Kircheis (1994) appears to be the result of random genetic drift rather than the long-term evolution of a char lineage distinct from that of all other Maine populations.

Floods Pond was unique in the present study in that it was the only lake where a highly significant deficit in heterozygotes was observed across loci. A factor commonly invoked to explain heterozygote deficiency at microsatellite loci is the occurrence of null alleles, which may be due to a failure to amplify large alleles and/or primer mismatch. We cannot entirely rule out this possibility. Nevertheless, the existence of null alleles would be more likely to generate heterozygote deficiency across many populations at a given locus than across many loci within a given population, as was observed here. An alternative and more likely explanation is that the heterozygote deficiency in Floods Pond is the consequence of population admixture (Walhund's effect). Although not clearly documented in North American lakes, the occurrence of genetically differentiated sympatric Arctic char populations is relatively common in Eurasian lakes (Hindar 1994; Jonsson and Jonsson 2001). Moreover, the occurrence of sympatric populations in another salmonid, the lake whitefish *Coregonus clupeaformis*, is documented in other Maine lakes (Bernatchez and Dodson 1990; Pigeon et al. 1997). Therefore, further investigation into the possible existence of sympatric Arctic char populations in Floods Pond is warranted.

Implications for Conservation and Management

Our results have several implications for the conservation and management of Maine's Arctic char. First, the results of microsatellite analysis are compatible with those of mtDNA studies that indicate by reciprocal monophyly that all populations from Maine belong to a single Laurentian lineage that evolved independently from other evolutionary groups of Arctic char found elsewhere in the United States (Wilson et al. 1996; Brunner et al. 2001). As such, all Arctic char from Maine should be considered a distinct ''evolutionarily significant unit'' (ESU) as defined by Moritz (1994). Second, the previous conclusion of Kornfield and Kircheis (1994) that ''all blueback char populations may be viewed as genetically homogeneous and do not justify individual protection'' needs to be revised. Our results indicate that evolutionary forces acting independently on Arctic char from different lakes led to the evolution of unique genetic compositions for most populations. Admittedly, this conclusion must be taken cautiously since it is derived from patterns observed at neutral loci and some samples were very limited in size. Yet the observation of genetic divergence, coupled with distinctive phenotypes for several of these populations, suggests that they may have independently evolved unique adaptations. For example, the dwarf char found in Green Lake (Kircheis 1985) have adapted to a very deep (>60 m), benthic environment where they spawn in midsummer, while those in Wadleigh Pond thrive in a shallow (approximately 15 m) lake where they lead a pelagic lifestyle and spawn in late fall. The small, silvery char in Wassataquoik Lake contrast sharply with those of similar size in Big Black Pond, which retain their bright-orange spawning colors in the paired fins all year. Thus, each Arctic char population from Maine should be treated as a separate management unit in order to maximize the preservation of genetic variance within this ESU (Fraser and Bernatchez 2001). Consequently, Maine

fisheries managers should continue to protect these populations from genetic degradation, excessive competition, overfishing, and introductions (Kircheis 1981, 1989). Furthermore, as these 12 populations represent the only ones of Laurentian lineage, protecting them justifies a conservative approach in order to avoid their extirpation in the United States. However, given that all these populations are significantly divergent from each other but that no one stands out as being particularly unique genetically (or as belonging to a distinct evolutionary group), no single population should require special protection based on its genetic makeup alone. If all 12 populations are taken to be equally unique, then priority for protection should be given to those that are particularly threatened by human impact (Bernatchez 1995).

The long-term survival of the silver char population from Floods Pond may be most at risk because the lake is the main source of domestic water in a region of rapid population growth. The other Maine char populations occur in undeveloped areas. Therefore, the Floods Pond population may be considered the most threatened and should be given priority for preservation. Based on the belief that this population represents a unique evolutionary lineage, the Maine Char Management Plan required the introduction of five new populations of silver char from Floods Pond. However, this may not be appropriate since both the genetic and phenotypic uniqueness of the Floods Pond population is most likely the product of local genetic drift and adaptation. Thus, it is likely that fish from Floods Pond that are dispersed to other lakes will diverge away from their founding population by the same processes. In this context, the most rational approach is to conserve the genetic integrity of the silver char by preventing the demographic decline in Floods Pond rather than transplanting them to other lakes.

Acknowledgments

Assistance with sample collections was provided by C. Adams, D. Basley, J. Boland, D. Cote, S. King, D. Page, R. Saunders, I. Sikorsky, and J. Trial. We are also grateful to S. Martin for technical assistance. Financial assistance for this study was provided by the Bradford Camps (Ashland, Maine), the Webber Oil Co. (Bangor, Maine), the Maine Department of Inland Fisheries and Wildlife, the Ontario−Quebec science exchange program, and the Maine Outdoor Heritage Fund.

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