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HOLARCTIC PHYLOGEOGRAPHY OF ARCTIC CHARR (*SALVELINUS ALPINUS* L.) INFERRED FROM MITOCHONDRIAL DNA SEQUENCES

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Abstract.—This study evaluated mitochondrial DNA (mtDNA) sequence variation in a 552-bp fragment of the control region of Arctic charr *(Salvelinus alpinus)* by analyzing 159 individuals from 83 populations throughout the entire range of the complex. A total of 89 (16.1%) nucleotide positions were polymorphic, and these defined 63 haplotypes. Phylogenetic analyses supported the monophyly of the complex and assigned the observed haplotypes to five geographic regions that may be associated with different glacial refugia. Most notably, a formerly defined major evolutionary lineage *(S. a. erythrinus)* ranging from North America across the Arctic archipelago to the Eurasian continent has now been partitioned into the Arctic group and the newly identified Siberian group. The Beringian group, formed entirely by specimens assigned to *S. malma* (Dolly Varden), encompassed the area formerly assigned to *S. a. taranetzi.* The latter, due to a unique haplotype, became the basal member of the Arctic group. Overall, the *S. alpinus* complex reflects divergent evolutionary groups coupled with shallow intergroup differentiation, also indicated by an analysis of molecular variance that attributed 73.7% ($P < 0.001$) of the total genetic variance among groups. Time estimates, based on sequence divergence, suggest a separation of the major phylogeographic groups during early to mid-Pleistocene. In contrast, colonization of most of today's range started relatively recently, most likely late Pleistocene during the last retreat of ice sheets some 10,000–20,000 years ago. This time scale obviously is too shallow for detecting significant variation on a smaller scale using mtDNA markers. However, other studies using nuclear microsatellite DNA variation strongly suggested ongoing evolution within groups by revealing strong population-genetic substructuring and restricted gene flow among populations. Thus, Arctic charr could serve as a model organism to investigate the linkage between historical and contemporary components of phylogeographic structuring in fish, and, with a global perspective of the distribution of genetic variation as a framework, meaningful comparisons of charr studies at a smaller geographic scale will now be possible.

Key words.—Arctic charr, control region, mitochondrial DNA, phylogeography, *Salvelinus alpinus*.

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Pleistocene glaciations had enormous impacts on the phylogeographic structure and evolution of circumpolar biota (Hewitt 1996). Habitat and range alterations forced species into refugia during colder conditions and allowed recolonization and dispersal during warmer interglacials (Bernatchez and Dodson 1991; O'Reilly et al. 1993; Wilson et al. 1996). Primarily, historic patterns of vicariance and dispersal (combined with current ecological interactions) govern the geographic distribution of genetic diversity in polar taxa. Whereas geographic isolation, genetic drift, or selection for local adaptation contribute to pronounced intraspecific phylogeographic structure, gene flow retards the genetic divergence of populations (Avise et al. 1987). Compared to more southerly regions, formerly glaciated polar regions are younger, having only recently been open for recolonization, and are therefore further from an equilibrium condition (Bernatchez and Wilson 1998). Thus, taxa primarily inhabiting these areas are ideal model organisms to study forces that shaped their population genetics and phylogeography.

Despite the tremendous impact of the Pleistocene glaciations, few studies have examined the genetic differentiation and phylogeography of taxa at large geographic scales. The Arctic charr (*Salvelinus alpinus;* Teleostei: Salmonidae) is particularly suited to address questions relating to impacts of population bottlenecks, genetic and morphological divergence in isolated refugia, and subsequent dispersal and gene flow. This salmonid has a Holarctic distribution (Behnke 1972) and is adapted and restricted to cold-water habitats, resulting in a distribution at higher latitudes than any other freshwater or anadromous fish. Consequently, the range of this pioneer species was repeatedly impacted during Pleistocene glaciations as it colonized habitats created by advancing or retreating ice margins (Johnson 1980). A second motivation for this study was the complex mosaic of variability in morphology, coloration, and ecology that Arctic charr exhibits throughout its range. For example, Arctic charr morphs in Thingvallavatn (the largest lake in Iceland) fall into a benthic type and a limnetic type that show distinct differences in trophic morphology, life history, reproductive behavior, and habitat use (Hindar and Jonsson 1982; Skúlason et al. 1989; Malmquist et al. 1992). Arctic charr also show varying degrees of anadromy, forming both landlocked resident populations in freshwater and anadromous populations that may exist sympatrically (Nordeng 1983). Because taxonomic recognition is based primarily on these characters, systematic and evolutionary scenarios to explain diversity in this species complex have been controversial (Behnke 1980; Savvaitova 1980; Nyman 1989). For example, in their field-guide for freshwater fishes of Europe, Ladiges and Vogt

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(1979) list 21 subspecies and forms of Arctic charr (see also Kottelat 1997, pp. 144–156). Many of these phenotypically and ecologically distinct populations live in sympatry (Behnke 1972). The situation has been further complicated by an unusual low degree of genetic variation among morphs, distant populations, and even subspecies (allozymes: Kornfield et al. 1981; Andersson et al. 1983; RFLP and sequence analysis of mtDNA: Danzmann et al. 1991; Hartley et al. 1992; Volpe and Ferguson 1996; minisatellite fingerprinting: Volpe and Ferguson 1996; but see Brunner et al. 1998).

To date, deeper phylogenetic relationships in the *S. alpinus* complex have been obscured by a lack of comparative genetic studies throughout the Northern Hemisphere, and by the use of genetic markers with low resolving power. The evolution of molecular genetic techniques during the last two decades, particularly analysis of mitochondrial DNA (mtDNA: Moritz et al. 1987; Meyer 1993) has substantially contributed to an understanding of natural genetic diversity and speciation issues (Avise 1994; Templeton 1994). This is especially helpful in such puzzling groups as salmonids, which show a mosaic of morphological and ecological traits. This study presents a Holarctic survey covering the whole range of the *S. alpinus* complex based on sequence variation of the fast evolving mitochondrial control region. The general goals are, first, to provide a global atlas of mtDNA diversity in the *S. alpinus* complex. Such comparisons are essential, otherwise restricted results from previous observations cannot be placed within a more general evolutionary framework, and thus must remain anecdotal. Second, to evaluate congruence of genetic findings with evolutionary lineages and current taxonomy based on morphological and zoogeographic evidence. Finally, in addition to studies from other Holarctic taxa such as shorebirds (Wenink et al. 1996) or passively dispersed *Daphnia* (Weider et al. 1999), this study can serve as a model for an actively dispersed freshwater and anadromous Holarctic taxa.

Current Taxonomic Status of the Salvelinus alpinus Complex

Although biologists disagree on many aspects of charr phylogeny and classification, the monophyly of the genus is supported both morphologically (Sanford 1990) and genetically (Grewe et al. 1990). Three distinct taxa are generally recognized: brook charr *(Salvelinus fontinalis),* lake charr *(S. namaycush),* and the Arctic charr *(Salvelinus alpinus)* species complex. The first two are endemic to North America, where they diverged from a common ancestor during the Pliocene (Behnke 1972). Phylogenetic relationships within *Salvelinus* are controversial (Behnke 1980; Savvaitova 1980). Some consider the Far Eastern Japanese charr *(S. leucomaenis)* as an additional, distinct taxon and the probable sister group to well-characterized North American species (i.e., *S. fontinalis* and *S. namaycush*). This hypothesis is supported by chromosomal (Phillips et al. 1989), rDNA (Phillips and Pleyte 1991), and morphological data (Stearley and Smith 1993). The Arctic charr complex, which is circumpolar in distribution, includes all remaining members of the genus and would form the sister taxon to these. It is here that most taxonomic confusion exists.

Behnke (1984) postulated an early Pleistocene divergence into Arctic charr *(S. alpinus)* and the Dolly Varden *(S. malma)* groups. The latter lives on the eastern and western sides of the Pacific Ocean. Within Arctic charr, Behnke (1980, 1984) recognized three evolutionary lineages: (1) European, containing northern *S. a. alpinus* and central *S. a. salvelinus;* (2) Arctic, consisting of the vast and diverse *S. a. erythrinus* group (from Asiatic drainages as well as those from North America east of the Mackenzie River); (3) and Eastern Asiatic/Western North America, containing *S. a. taranetzi* (Fig. 1). This Arctic charr from Chukotsk and Bering Seas is considered a distinct form, based on its peculiar coloration (Behnke 1984). It is the only widely accepted subspecies from Central and Eastern Asia, whereas numerous other nominate species and subspecies from this region are controversial. An additional group, based on evidence from allozyme and restriction fragment length polymorphism (RFLP) of mtDNA studies (Kornfield et al. 1981; Kornfield and Kircheis 1994), has been designated *S. a. oquassa* and contains disjunct relict populations in Maine and southern Quebec.

Materials and Methods

Eighty-three populations representing at least 11 named species and subspecies were sampled from the entire geographic range of the *S. alpinus* complex (Fig. 1). Previous research demonstrates that mtDNA variation in *S. alpinus* is distributed primarily among rather than within populations (Wilson et al. 1996). Therefore, to maximize numbers of populations analyzed, two individuals were usually sequenced per population. Two specimens each from lake trout *(S. namaycush)* and Japanese charr *(S. leucomaenis)* served as an intraspecific comparison. Brook charr *(S. fontinalis)* was used as the outgroup because it is considered the most basal taxon in the genus (Grewe et al. 1990; Crane et al. 1994). Exact sampling localities, taxonomic designation, and sample sizes are presented in the Appendix. Samples labeled AP1–13 reflect populations sampled in Brunner et al. (1998). North American samples AL1–2, CD6, CD8–10, CD13–14, CD17, MN4, and MN7 are from Wilson et al. (1996). Osinov et al. (1996) recently described samples FI2–3, NO3, NO5–8, and TM1–4, whereas Labrador samples CD1 and CD4–6 are from Bernatchez et al. (1998). Re-analysis of these samples by direct sequencing will allow comparison with results from previous studies using other genetic markers.

Total genomic DNA was extracted from liver or muscle using a proteinase K/phenol-chloroform procedure with subsequent ethanol precipitation (described in Bernatchez et al. 1992). Mitochondrial DNA variation was analyzed by sequencing performed on products amplified via polymerase chain reaction (PCR), as outlined by Bernatchez et al. (1992). Primers used to amplify the entire control region were HN20 (5′-GTG TTA TGC TTT AGT TAA GC-3′), and Tpro2 (5′-ACC CTT AAC TCC CAA AGC-3′), located in the phenylalanine and proline tRNAs, respectively. Low-melting-point agarose gel purification of the double-stranded PCR product followed instructions from a commercial kit (QIAquick, QIAGEN, Inc., Valencia, CA). DNA sequences were generated directly using an Applied Biosystems, (Foster City, CA) automated sequencer. All DNA was sequenced in the

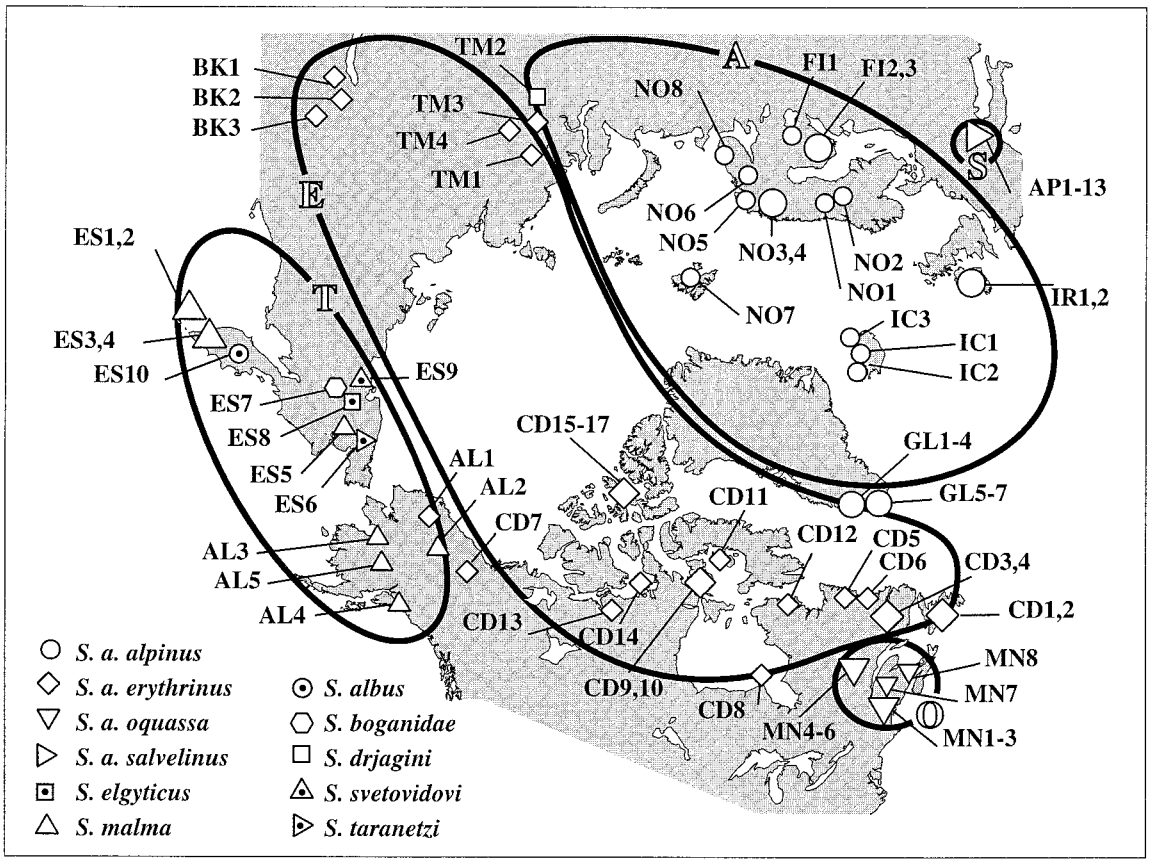

FIG. 1. Sample localities for Arctic charr *(Salvelinus alpinus)*. For exact sample sites and sample sizes, see Appendix. Gray lines denote putative ranges of the major evolutionary lineages recognized by Behnke (1984): A, *S. alpinus alpinus;* E, *S. a. erythrinus;* O, *S. a. oquassa;* S, *S. a. salvelinus;* T, *S. a. taranetzi*. Larger symbols represent multiple sample sites.

forward direction. Additionally, one representative sample for each haplotype was also sequenced in the reverse direction to assure the accuracy of nucleotide assignments. DNA sequences were aligned by eye with the multiple sequence editor CLUSTAL V (Higgins and Sharp 1988).

Mitochondrial DNA polymorphism within groups was estimated as haplotypic (nucleon) diversity (*h;* Nei and Tajima 1981), nucleotide diversity, (π, Nei 1987), and as net percent sequence divergence between haplotypes. The proportion of genetic diversity within and among the phylogeographic groups identified in the neighbor-joining tree was estimated with an analysis of molecular variance (Arlequin, vers. 2.0; Schneider et al. 2000). Three different methods to assess phylogenetic relationships were compared. A neighbor-joining tree was constructed based on Kimura's two-parameter model (transversions were empirically weighted 1.7 to transitions) with MEGA version 2.0 (Kumar et al. 1994). Parsimony and maximum-likelihood analyses were conducted with PAUP version 4.0 (Swofford 1999). To test for statistical significance of the generated trees, data were resampled 1000 times to obtain bootstrap *P*-values (Felsenstein 1985). Finally, Mantel tests were performed using the R-Package version 4.0 (P. Casgrain, Univ. de Montreal) to determine possible associations between geographic distance (km) and genetic distance (net sequence divergence) among sampling regions.

RESULTS

For each of 159 Arctic charr individuals, 552 bp of mtDNA sequence from the 5′ end of the control region were obtained. Except for seven populations, all individuals from the same population had identical sequences (Appendix). All observed variation was in the form of single base-pair substitutions. A total of 89 (16.1%) nucleotide positions were polymorphic, and these defined 63 haplotypes (Fig. 2). The most divergent haplotypes (ARC13 and ACD5) differed by 30 substitutions (5.4%). Alignment of Arctic charr sequences with those of *S. fontinalis* revealed variability at an additional 27 nucleotide positions. Twenty-four of these underwent single base-pair substitutions and three were in the form of single base-pair additions/deletions. Average sequence divergence between genotypes of the Arctic charr complex and those of congeners was 5.82% (from *S. fontinalis*), 4.26% (from *S. namaycush*), and 3.99% (from *S. leucomaenis*), respectively. Although most haplotypes show restricted distributions, some have dispersed across immense geographic distances. For example, haplotype ATL8 is found in Scandinavia, Ireland, and also across the Atlantic ocean in Labrador and New Foundland. On the North American continent, haplotype ARC4 spans the Arctic from Cornwallis and King William Islands eastward to Greenland.

The three methods of phylogenetic inference produced

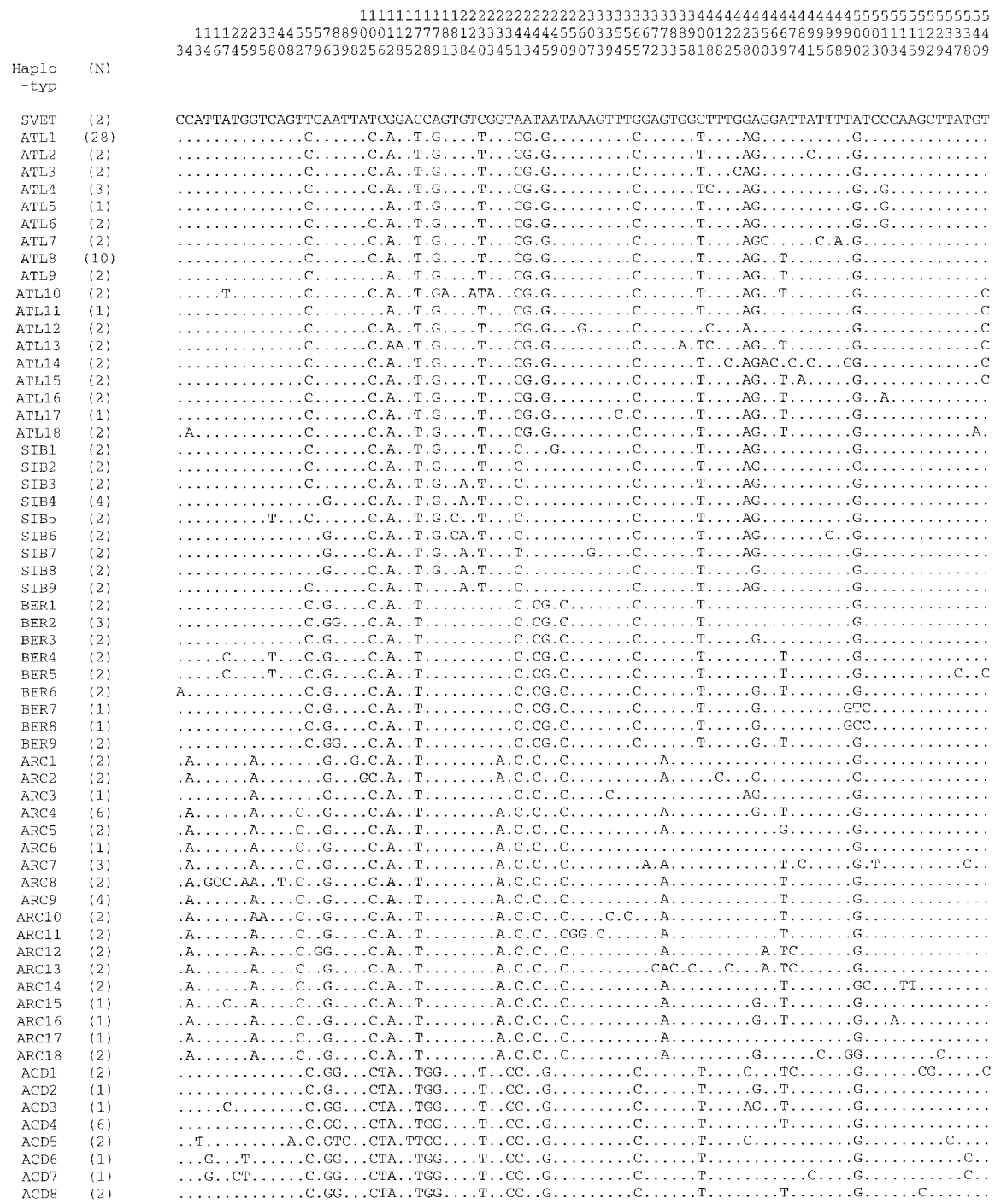

FIG. 2. Mitochondrial sequence variation from 552 bp of the control region of 159 specimens from the *Salvelinus alpinus* complex. Sites are numbered according to their position in the control region sequence of *Oncorhynchus mykiss* (rainbow trout) as given by Shedlock et al. (1992).

trees with identical topologies and similar bootstrap values. Figure 3 shows the neighbor-joining tree for the *Salvelinus* haplotypes. Although placed in the *S. alpinus* complex by Behnke (1980), *S. leucomaenis* is closer to *S. namaycush* than *S. fontinalis*, thus corroborating previous genetic and morphological studies (Phillips et al. 1989; Phillips and Pleyte 1991; Stearley and Smith 1993). The other *S. alpinus* haplotypes show a combination of divergent evolutionary groups and shallow intergroup differentiation. They form a relatively well-supported monophyletic clade, as indicated by bootstrap values varying from 87% to 90% depending on the clustering method. Within this clade, phylogenetic analyses assigned haplotypes to five major lineages that relate to geographic regions, hereafter referred to as Atlantic (northern and central Europe, Atlantic islands, Labrador, and Newfoundland), Siberia (Baikal region to Taimyr Peninsula), Bering (eastern

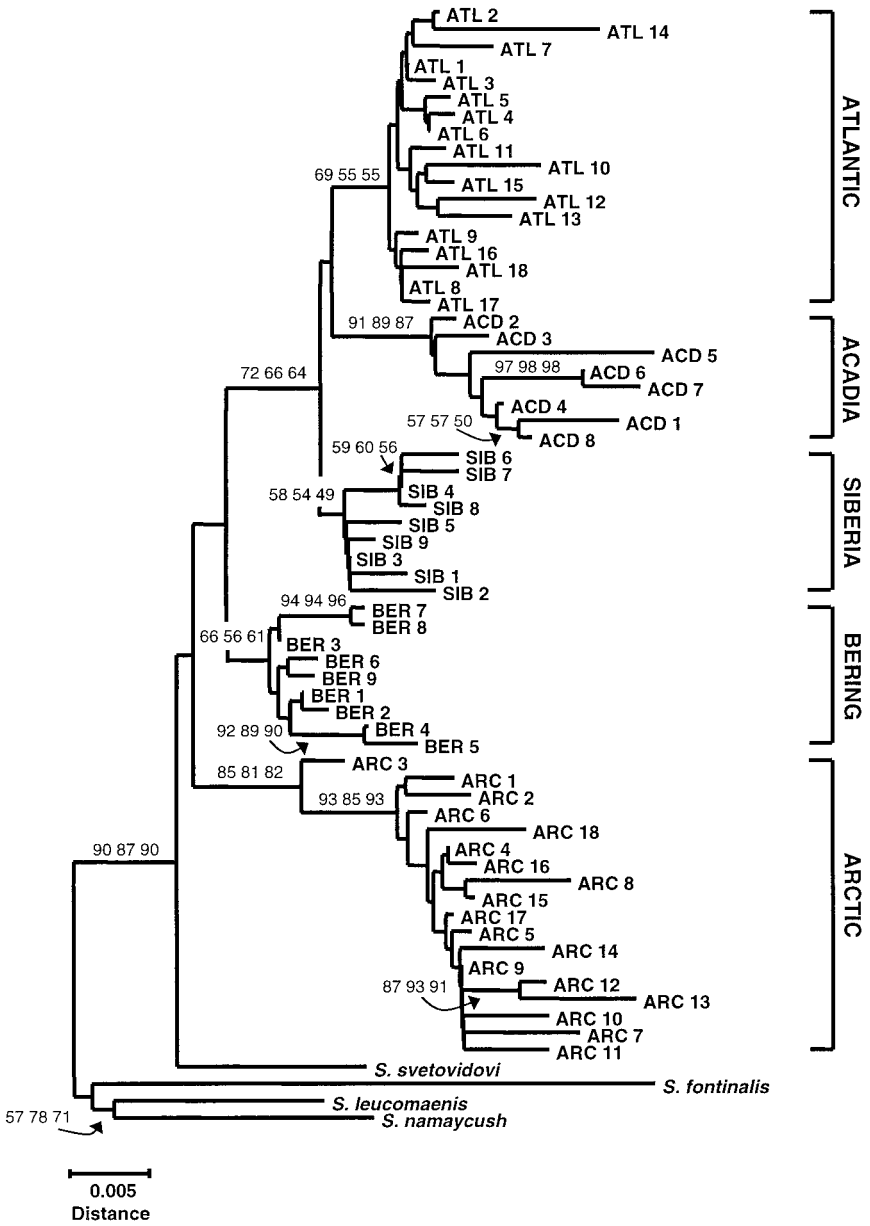

FIG. 3. Neighbor-joining tree summarizing geographic locations and relationships among 63 haplotypes detected in 159 individual *Salvelinus alpinus* and three outgroups. Numbers are bootstrap values computed by 1000 replications using Kimura's two-parameter distance, followed by values obtained with maximum parsimony and by values using the maximum-likelihood clustering method.

Siberia and western Alaska), Arctic (Canadian Arctic and northern Alaska), and Acadia (southern Quebec and New England; Table 1; Fig. 3). Analysis of molecular variance indicates that 73.7% ($P < 0.001$) of the total genetic variance is distributed among regions, supporting strong geographic structuring of mtDNA polymorphism. This substantial lineage sorting between attributed regions suggests that major vicariance events are not recent (Avise et al. 1984). Recently, a morphologically aberrant charr from Lake Elgygytgyn (Chukotka Peninsula) was described as *Salvethymus svetovidovi* (Chereshnev and Skopetz 1990) representing a new genus. Phylogenetic analyses placed haplotype SWET, found only in *Salvethymus svetovidovi,* basal to all Arctic charr, supporting its distinct taxonomic status and reflecting its probable sister-group relationship to the *S. alpinus* complex (Fig. 3).

Diversity values are summarized by sample localities and major geographic regions in Table 2. The greatest haplotype diversity (i.e., $n = 16$, $h = 0.952$) was observed among the Arctic samples, whereas extensive sampling revealed only three haplotypes in the Alpine region of Europe ($h = 0.808$). In terms of nucleotide differences per site π, or nucleotide diversity, greatest values were calculated for the Arctic group ($\pi = 0.0093$), while Atlantic had the lowest value ($\pi = 0.0047$). Members of two lineages can also be geographically interspersed in contact regions. Bering and Arctic lineages are interspersed on the Kamchatka Peninsula in eastern Siberia and Acadian and Arctic lineages in Greenland and Lab-

TABLE 1. Geographic distribution of 63 observed mitochondrial DNA haplotypes in the *Salvelinus alpinus* complex. Sample sizes are given below their respective locale abbreviation, as defined in the Appendix.

	Sample locations											
Haplo-type	AP (26)	IR (4)	NO (16)	FI (8)	IC (6)	GL (9)	TM (8)	BK (6)	ES (17)	AL (9)	CD (35)	MN (16)
ATL1	22	—	6	—	—	1	—	—	—	—	—	—
ATL2	2	—	—	—	—	—	—	—	—	—	—	—
ATL3	2	—	—	—	—	—	—	—	—	—	—	—
ATL4	—	—	—	—	3	—	—	—	—	—	—	—
ATL5	—	—	—	—	1	—	—	—	—	—	—	—
ATL6	—	—	—	—	2	—	—	—	—	—	—	—
ATL7	—	—	2	—	—	—	—	—	—	—	—	—
ATL8	—	4	4	—	—	—	—	—	—	—	2	—
ATL9	—	—	—	—	—	2	—	—	—	—	—	—
ATL10	—	—	—	—	—	1	—	—	—	—	—	—
ATL11	—	—	—	—	—	1	—	—	—	—	—	—
ATL12	—	—	—	2	—	—	—	—	—	—	—	—
ATL13	—	—	—	2	—	—	—	—	—	—	—	—
ATL14	—	—	2	—	—	—	—	—	—	—	—	—
ATL15	—	—	—	—	—	—	—	—	—	—	2	—
ATL16	—	—	—	—	—	—	—	—	—	—	2	—
ATL17	—	—	—	—	—	—	—	—	—	—	1	—
ATL18	—	—	—	—	—	—	—	—	—	—	2	—
SIB1	—	—	—	2	—	—	—	—	—	—	—	—
SIB2	—	—	—	2	—	—	—	—	—	—	—	—
SIB3	—	—	2	—	—	—	—	—	—	—	—	—
SIB4	—	—	—	—	—	—	2	2	—	—	—	—
SIB5	—	—	—	—	—	—	2	—	—	—	—	—
SIB6	—	—	—	—	—	—	—	2	—	—	—	—
SIB7	—	—	—	—	—	—	2	—	—	—	—	—
SIB8	—	—	—	—	—	—	—	2	—	—	—	—
SIB9	—	—	—	—	—	—	2	—	—	—	—	—
BER1	—	—	—	—	—	—	—	—	2	—	—	—
BER2	—	—	—	—	—	—	—	—	3	—	—	—
BER3	—	—	—	—	—	—	—	—	2	—	—	—
BER4	—	—	—	—	—	—	—	—	2	—	—	—
BER5	—	—	—	—	—	—	—	—	2	—	—	—
BER6	—	—	—	—	—	—	—	—	—	2	—	—
BER7	—	—	—	—	—	—	—	—	—	1	—	—
BER8	—	—	—	—	—	—	—	—	—	1	—	—
BER9	—	—	—	—	—	—	—	—	—	2	—	—
SVET	—	—	—	—	—	—	—	—	2	—	—	—
ARC1	—	—	—	—	—	—	—	—	2	—	—	—
ARC2	—	—	—	—	—	—	—	—	2	—	—	—
ARC3	—	—	—	—	—	—	—	—	1	—	—	—
ARC4	—	—	—	—	—	2	—	—	—	—	4	—
ARC5	—	—	—	—	—	—	—	—	—	2	—	—
ARC6	—	—	—	—	—	—	—	—	—	—	1	—
ARC7	—	—	—	—	—	—	—	—	—	—	3	—
ARC8	—	—	—	—	—	—	—	—	—	—	2	—
ARC9	—	—	—	—	—	—	—	—	—	—	4	—
ARC10	—	—	—	—	—	—	—	—	—	—	2	—
ARC11	—	—	—	—	—	—	—	—	—	—	2	—
ARC12	—	—	—	—	—	—	—	—	—	—	2	—
ARC13	—	—	—	—	—	—	—	—	—	—	2	—
ARC14	—	—	—	—	—	—	—	—	—	—	2	—
ARC15	—	—	—	—	—	1	—	—	—	—	—	—
ARC16	—	—	—	—	—	1	—	—	—	—	—	—
ARC17	—	—	—	—	—	—	—	—	—	1	—	—
ARC18	—	—	—	—	—	—	—	—	—	—	2	—
ACD1	—	—	—	—	—	—	—	—	—	—	—	2
ACD2	—	—	—	—	—	—	—	—	—	—	—	1
ACD3	—	—	—	—	—	—	—	—	—	—	—	1
ACD4	—	—	—	—	—	—	—	—	—	—	—	6
ACD5	—	—	—	—	—	—	—	—	—	—	—	2
ACD6	—	—	—	—	—	—	—	—	—	—	—	1
ACD7	—	—	—	—	—	—	—	—	—	—	—	1
ACD8	—	—	—	—	—	—	—	—	—	—	—	2

TABLE 2. Values for haplotype diversity (h) and nucleotide diversity (π) of Arctic charr populations grouped by sampling locations and the five major phylogeographic groups identified in Figure 3. Mantel tests examined the association between geographic distance (km) and genetic distance (% sequence divergence).

Location (phylogeographic group)	N	Number of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Mantel test		
					z	t	P
Alpine Europe (AP)	26	3	0.283	0.0005	11.50	0.935	0.175
Ireland (IR)	4	1	—	—	—	—	—
Norway (NO)	16	5	0.835	0.0069	146.94	0.117	0.454
Finland (FI)	8	4	0.857	0.0096	21.98	1.547	0.061
Iceland (IC)	6	3	0.733	0.0017	1.44	−0.944	0.173
Greenland (GL)	9	7	0.944	0.0161	2.07	0.049	0.481
Taimyr region (TM)	8	4	0.889	0.0074	12.53	−0.286	0.387
Baikal region (BK)	6	3	0.800	0.0029	4.25	2.146	0.016
Eastern Siberia (ES)	17	9	0.925	0.0125	714.79	3.549	<0.000
Alaska (AL)	9	6	0.917	0.0140	146.91	2.325	0.010
Arctic Canada (CD)	35	16	0.956	0.0198	6736.46	6.484	<0.000
Maine region (MN)	16	8	0.850	0.0087	120.99	1.199	0.115
Atlantic (ATL)	68	18	0.808	0.0047	9372.38	2.143	0.016
Siberia (SIB)	20	9	0.926	0.0057	648.40	1.149	0.125
Bering (BER)	17	9	0.934	0.0065	706.02	4.488	<0.000
Arctic (ARC)	38	18	0.952	0.0093	4648.66	1.417	0.078
Acadia (ACD)	16	8	0.850	0.0087	120.99	1.199	0.115
Total	159	63	0.959	0.0203	358,300.0	6.485	<0.000

rador (Table 1; Fig. 4). The presence of different lineages in these regions is also reflected by high nucleotide diversity. Overall haplotype and nucleotide diversities in Arctic charr were $h = 0.947$ and $\pi = 0.0209$, respectively.

Mantel tests revealed associations between geographic distances (km) and genetic distances (net sequence divergence) were significant for four (of 11) sampling locations (i.e., Baikal, eastern Siberia, Alaska, and Arctic Canada) and for two (of five) phylogeographic groups (i.e., Atlantic and Bering; Table 2).

DISCUSSION

Mitochondrial DNA Diversity and Population Structure

In fishes, tempo and mode of control region evolution may vary considerably among different lineages, nevertheless, it has proven well suited to examine relationships among populations (Bernatchez et al. 1992), species (Shedlock et al. 1992), and genera (Sturmbauer and Meyer 1992; Lee et al. 1995). In this study, mutation sites were distributed unevenly in *S. alpinus;* several regions of conserved sequence were observed. The most obvious is a 100–150-bp region starting approximately at nucleotide position 250 (Fig. 2). This pattern corroborates the observation of a ''central conserved region'' across several other teleost families (Lee et al. 1995). Genetic diversity at the variable parts of the control region of *Salvelinus* was much higher than those found in previous studies using other genetic markers. For instance, mean genetic divergence between North American Arctic populations was 0.79 ± 0.39% compared to 0.11 ± 0.04% detected in the same samples using RFLP analysis (Wilson et al. 1996). Similarly, mtDNA diversity observed in samples from the Taimyr, Norway, and Finland region largely exceed values reported in allozyme studies (Osinov et al. 1996). Thus, results presented here allow a more detailed examination of relationships within the Arctic charr complex than provided by previous studies.

Despite the improved resolution, our data allow only qualitative conclusions regarding population structure. Although there is a presumably high mutation rate in the control region, there may be little microgeographic and intrapopulation variation. All samples from the same population, except three, yielded identical sequences. Also, despite extensive sampling, only three haplotypes were detected in the Alpine region of Europe, resulting in the lowest diversity values of all sampled regions (Table 2). The Alpine region has been ice-free for only the last 10,000–20,000 years (Hantke 1978). Thus, it can be argued that reduced genetic variability is the result of recent recolonization from a genetically depauperate glacial refuge or a founder effect. However, examination of the same samples using microsatellite DNAs showed significant hydrogeographic structure and among-population differentiation (Brunner et al. 1998). Bernatchez et al. (1998) reported similar differentiation among anadromous charr populations from Labrador, indicating reduced gene flow due probably to hatchery site homing behavior and limited dispersal at sea. Present-day genetic structure is a result of both historical (e.g., Pleistocene glaciations) and contemporary components (such as natal homing). Therefore, Arctic charr (and taxa with similar ecological features, such as homing of shorebirds to breeding sites; Wenink et al. 1996) are particularly suited to address phylogeographic questions. These ecological features limit contemporary gene flow among populations, a prerequisite for maintaining historical geographic structuring over time and space even when physical barriers are absent or permeable.

Phylogeography and Dispersal

The most pronounced phylogeographic pattern in Arctic charr is the resolution of five primary lineages corresponding

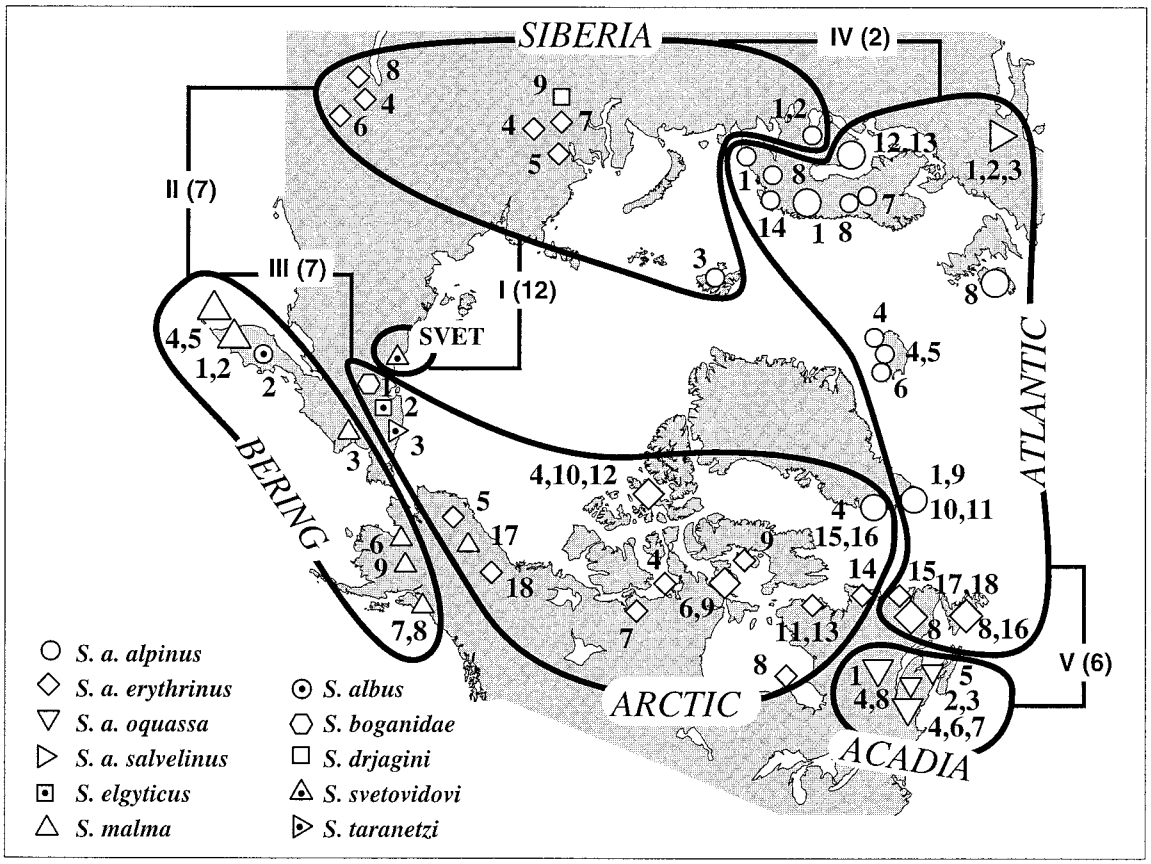

FIG. 4. Holarctic distribution of 63 mitochondrial haplotypes found in the Arctic charr *(Salvelinus alpinus)* complex. For clarity, the superimposed parsimony network is linking only the major phylogeographic groups identified in this study (see also Fig. 3). Minimum number of mutations between groups are given in parentheses, and roman numerals denote linked haplotypes: I, SVET-SIB2; II, SIB2-BER3; III, BER3-ARC3; IV, SIB2-ATL1; V, ATL8-ACD2. An alternative configuration linking the Atlantic with the Bering phylogeographic group (ATL1-BER3) cannot be eliminated but is discounted based on geographical considerations and estimates of separation times (see text). See Appendix for further details on exact sample sites and attributed haplotypes.

to distinct regions of the Northern Hemisphere. Further, it is clear that these five lineages have been modified by the idiosyncrasies of geography and climate within each region.

Individuals of the Atlantic phylogeographic lineage are not restricted to continental Europe but are spread across the Atlantic to the North American continent. Assuming no contemporary trans-Atlantic migrations, this pattern is consistent with the conclusion lineage extinctions are dramatically slowed in an expanding population (Avise et al. 1984). Ice cover during the Late Wisconsinan maximum spanned the North Atlantic and could have provided reduced salinity for trans-Atlantic dispersers. The positive correlation in the Atlantic clade between genetic divergence and geographic distances implies isolation by distance and supports the scenario of an Atlantic-island-hopping dispersal from the edge of a rapidly expanding source population. The recent nature of this dispersal event is further supported by the occurrence of haplotype ATL1 in several populations of Scandinavia, Greenland, and in the landlocked populations of the central Alpine region of Europe. The latter region was completely covered by ice until 10,000–20,000 years ago (Hantke 1978), and this time frame is congruent with the ice retreat in Labrador (11,000 years ago; Dyke and Prest 1987) that would have enabled establishment of charr from Arctic refugia. This scenario is consistent with the recent colonization of the Atlantic Basin from the Arctic, as suggested for sticklebacks (Ortí et al. 1994). An almost identical pattern was observed in migratory shorebirds. Wenink et al. (1996) reported that a haplotype ubiquitous in continental Europe was also present in a Greenland population. In spite of an otherwise strong global phylogeographic structuring, they advocated a relatively recent expansion of European birds into Greenland.

The Arctic group is characterized by broad distribution as well. However, in contrast to the Atlantic group, it did not cross the North Atlantic to colonize sites in northern Europe. Its easternmost distribution seems to be Greenland, where individuals are found in close geographic proximity to charr belonging to the Atlantic group. This pattern of phylogenetic discontinuity, which is not associated with spatial separation, has generally been attributed to secondary contact (Avise et al. 1987). No significant indices for isolation by distance are present in the Arctic group, suggesting that migrants came repeatedly from a refugial source population rather than from the edge of a constantly expanding population. Again, recent and rapid dispersal is strongly indicated by widespread haplotypes. The presence of Arctic haplotypes in eastern Siberia

can be explained by the Bering land bridge and its associated freshwater habitats; these would have enabled continental exchange even during glacial maxima.

The major phylogeographic groups identified herein may be associated with different glacial refugia. The Mississippian refugium southwest of Lake Erie is generally accepted as the area from which most freshwater fish species dispersed to recolonize the upper North American continent (Briggs 1986). Nevertheless, the widespread northern distribution of haplotype ARC4 may instead suggest the existence of an Arctic island refugium, a hypothesis corroborated by parasite distribution. *Cystidicola stigmatura* is a Mississippian-refuge parasite that dispersed north and infected Arctic charr populations throughout the central Arctic archipelago (Black 1983). The absence of the parasite from Banks and Ellesmere Island, which remained partially unglaciated during Pleistocene, suggests these populations where established prior to the arrival of *C. stigmatura* in the Arctic and probably prior to other infected populations from this region.

The pattern of haplotype distribution in the Acadian group shows a geographically very restricted distribution, quite different from the two previously discussed. A virtually identical pattern (i.e., the Laurentian group) resulted from allozyme analyses of Wilson et al. (1996). Most interestingly, Bernatchez and Dodson (1991) observed a similar restricted distribution in their Acadian race of whitefish, which exhibited high genetic diversity. Diversity in the Acadian group of *Salvelinus* was also high and comparable to that found in the wide-ranging Arctic group. Neither clustering nor analysis of molecular variance revealed a close relationship of Acadian with charr from the Arctic group, suggesting a deeper divergence of these two groups. Despite geographic proximity and the capability of charr to rapidly recolonize new areas, gene flow and mixing of phylogeographic groups have apparently been limited. The reason for this may reside within the ecological plasticity of *S. alpinus* that allows diverse niches to be occupied and different life-history strategies to be adopted (Hindar and Jonsson 1982; Skúlason et al. 1989; Malmquist et al. 1992). Both aspects reduce intraspecific competition and are prerequisites for maintaining reproductive isolation in sympatry.

Bernatchez and Dodson (1991) proposed that the currently submerged Northeastern Banks were a possible coastal refuge for the Acadian whitefish lineage. From this Atlantic refugium, whitefish would have recolonized northward through coastal dispersal, facilitated by low-salinity conditions produced by melting glaciers. Given the species' higher salinity tolerance and similar distribution pattern, this scenario is also readily adoptable for Acadian charr.

The fourth lineage identified within the Arctic charr complex spans the Bering Sea strait. No widely distributed haplotypes were observed in this group, and Mantel tests suggested a strong isolation by distance (Table 2). Assuming a once-continuous distribution across the Bering land bridge, opening of the Bering Sea could have produced independent genetic substructuring on both American and Eurasian continents.

Finally, the newly identified Siberia group that resulted from splitting of the former *S. a. erythrinus* lineage encloses populations from the Baikal and Taimyr region. Its extension to the east and possible contact zones with the Bering and Arctic group cannot be delineated because of sparse sampling in this region. To the west, Siberian haplotypes were found on Spitsbergen and in Finland, in close proximity with Atlantic haplotypes, indicating recent contact with a westerly expanding population. Drainage of postglacial lakes in Eurasia, which formed by the recession of the Scandinavian and Barents Sea Ice Domes, was in a northwesterly direction during late stages of the Pleistocene. *Salvelinus* could have used this pathway to disperse from Siberian and European refugia. A phylogeographically distinct central Siberian group of shorebirds has also been detected (Wenink et al. 1996), contacting the European group further east, on the Yamal Peninsula.

Divergence Times and Congruence of Genetic Findings with Current Taxonomy

Genetic variation revealed in the mtDNA control region did not entirely reflect the magnitude of phenotypic and ecological polymorphism in the *S. alpinus* complex. Patterns on a macrogeographic scale can be recognized, but not all taxa suggested by current taxonomy could be confirmed. Although major groups (as defined by Behnke [1984] and others) could be observed, additional distinct lineages were also identified.

The proposed common lineage for northern European *S. a. alpinus* and central Alpine *S. a. salvelinus* is supported. This is especially interesting when compared to findings of Bernatchez and Dodson (1994) for European whitefish (*Coregonus*). These researchers suggested a separate postglacial colonization of Northern and Central Europe from two allopatric refuge populations. Our results also showed a clear distinction between haplotypes of Acadian (Maine, southern Quebec) and Arctic (Arctic North America, Alaska and Kamchatka Peninsula) *S. alpinus* populations. This supports current taxonomy, which recognizes them as distinct subspecies, *S. a. oquassa* and *S. a. erythrinus*, respectively (Grewe et al. 1990; Danzmann et al. 1991).

Most interestingly, almost identical sequence divergences suggest an ancient supergroup including Acadia, Atlantic, and Siberia that split from the Arctic group, followed by a subsequent split into Acadia and a Eurasian group. Finally, the most recent separation was when the Eurasian group split into Atlantic and Siberia, respectively. A widely used divergence time for mtDNA in bony fishes is 2% sequence divergence between lineages per million years (Bermingham and Avise 1986; Thomas et al. 1986; Grewe et al. 1990). Based on salmonid fossil evidence, Smith (1992) estimated salmonid mtDNA to have diverged at an approximate rate of 1% per million years. This is also the rate applied in the mtDNA RFLP survey in Arctic charr of Wilson et al. (1996), in that resulting time estimates seemed plausible and showed good congruence with Pleistocene events. However, these mutation rates are in fact average estimates of the entire mtDNA molecule, whereas the specific mutation rate for the noncoding control region is generally much faster. In humans it is 8.4% (Vigilant et al. 1989) and comparably high (or higher) mutation rates have also been reported for the control region in fish such as sturgeon (Brown et al. 1993), African cichlids (Fajen and Breden 1992), and sardines (Bowen and

Grant 1997). An important argument in favor of applying a faster molecular clock is that sequence divergences in the control region of Arctic charr are five to ten times higher than those observed (in the same samples) in the RFLP survey by Wilson et al. (1996). According to this molecular clock of 5–10% per million years between lineages, the deepest differentiation between Arctic charr phylogeographic groups (i.e., between Arctic and the supergroup) corresponds to approximately 300,000–700,000 years. While these time estimates must be interpreted with caution, RFLP analysis (Grewe et al. 1990) and morphological/zoogeographical evidence (Behnke 1980) are concordant in indicating that the five phylogeographic groups trace back to a common ancestor within the early to mid-Pleistocene. Thus, these results refute a close relationship between Acadia and Arctic groups, a conclusion also supported by the neighbor-joining tree (Fig. 3) and the parsimonious network (Fig. 4). This contrasts with findings of Bernatchez and Dodson (1991) that suggested a close relationship and recent separation of Acadian and Arctic races of whitefish.

The relationship of North American and Asiatic *S. a. erythrinus* populations was confirmed only for some eastern (Beringian) populations. Behnke's (1980) argument that Taimyr charr and North American Arctic charr share a common ancestry and their continuous distribution was interrupted by the last glacial epoch could not be supported. The Taimyr charr instead clusters with central Siberian charr to form a distinct Siberian group, with an early separation from the Arctic group (see above). Thus, repeated appearance of stereotypic morphologies and ecotypes does not necessarily indicate sister relationship. M. R. Douglas and P. C. Brunner (unpubl. ms) noted that for European whitefish, a species complex that in many aspects parallels the situation in *Salvelinus,* occurrence of identical forms in central Alpine lakes reflects convergent evolution in allopatry. These authors proposed ''phylogenetic constraint'' (sensu Douglas and Matthews 1992), such that radiation can only take place along particular morphological pathways due to structural limitations imposed by a particular ''bauplan.'' Thus, diversification within that bauplan will occur repeatedly, but often along the same trajectories.

Although included in the Arctic cluster, *S. a. taranetzi* exhibits a distinct and unique haplotype (Arctic 3) which makes it a possible sister group to the cluster containing all other Arctic haplotypes. Therefore, Behnke's (1984) suggestion to recognize it as a distinct taxon is supported. Surprisingly, the proposed geographic distribution of *S. a. taranetzi* (Fig. 1) was observed in populations assigned to *S. malma.* All of them cluster together in a distinct Beringian group (Fig. 4). However, relationship amongst the five major phylogeographic lineages remain unresolved. Therefore, the species status of *S. malma* is questionable and it cannot be confirmed as a sister taxon to all other *S. alpinus* (Behnke 1984).

In contrast, *S. leucomaenis* clustered together with *S. namaycush.* These two form the sister group of *S. fontinalis.* All three should be considered as distinct species and are well separated from all other species in the *S. alpinus* complex. A similar pattern of disagreement between taxonomic designation and genetic evidence was observed in the Siberian lineage. Here, populations from central and northern Siberia clustered in a previously undescribed Siberian group. Five subspecies fell within this cluster and all were genetically undifferentiated. Such patterns may be consistent with an evolutionary scenario that assumes major geographic patterns of mtDNA distribution were established first, followed by morphological differentiation and ecological specialization (Riget et al. 1986; Walker et al. 1988) producing an overlying pattern of morphological differentiation among trophic structures (Skúlason et al. 1989; Skúlason and Smith 1995). Therefore, it can generally be argued that traditional characters used for *S. alpinus* taxonomy (such as gill rakers and pyloric caecae) may be affected by natural selection and environmental conditions (Lindsey 1981). The presence of morphological differences within mtDNA lineages is open to alternative interpretations, for instance, the potential of genetically based, localized changes. Due to the recency of colonization of most of today's range, the time scale could be too shallow for accumulating de novo mtDNA mutations through selection or genetic drift.

Conclusion

Analysis of mtDNA diversity has revealed unexpectedly clear phylogeographic patterns in the morphologically and ecologically polymorphic Arctic charr species complex. Unrelated taxa inhabiting areas not affected by Pleistocene glaciations often show uniform, concordant phylogeographies (Avise 1994). In contrast, this study provides evidence that even within a closely allied species complex, unique postglacial histories exist. Thus, Holarctic taxa like *S. alpinus* provide a exceptional opportunity to study those mechanisms that sculpture population genetic structure, such as bottlenecks, dispersal corridors, or reproductive isolation.

Not all of the previously proposed evolutionary lineages and subspecies of charr are corroborated. Two main hypotheses might explain the discrepancy between morphological and genetic findings: (1) current *S. alpinus* taxonomy may bear little relation to evolutionary reality because it is based on homoplasious morphological characters that evolved independently due to phylogenetic constraints; and (2) today's morphological and ecological diversity originated very recently (i.e., after the last Pleistocene glaciation), a time span too short to produce differences detectable with mtDNA sequences.

With this global view as a framework, meaningful comparisons at a smaller scale are now possible. For example, all Arctic haplotypes on Greenland are found in anadromous populations, whereas Atlantic haplotypes are restricted to resident lake populations (Appendix). Could this indicate contemporary gene flow with nearby Baffin Island or the Labrador coast? It would be also interesting to compare mechanisms underlying reproductive isolation and similar distribution pattern of Acadian lineages of *Salvelinus* and whitefish, given their different evolutionary histories, ecologies, and life-history strategies. Clearly, these and related questions have to be addressed using larger sample sizes and markers such as microsatellite DNAs that provide greater resolution on smaller geographic scales (e.g., Bernatchez et al. 1998; Brunner et al. 1998). We hope this study will stim-

ulate ongoing and future projects to test hypotheses about speciation and isolating mechanisms, morphological divergence, and postglacial distribution in this most enigmatic species group.

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Appendix

Population abbreviations (as Fig. 1), current taxonomy, exact sample location, life-history status (landlocked or anadromous), haplotype abbreviations (as Figs. 2, 3, 4) and frequencies, and Genbank (GB) accession numbers for *Salvelinus alpinus* sequences. Genbank accession numbers for the outgroup taxa are: *S. fontinalis*, AF297987; *S. leucomaenis*, AF297988; *S. namaycush*, AF297989.

Pop.	Taxon (Form)	Locality	Status	Haplotype	Freq.	GB
AP1	*Salvelinus alpinus salvelinus*	Thun Lake, Switzerland	landlocked	ATL1	(1)	AF297991
AP2	*S. a. salvelinus*	Brienz Lake, Switzerland	landlocked	ATL2	(2)	AF297992
AP3	*S. a. salvelinus*	Walenstadt Lake, Switzerland	landlocked	ATL1	(1)	AF297991
AP4	*S. a. salvelinus*	Zug Lake, Switzerland	landlocked	ATL1	(2)	AF297991
AP5	*S. a. salvelinus*	Zürich Lake, Switzerland	landlocked	ATL1	(2)	AF297991
AP6	*S. a. salvelinus*	Constance Lake, Switzerland	landlocked	ATL1	(4)	AF297991
AP7	*S. a. salvelinus*	Luzern Lake, Switzerland	landlocked	ATL1	(2)	AF297991
AP8	*S. a. salvelinus*	Lac Neuchâtel, Switzerland	landlocked	ATL1	(1)	AF297991
AP9	*S. a. salvelinus*	Lac Léman, Switzerland	landlocked	ATL1	(3)	AF297991
AP10	*S. a. salvelinus*	Lac Du Bourget, France	landlocked	ATL3	(2)	AF297993
AP11	*S. a. salvelinus*	Königssee, Germany	landlocked	ATL1	(2)	AF297991
AP12	*S. a. salvelinus*	Ammersee, Germany	landlocked	ATL1	(2)	AF297991
AP13	*S. a. salvelinus*	Grundelsee, Austria	landlocked	ATL1	(2)	AF297991
IR1	*S. a. alpinus*	Lough Veagh, Ireland		ATL8	(2)	AF297998
IR2	*S. a. alpinus*	Lough Dunlewy, Ireland		ATL8	(2)	AF297998
IC1	*S. a. alpinus*	Svinavatn, Iceland		ATL4	(1)	AF297994
		Svinavatn, Iceland		ATL5	(1)	AF297995
IC2	*S. a. alpinus*	Vatnshlidarvatn, Iceland		ATL6	(2)	AF297996
IC3	*S. a. alpinus*	Stóravidarvatn, Iceland		ATL4	(2)	AF297994
GL1	*S. a. alpinus*	Lake near Isortoq Fjord, Greenland	landlocked	ATL9	(2)	AF297999
GL2	*S. a. alpinus*	Lake near Isortoq Fjord, Greenland	landlocked	ATL10	(1)	AF298000
GL3	*S. a. alpinus*	Lake near Isortoq Fjord, Greenland	landlocked	ATL11	(1)	AF298001
GL4	*S. a. alpinus*	Lake near Isortoq Fjord, Greenland	landlocked	ATL1	(1)	AF297991
GL5	*S. a. alpinus*	River near Isortoq Fjord, Greenland	anadromous	ARC15	(1)	AF298041
GL6	*S. a. alpinus*	River near Isortoq Fjord, Greenland	anadromous	ARC16	(1)	AF298042
GL7	*S. a. alpinus*	River near Isortoq Fjord, Greenland	anadromous	ARC4	(2)	AF298030
NO1	*S. a. alpinus*	Grønningen Lake, Norway		ATL8	(2)	AF297998
NO2	*S. a. alpinus*	Femund Lake, Norway		ATL7	(2)	AF297997
NO3	*S. a. alpinus*	Mekkelandsvatnet, Norway		ATL1	(2)	AF297991
NO4	*S. a. alpinus*	Hals Lake, Norway		ATL1	(2)	AF297991
NO5	*S. a. alpinus*	Fjellfrosvatnet, Norway		ATL14	(2)	AF298004
NO6	*S. a. alpinus*	Vassdalsvatnet, Norway		ATL8	(2)	AF297998
NO7	*S. a. alpinus*	Spitsbergen, Norway		SIB3	(2)	AF298011
NO8	*S. a. alpinus*	Viashenskoe Lake, Kol'sky Peninsula		ATL1	(2)	AF297991
FI1	*S. a. alpinus*	Kuolimo Lake, Finland		SIB1	(2)	AF298009
		Kuolimo Lake, Finland		SIB2	(2)	AF298010
FI2	*S. a. alpinus*	Luomusjarvi Lake, Finland		ATL12	(2)	AF298002
FI3	*S. a. alpinus*	Kanes Laddu Lake, Finland		ATL13	(2)	AF298003
TM1	*S. alpinus*	Arilakh Lake, Taimyr Peninsula, central Siberia		SIB5	(2)	AF298013
TM2	*S. a. drjagini*	Lama Lake, Taimyr Peninsula		SIB9	(2)	AF298017
TM3	*S. a. alpinus* (Pucheglazka)	Lama Lake, Taimyr Peninsula		SIB7	(2)	AF298015
TM4	*S. a. alpinus* (Putoranchic)	Ayan Lake, Taimyr Peninsula		SIB4	(2)	AF298012
BK1	*S. a. erythrinus* (Davatchan)	Frolikha Lake, Baikal region, central Siberia		SIB8	(2)	AF298016
BK2	*S. a. erythrinus* (Davatchan)	Goltsovoe Lake, Baikal region		SIB4	(2)	AF298012
BK3	*S. a. erythrinus* (Davatchan)	Leprindo Lake, Baikal region		SIB6	(2)	AF298014
ES1	*S. malma*	Kuma River, Paramushir Isle, eastern Siberia		BER4	(2)	AF298021
ES2	*S. malma*	Kakhmauri River, Paramushir Isle		BER5	(2)	AF298022
ES3	*S. malma* (Stone)	Kamchatka River, Kamchatka Peninsula		BER1	(2)	AF298018
ES4	*S. malma*	Kamchatka River, Kamchatka Peninsula		BER2	(2)	AF298019
ES5	*S. malma*	Seutakan River, Cukotka Peninsula		BER3	(2)	AF298020
ES6	*S. a. taranetzi*	Seutakan River, Cukotka Peninsula		ARC3	(1)	AF298029
ES7	*S. boganidae*	Elgygytgyn Lake, Cukotka Peninsula		ARC1	(2)	AF298027
ES8	*S. elgyticus*	Elgygytgyn Lake, Cukotka Peninsula		ARC2	(2)	AF298028
ES9	*Salvethymus svetovidovi*	Elgygytgyn Lake, Cukotka Peninsula		SVET	(2)	AF297990
ES10	*Salvelinus albus*	Kamchatka, Kamchatka Peninsula		BER2	(1)	AF298019
AL1	*S. a. erythrinus*	Horizon Lake, Alaska		ARC5	(2)	AF298031
AL2	*S. malma*	Galbraith Lake, Alaska		ARC17	(1)	AF298043
AL3	*S. malma*	Clear Hatchery, Alaska		BER6	(2)	AF298023
AL4	*S. malma*	Prince William Sound, Alaska		BER7	(1)	AF298024
		Prince William Sound, Alaska		BER8	(1)	AF298025

APPENDIX. Continued.

Pop.	Taxon (Form)	Locality	Status	Haplotype	Freq.	GB
AL5	S. malma	Auke Creek, Alaska		BER9	(2)	AF298026
CD1	*S. a. erythrinus*	Gander Lake, New Foundland	landlocked	ATL16	(2)	AF298006
CD2	*S. a. erythrinus*	Bluehill Pond, New Foundland		ATL8	(1)	AF297998
CD3	*S. a. erythrinus*	Ikadlivik Brook, Labrador	anadromous	ATL17	(1)	AF298007
		Ikadlivik Brook, Labrador		ATL8	(1)	AF297998
CD4	*S. a. erythrinus*	Kugluktokoluk Brook, Labrador	anadromous	ATL18	(2)	AF298008
CD5	*S. a. erythrinus*	Pangertok Inlet, Labrador	anadromous	ARC14	(2)	AF298040
CD6	*S. a. erythrinus*	Fraser River, Labrador	anadromous	ATL15	(2)	AF298005
CD7	*S. a. erythrinus*	P&N Lakes		ARC18	(2)	AF298044
CD8	*S. a. erythrinus*	Kasegalik Lake, Belcher Island		ARC8	(2)	AF298034
CD9	*S. a. erythrinus*	Hall Lake, Melville Peninsula		ARC6	(1)	AF298032
CD10	*S. a. erythrinus*	Avamuktulik, Melville Peninsula		ARC9	(2)	AF298035
CD11	*S. a. erythrinus*	Igloolik, Igloolik Island		ARC9	(2)	AF298035
CD12	*S. a. erythrinus*	Lac DuquetWeir, Québec		ARC11	(2)	AF298037
		Lac DuquetWeir, Québec		ARC13	(2)	AF298039
CD13	*S. a. erythrinus*	Nauyuk Lake, Mackenzie District		ARC7	(3)	AF298033
CD14	*S. a. erythrinus*	Swan Lake, King William Island		ARC4	(2)	AF298030
CD15	*S. a. erythrinus*	Charr Lake, Cornwallis Island		ARC10	(2)	AF298036
CD16	*S. a. erythrinus*	Resolut Lake, Cornwallis Island		ARC12	(2)	AF298038
CD17	*S. a. erythrinus*	Amituk Lake, Cornwallis Island		ARC4	(2)	AF298030
MN1	*S. a. oquassa*	Floods Pond, Maine		ACD4	(2)	AF298048
MN2	*S. a. oquassa*	Penobscot Lake, Maine		ACD4	(2)	AF298048
MN3	*S. a. oquassa*	Wassataquoik Lake, Maine		ACD6	(1)	AF298050
		Wassataquoik Lake, Maine		ACD7	(1)	AF298051
MN4	*S. a. oquassa*	Lac Rond, Québec		ACD4	(2)	AF298048
MN5	*S. a. oquassa*	Lac Sans Baie, Québec		ACD1	(2)	AF298045
MN6	*S. a. oquassa*	Lac Français, Québec		ACD8	(2)	AF298052
MN7	*S. a. oquassa*	Lac Chaudière, Québec		ACD2	(1)	AF298046
		Lac Chaudière, Québec		ACD3	(1)	AF298047
MN8	*S. a. oquassa*	Walton Lake, New Brunswick		ACD5	(2)	AF298049