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ORIGINAL ARTICLE

Post-glacial recolonization of the North American Arctic by Arctic char (*Salvelinus alpinus*): genetic evidence of multiple northern refugia and hybridization between glacial lineages

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ABSTRACT

Aims We investigated post-glacial recolonization of the North American Arctic by Arctic char (*Salvelinus alpinus*) and examined potential hybridization between different glacial lineages upon secondary contact.

Location North American Arctic and adjacent areas.

Methods We collected mtDNA sequence data from 1355 individuals from 110 sampling locations and data from nine microsatellite loci from 931 individuals from 37 locations. We assessed the phylogenetic relationships and geographical distribution of mtDNA haplotypes and conducted historical demographic analyses. We used a Bayesian clustering analysis method to detect potential hybridization between glacial lineages.

Results Two highly divergent mtDNA lineages were identified in the Arctic region with distinct but overlapping geographic distributions: one in Beringia and the other over the entire Arctic Archipelago and coastal mainland east of Alaska. The microsatellite data also implied the existence of these two lineages. Evidence of hybridization was detected between the Arctic lineage and an Atlantic lineage in eastern North America.

Main conclusions Our data suggested survival and recolonization from two northern glacial refugia: one in Beringia and another in a smaller refugium, perhaps in the Arctic Archipelago itself or a separate refugium within Beringia. Patterns of hybridization detected supported the presence of a secondary contact zone between glacial lineages in the eastern Canadian Arctic.

Keywords
Arctic, cryptic glacial refugia, gene flow, microrefugia, microsatellites, mismatch analysis, mitochondrial DNA, mito-nuclear discordance, North America, phylogeography

INTRODUCTION

For high latitude biota of the Northern Hemisphere, the Pleistocene glaciations had a dominant and well-documented role in shaping patterns of genetic diversity (Hewitt, 2000, 2004). The consensus view emerging from decades of phylogeographical study is that most northern species survived in refugia south of the ice sheets during the Last Glacial Maximum (LGM) before they recolonized their current range (Bernatchez & Wilson, 1998; Hewitt, 2000; Soltis *et al.*, 2006). Accumulating evidence, however, suggests that many species also survived the LGM in refugia in areas far north of the ice sheets (Provan & Bennett, 2008; Shafer *et al.*, 2010; Stewart *et al.*, 2010). Such refugia are known as 'cryptic refugia' or 'microrefugia', and are defined as small areas of favourable conditions outside of the species main (macro) refugia, often situated at different latitudes or longitudes than would normally be expected (Rull, 2009; Stewart *et al.*, 2010; Mee & Moore, 2014). In North America, the Beringian refugium was a major ice-free area where many species

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Journal of Biogeography

persisted during Pleistocene glaciations (Lindsey & McPhail, 1986; Bernatchez & Dodson, 1991; Abbott *et al.*, 2000; Harris & Taylor, 2010; Shafer *et al.*, 2011). A number of species, however, may have survived the LGM in the Arctic Archipelago itself. Indeed, some areas of the Canadian Arctic Archipelago were ice-free throughout the LGM (Fig. 1 and Dyke *et al.*, 2003), and phylogeographical evidence suggests they may have been used as refugia by mammals (Fedorov & Stenseth, 2002), birds (Holder *et al.*, 1999) and plants (Tremblay & Schoen, 1999; Abbott *et al.*, 2000; see Shafer *et al.*, 2010 for a review).

The Arctic char (*Salvelinus alpinus* (L.)) is a salmonid fish that shows a bewildering array of phenotypic diversity within what is described as a 'species complex' (McPhail, 1961; Klemetsen, 2010; Reist *et al.*, 2013). Arctic char is also the most northerly distributed species of freshwater fish in the world (Reist *et al.*, 2013), and large portions of its current distribution were covered by ice during the LGM. Being an anadromous species capable of extensive dispersal (Moore *et al.*, 2013), recolonization most likely occurred via marine routes (Crossman & McAllister, 1986). Our current understanding of the effects of Pleistocene glaciations on the distribution of genetic variation in Arctic char is mainly based on the Holarctic study of Brunner *et al.* (2001; see also Wilson *et al.*, 1996). Brunner *et al.* (2001) used mitochondrial (mtDNA) control region sequences to identify five distinct glacial lineages of Arctic char worldwide (Fig. 1). While global in scope, sampling resolution in Brunner *et al.* (2001) did not allow inferences on the location of these glacial refugia. Of particular interest is the hypothesis formulated by Crossman & McAllister (1986), and reiterated by Brunner *et al.* (2001), that North American Arctic char may have survived the last glaciation in a high Arctic 'cryptic refugium'. The presence of four highly divergent mtDNA lineages (*sensu* Brunner *et al.*, 2001) in North America also suggested the possibility of secondary contact and introgression between lineages. In fact, Wilson *et al.* (1996) showed that two mtDNA lineages co-existed in populations of Arctic char from Northern Labrador.

Our study sought to better understand the recolonization of Arctic North America by Arctic char following the last glaciation. To do so, we used mtDNA control region sequence data and built upon previous North American (Wilson *et al.*, 1996) and global (Brunner *et al.*, 2001) studies by increasing the number of locations sampled and the number of samples per location. In addition, we surveyed variation in nine microsatellite markers to provide increased resolution for historical inference given that they have proven useful in many other phylogeographical studies of northern salmonid fishes from recently deglaciated areas (Angers & Bernatchez, 1998; Koskinen *et al.*, 2002; Harris & Taylor, 2010). Microsatellite data also provided nuclear genetic markers independent from mtDNA. We assessed two alternative hypotheses regarding refugial origins of Arctic char in the North American Arctic: (1) that the North American Arctic was recolonized only from a Beringian refugium, or

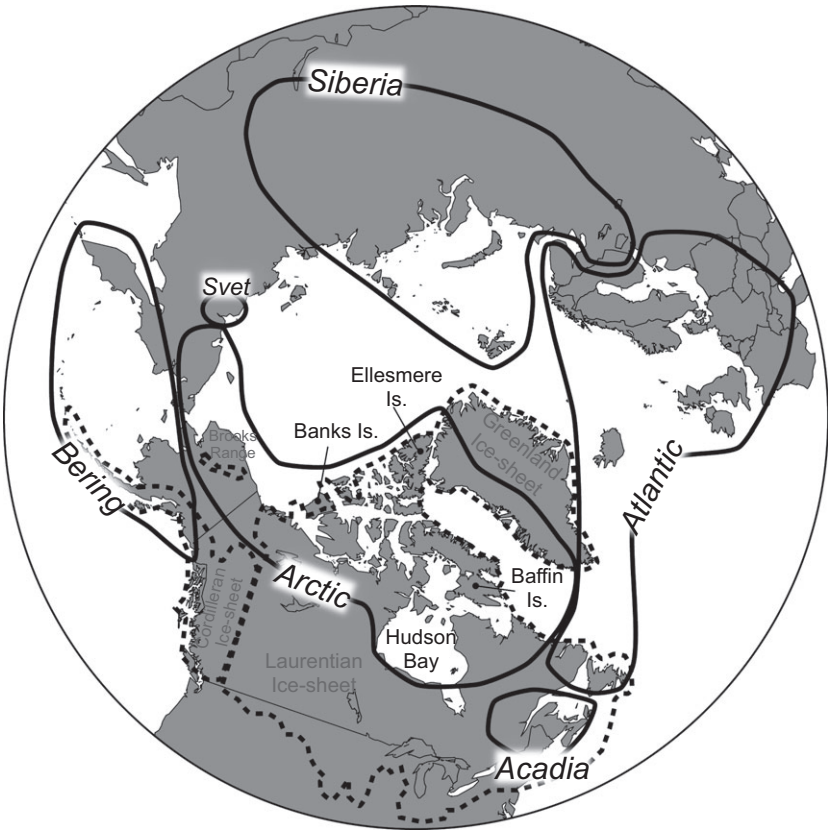

Figure 1 North polar projection showing the distribution and names of the five major mitochondrial DNA lineages of Arctic char (*Salvelinus alpinus*) identified in the Holarctic phylogeography study of Brunner *et al.* (2001) (Acadia, Arctic, Atlantic, Bering, Siberia), and an isolated sample of *Salvethymus svetovidovi* (Svet); see Brunner *et al.* (2001) for details. Dotted lines show the approximate extent of the three major ice-sheets that covered North America at the Last Glacial Maximum (Dyke *et al.*, 2003).

(2) that recolonization occurred from two separate refugia. If Arctic char did survive in two refugia, we predicted that there would be evidence of two separate genetic lineages within the North American Arctic using both the mtDNA and microsatellite data. Further, because a second northern refugium was probably much smaller in area than the > 1 million km^2 Beringian refugium, we predicted that we would find comparatively lower genetic diversity, and signatures of recent population expansion. Our second objective was to assess the possibility of hybridization between glacial lineages where they come into secondary contact. If hybridization occurred, we predicted that distinct mitochondrial haplotypes should co-exist in areas of contact, and that admixture would be evident at microsatellite markers.

MATERIALS AND METHODS

Samples

We collected mtDNA sequence data from a total of 1355 individuals from 110 sampling locations distributed across eastern Siberia and the North American Arctic (see Table S1 in Appendix S1 in Supporting Information). Sequences generated for this study were combined with sequences from Brunner *et al.* (2001), Taylor *et al.* (2008), Power *et al.* (2009) and Alekseyev *et al.* (2009) obtained from GenBank. As in previous studies, we also included samples of Dolly Varden (*Salvelinus malma*). The taxonomic status of Arctic char and Dolly Varden as separate species has been contentious (Reist *et al.*, 1997; Brunner *et al.*, 2001), but recent work strongly suggests they are distinct biological species (Taylor *et al.*, 2008). Nevertheless, we included samples of Dolly Varden (1) to allow direct comparisons with previous studies (Brunner *et al.*, 2001; Taylor *et al.*, 2008; Alekseyev *et al.*, 2009), and (2) because mtDNA haplotypes have been shown to introgress between the two species (Brunner *et al.*, 2001; Taylor *et al.*, 2008), and their inclusion can thus help understand the history of mtDNA lineages in this region. We also added samples from locations where other lineages of Arctic char (i.e. Siberian, Atlantic and Acadian) were identified by Brunner *et al.* (2001) to place our study within this broader evolutionary framework. Finally, we used homologous sequences from brook trout (*Salvelinus fontinalis*) and lake trout (*Salvelinus namaycush*) as outgroup taxa. GenBank accession numbers for all haplotypes can be found in the online supplementary materials (see Table S2 in Appendix S1).

A different set of samples was used for the microsatellite analysis (see Table S3 and Fig. S1 in Appendix S1). Samples came from a total of 37 sampling locations that covered most of the distribution of Arctic char in the Canadian Arctic. We also included three sampling locations from Alaska and one sampling location from Labrador so that representatives of the three mtDNA lineages found in the North American Arctic (*sensu* Brunner *et al.*, 2001) would be included in the microsatellite analysis. The number of individuals sampled per population varied from seven to 56 (mean = 25.2; total = 931). Because of the difficulty of obtaining samples from the high Arctic, some populations were represented by a small number of individuals. Population-based statistics should thus be interpreted cautiously, although the STRUCTURE analysis should not be as affected.

DNA methods

Samples consisted of either dorsal muscle tissue, liver, or fin preserved in a 20% DMSO/NaCl solution or in 95% ethanol and were kept frozen until DNA extraction. The entire mtDNA control region was amplified with primers *Tpro2* (Brunner *et al.*, 2001) and *SalpcrR* (Power *et al.*, 2009). We sequenced 502 base pairs of the control region left domain region according to methods outlined in Power *et al.* (2009). Individual genotypes were obtained at nine microsatellite loci (details in Appendix S3).

mtDNA analysis

Control region sequences were aligned with the GENEIOUS (6.1.8; www.geneious.com) alignment tool with a gap penalty of 5 (other parameters were set to default). We calculated haplotype/gene (h) and nucleotide diversity (π) using ARLEQUIN 5.0 (Excoffier *et al.*, 2005). We used JMODELTEST 2.4.1 (Darriba *et al.*, 2012) to identify the most likely model of evolution on an alignment containing one copy of all haplotypes (including the outgroup) limiting the search to five substitution models. The most likely model according to both Akaike and Bayesian information criteria was the HKY85+I+G model. We used the PhyML (Guindon & Gascuel, 2003) plugin in GENEIOUS to infer phylogenetic relationships among haplotypes. We used the Nearest Neighbour Interchange topology search algorithm under the HKY85+I+G model. Support for the nodes was assessed with 1000 bootstrap replicates. Percentage sequence divergence estimates corrected for multiple hits were obtained from branch lengths. A rough estimate of divergence times was obtained using a standard molecular clock of 5–10% per million years divergence rate (see Brunner *et al.*, 2001 for a detailed discussion of this choice of clock rate). We also reran the same analysis on a sequence alignment excluding all haplotypes that were only identified in Dolly Varden.

Historical demography

The hypothesis that the Arctic and Bering lineages experienced recent population expansion following the LGM was evaluated using a variety of tests. First, departures from neutrality of the mtDNA sequence data (frequency data of haplotypes included) were tested using Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997). Significant negative values for both of these statistics would be indicative of recent population expansion. The statistics were estimated and their significance was assessed using 1000 bootstrap replicates in

ARLEQUIN. Second, we performed mismatch analysis (Rogers & Harpending, 1992) to test whether mitochondrial DNA variation in Arctic and Bering lineages conform to a model of recent population expansion (details in Appendix S2). We repeated these analyses on a data set excluding haplotypes found exclusively in Dolly Varden samples to avoid influencing results by the inclusion of two taxa.

Microsatellite analysis

We used FSTAT 2.9.3.2 (Goudet, 2001) to test for Hardy–Weinberg equilibrium and genotypic disequilibrium using 10,000 permutations for both analyses, and setting the nominal significance level at $\alpha = 0.05$. We used MICROSATELLITETOOLKIT (3.1.1; Park, 2001) to generate estimates of observed heterozygosity (H_O), and expected heterozygosity (H_E) corrected for sample size. FSTAT was used to calculate allelic richness (A_R) and pairwise F_{ST} (Weir & Cockerham, 1984) between each sample, and significance was assessed with 10,000 permutations (experiment-wide $\alpha = 0.05$ after Bonferroni correction). We used PHYLIP 3.68 (Felsenstein, 1993) to generate a neighbour-joining tree of all samples based on Cavalli-Sforza's chord measure (Cavalli-Sforza & Edwards, 1967) employing 1000 bootstrap replicates to test support for each node.

We used the program STRUCTURE (Pritchard *et al.*, 2000) to confirm that the same genetic groups identified with the mtDNA are also observed in the nuclear genome and to assess whether hybridization occurred among glacial lineages. The analysis included all the samples (931 individuals), and K values ranging from one to ten were tested using 500,000 burn-in and 1,000,000 Markov chain Monte Carlo repetitions under the admixture and correlated allele frequency models without location priors. Twenty independent runs were performed for each K. The ΔK method of Evanno *et al.* (2005) was used to determine the most likely value of K. The program CLUMPP (1.1.2; Jakobsson & Rosenberg, 2007) was used to combine the results of the 20 independent runs using the *Greedy* algorithm, and program DISTRUCT (1.1; Rosenberg, 2004) was used to visualize the results.

To test the prediction that genetic diversity should decline away from the putative high Arctic glacial refugium, we regressed values of expected heterozygosity (Nei's unbiased gene diversity; Nei, 1987) and allelic richness (as calculated by FSTAT; details presented above) against distance from Banks Island, the most likely high Arctic putative glacial refugium. The distances were waterway distances generated using GOOGLE EARTH (5.2.1.1588). When more than one route was possible, we chose the most likely route based on patterns of glacial retreat (Dyke *et al.*, 2003). Note that the patterns would have been nearly identical if distance from the eastern boundary of Beringia had been used because Banks Island is located relatively close to Beringia. This analysis therefore does not allow falsification of any of the alternative hypotheses regarding refugial origins.

RESULTS

mtDNA variation

Our mtDNA sequencing results uncovered many inconsistencies with the results reported by Brunner *et al.* (2001). A total of 34 samples from 13 sampling locations used by Brunner *et al.* (2001) were graciously made available to us by L. Bernatchez, and resequencing of these samples confirmed that many haplotypes reported in Brunner *et al.* (2001) resulted from sequencing artefacts (see Table S1 in Appendix S1). We therefore excluded all haplotypes from Brunner *et al.* (2001) from subsequent analyses unless the haplotypes were corroborated by another study (i.e. by this study, Taylor *et al.*, 2008, Alekseyev *et al.*, 2009, and Power *et al.*, 2009).

We found two distinct groups of mtDNA haplotypes in the North American Arctic that roughly corresponded to the Beringian and Arctic lineages identified in Brunner *et al.* (2001; Fig. 1). The Arctic lineage formed a well-supported (97.1% bootstrap support) monophyletic group (Fig. 2). In contrast to previous studies (Brunner *et al.*, 2001; Taylor *et al.*, 2008; Alekseyev *et al.*, 2009), the Bering group did not form a reciprocally monophyletic clade or lineage when all samples were included (Fig. 2), but did when the haplotypes found only in Dolly Varden were excluded (see Fig. S2 in Appendix S2). The Arctic and Bering lineages had seven fixed differences between them (when the Dolly Varden haplotypes were included), which led to a 7.16% estimated divergence between the Arctic lineage and the nearest Bering haplotype after correction for multiple hits. Assuming a clock rate of 5–10% sequence divergence per million years, this leads to an estimated divergence time of 716,000–1,432,000 years ago.

Although we identified several new haplotypes not previously described from the Canadian Arctic (see Table S2 in Appendix S1), the overall level of genetic variation in mtDNA haplotypes in the Arctic lineage was very low; 1087 of 1141 (95.3%) individuals shared the ARC19 haplotype (which was not uncovered in Brunner *et al.*, 2001). The phylogenetic relationship among the Arctic haplotypes formed a 'star-phylogeny' centred on the ARC19 haplotype, which was found at all sampling locations where Arctic char (not Dolly Varden) were collected in North America (Fig. 2). Haplotype ARC20 was the only other widely distributed haplotype, being found at low frequency from the Kent Peninsula to Ellesmere Island to Baffin Island in the east, but was not found west of ~107 °W longitude. The other Arctic lineage haplotypes had no clear pattern of geographical distribution and tended to be found in a single sampling site, or in multiple sampling sites that were geographically proximate to each other (Fig. 2 and Table S1 in Appendix S1 for details). The Bering group contained more genetic diversity: we uncovered almost the same number of haplotypes in the Bering group (13) and the Arctic group (16) despite a much larger sample size for the Arctic group, and the most common

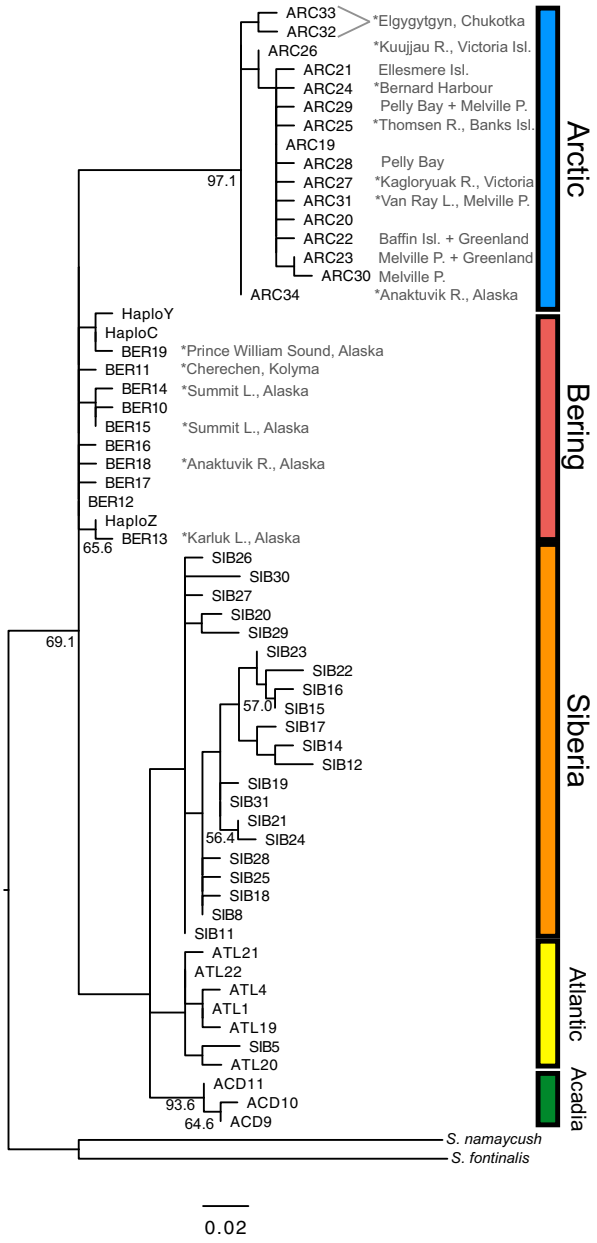

Figure 2 Maximum likelihood phylogenetic tree of mtDNA haplotypes of Arctic char (*Salvelinus alpinus*) generated using PhyML (Guindon & Gascuel, 2003). Bootstrap support values (1000 replicates) of more than 50% are shown. Lineages as discussed in the text are identified on the right. Grey text summarizes the geographical distribution of haplotypes from the Arctic and Bering lineages which are the focus of the current study, where the labels preceded by an asterisk represent haplotypes that were restricted to a single sampling location. Haplotypes without labels are either geographically widespread or do not occur in the focal area of the study. See Fig S2 in Appendix S2 for an equivalent phylogenetic analysis excluding haplotypes restricted to Dolly Varden (*Salvelinus malma*).

haplotype in the Bering group (BER12) was shared by only 40.0% of the individuals (see Table S2 in Appendix S1). Estimates of haplotype and nucleotide diversity for the Arctic lineage (h = 0.106, SD = 0.1761; π = 0.000213, SD = 0.00039) were both an order of magnitude lower than those for the Bering lineage (h = 0.7621, SD = 0.0244; π = 0.00311, SD = 0.0021). In contrast, the values reported in Brunner *et al.* (2001) were considerably higher (especially for the Arctic lineage) and did not differ between the two lineages (Arctic: h = 0.952, π = 0.009; Bering h = 0.934, π = 0.007).

We found that the Arctic lineage was distributed throughout the Arctic Archipelago, the North American Arctic Coast, Greenland and in one location from the Chukotka Peninsula (Fig. 3). In contrast with Brunner *et al.* (2001), however, we found that the Bering haplotype was distributed throughout Beringia (Fig. 3), while Brunner *et al.* (2001) only identified it in southern Beringia (Fig. 1). No Arctic char with Bering lineage haplotypes, however, were identified from northern Alaska. Of note is the co-existence of Arctic and Bering lineage haplotypes in Dolly Varden from two localities on the North Slope of Alaska (Anaktuvik River and Graylime Creek; Fig. 3, Table S1 in Appendix S1).

Historical demography

Historical demographic analyses were consistent with the hypothesis of a recent population expansion from a small population size in the Arctic lineage, but not in the Bering lineage. Estimates of both Fu's F_S and Tajima's D are significantly negative for the Arctic lineage, while they are not significantly different from zero for the Bering lineage both with and without Dolly Varden haplotypes included (Table 1). The mismatch distribution analysis indicated that the patterns of mtDNA substitutions in the Arctic lineage could not be distinguished from those expected under a model of sudden population expansion (Table 1, Fig. 4). On the contrary, the patterns of substitution in the Bering lineage were usually significantly different than expected under this model (for both sum-of-square deviations and Raggedness when including Dolly Varden haplotypes; when excluding Dolly Varden, it was marginally significant only for Raggedness; see Table 1). The shape of the mismatch distribution for the Arctic lineage, however, was also similar to what would be expected under a constant population size model (see Appendix S2).

Microsatellite DNA

We found a high level of polymorphism at the microsatellite loci, with the number of alleles per locus ranging from 3 (for *Omm1128*) to 40 (for *Sco220*) with a mean of 22.9 (population statistics in Table S4 in Appendix S3). Before correction for multiple comparisons, 14 locus/population combinations displayed significant heterozygote deficit and eight displayed significant heterozygote excess. No population/locus combination remained significant after correction for multiple comparisons, and no marker consistently displayed departure from Hardy–Weinberg equilibrium across multiple

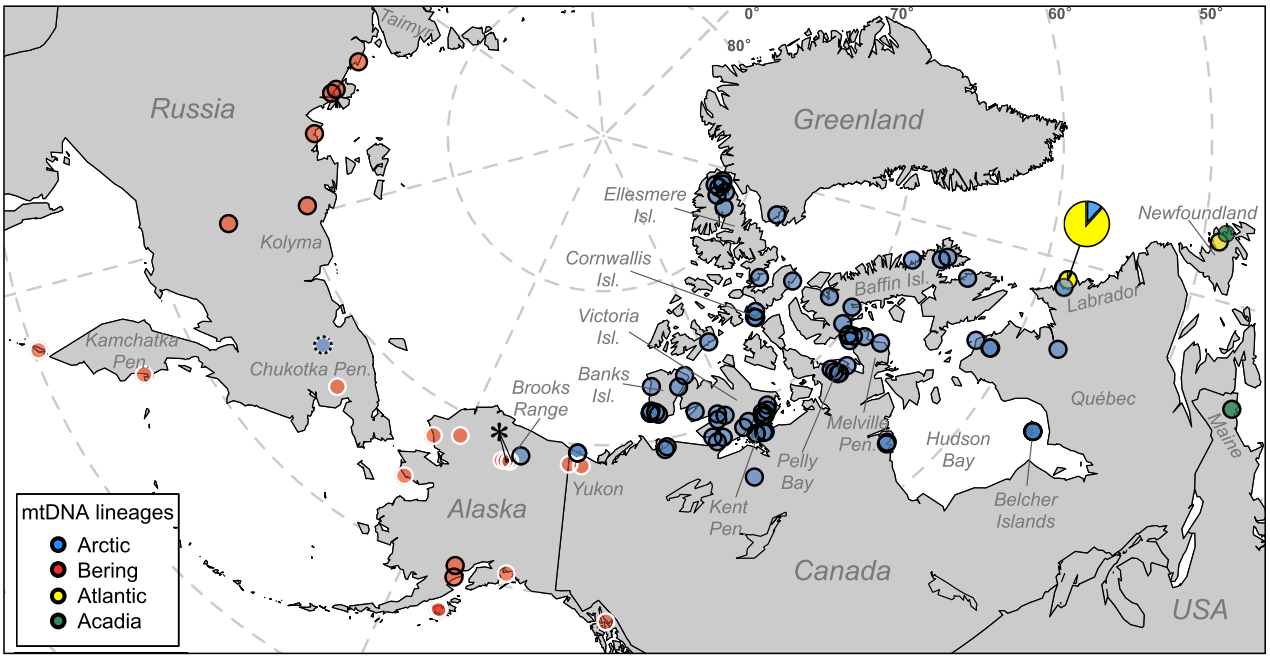

Figure 3 Geographical distribution of the mtDNA lineages of Arctic char (*Salvelinus alpinus*) identified in this study. Each point is a sampling location. The colours denote the haplotype composition of each sampling location: blue for Arctic lineage haplotypes, red for Bering lineage, yellow for Atlantic and green for Acadian (no Siberian haplotypes were found in the area covered by the map). Only three localities had a mixture of two lineages: a sampling location in Labrador shown by the enlarged pie chart, and two locations in the Brooks Range (identified by the asterisk) where a single Arctic haplotype was documented in each. Circles with a black outline represent locations where Arctic char were sampled, circles with a white outline represent locations where Dolly Varden char were sampled, and the dotted circle represents a location resequenced from Brunner *et al.* (2001), Lake Elgygytgyn, for which samples were identified as *S. boganidae* and *S. elgyticus*.

Table 1 Results of the historical demography analyses using neutrality tests (Fu's F_S and Tajima's D) and mismatch distribution analysis on mtDNA data from North American Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*). The two lineages identified (Arctic and Bering) are analysed separately. An additional analysis was performed on Bering haplotypes excluding all samples from Dolly Varden ('DV removed'). n is the sample size for each analysis. For the neutrality tests, significant P-values indicate departures from neutrality (i.e. either population expansion or positive selection). For the mismatch distribution analysis, τ is the estimated time of population expansion and θ is the populations size index (i.e. scaled by mutation rate) before (0) and after (1) the population expansion. Significant P-values indicate departure from a sudden population expansion model based on sum-of-square deviations (SSD) or based on the raggedness criteria (Rag.).

	Neutrality tests				Mismatch distribution analysis					
Lineage	Fu's F_S	P	Tajima's D	P	τ (95% CI)	θ_0 (95% CI)	θ_1 (95% CI)	P (SSD)	Raggedness	P (Rag.)
Arctic (n = 1087)	−34.03	< 0.005	−2.060	< 0.005	3.0 (0.48–3.50)	0.000 (0–0.007)	0.099 (0–∞)	0.370	0.690	0.720
Bering (n = 150)	−3.59	0.09	−0.552	0.353	1.969 (0.713–3.330)	0.000 (0–0.842)	11.685 (2.413–∞)	0.034	0.168	0.003
Bering (DV removed; n = 62)	0.77	0.707	1.032	0.866	2.62 (0.0–4.44)	0.000 (0–1.025)	2.46 (1.435–∞)	0.124	0.36	0.049

populations. Nine pairs of loci displayed significant linkage disequilibrium before correction for multiple comparisons, but only one remained significant after correction: *OtsG253b* × *Ssosl456*. The two Yukon sampling locations, Lake 103 and Lake 104, showed considerably lower variation at the microsatellite loci than any other populations and were fixed (or nearly fixed) for one allele at four of the nine otherwise polymorphic loci (*Omm1105*, *Omm1128*, *OtsG253b* and *Ssosl456*). These samples were therefore excluded from some analyses because their extreme low genetic variation could bias results. The neighbour-joining tree (see Fig. S3 in Appendix S3) showed that, within the Arctic Archipelago, the internal branches were short and poorly supported by bootstrap analysis. The separation of the Alaska and Labrador sampling

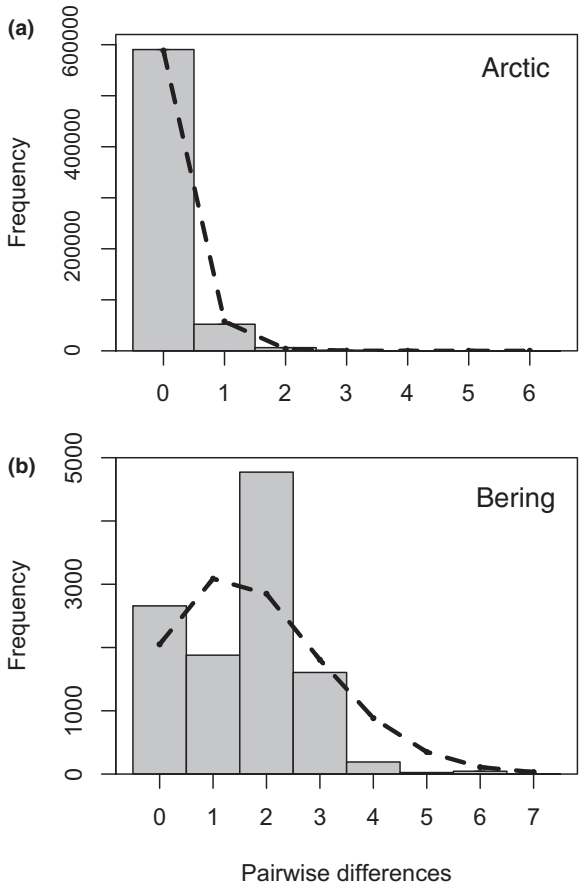

Figure 4 Results of the pairwise mismatch analysis on the mtDNA data from Arctic char (*Salvelinus alpinus*). The grey bars represent the empirically determined distribution of pairwise sequence differences in the Arctic (a) and Bering (b) lineages. The dashed lines represent theoretical expectations for a model of sudden population expansion (Rogers & Harpending, 1992) simulated in ARLEQUIN.

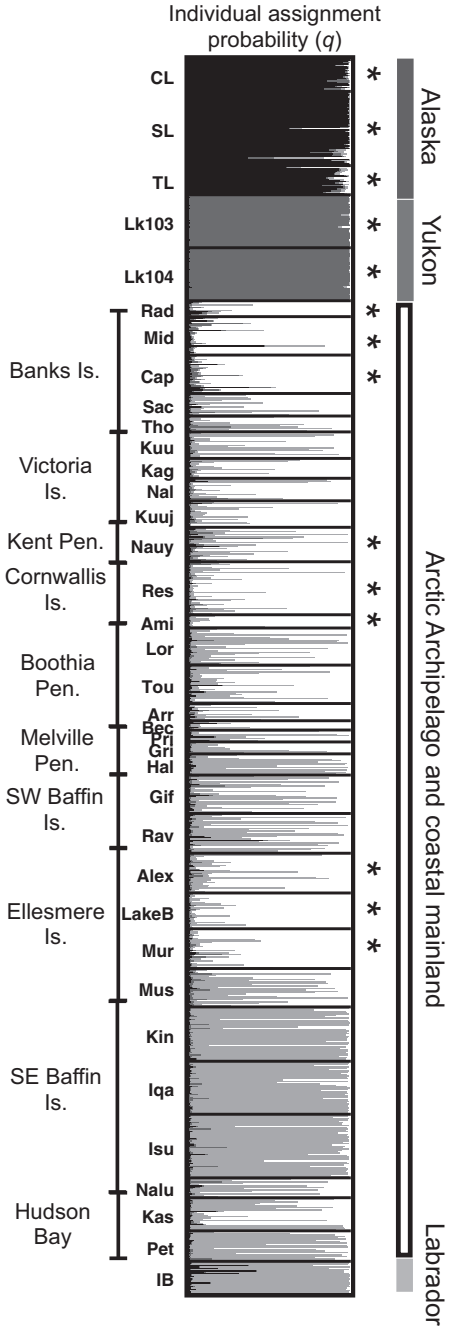

Figure 5 Results of the STRUCTURE analysis conducted on microsatellite data from 931 North American Arctic char (*Salvelinus alpinus*) based on the most likely number of genetic cluster: $K = 4$. Each coloured horizontal bar represents the probability of assignment of each individual to the four genetic clusters. The location code for each sampling location is shown on the left, along with regional groupings to help interpretation. The sampling locations are arranged approximately from west (top) to east (bottom). Larger scale regional groupings based roughly on the STRUCTURE results are shown on the right. Asterisks denote landlocked populations.

locations from the Arctic ones, on the other hand, was well supported by high bootstrap values (see Fig. S3 in Appendix S3).

Results of the STRUCTURE analysis provided evidence that genetic variation in the nuclear genome paralleled that observed in the mitochondrial genome. The ΔK method of Evanno *et al.* (2005) identified $K = 4$ as the most likely number of genetic clusters (see Fig. S5 in Appendix S3). One cluster consisted of the three Alaska sampling locations, a second consisted of the two Yukon sampling locations, the Labrador sampling location formed a third cluster, and the Arctic Archipelago and coastal mainland sampling locations formed a fourth cluster with varying amounts of admixture with the 'Labrador cluster' (Fig. 5).

When all sampling locations were included, we found no evidence that expected heterozygosity decreased with increasing distance from Banks Island (d.f. = 31; $r^2 = 0.0356$; $P = 0.293$; Fig. S4 in Appendix S3). There was also no evidence of decreasing heterozygosity when only anadromous populations were included (d.f. = 20; $r^2 = 0.006$; $P = 0.743$). When only landlocked populations were included (excluding the two Yukon samples), there was a trend of decreasing

heterozygosity (see Fig. S4 in Appendix S3), but the relationship was not significant (d.f. = 9; $r^2 = 0.319$; $P = 0.113$). To avoid biases related to small sample sizes in the calculation of allelic richness, we excluded populations with fewer than 10 individuals from the calculation of allelic richness. The results still mirrored those observed with heterozygosity: allelic richness did not decrease with increasing distance from Banks Island when all samples were included (d.f. = 24; $r^2 = 0.040$; $P = 0.327$), when only anadromous samples were included (d.f. = 14; $r^2 = 0.072$; $P = 0.314$), or when only landlocked samples (excluding the two Yukon samples) were included (d.f. = 6; $r^2 = 0.075$; $P = 0.510$; Fig. S4 in Appendix S3).

DISCUSSION

Our analysis of genetic variation using both mtDNA and microsatellite DNA variation has contributed novel insights into the phylogeography of Arctic char from the North American Arctic. Both mtDNA and microsatellite data provided evidence for the presence of multiple glacial refugia for Arctic char in the North American Arctic and for widespread dispersal of a single mtDNA lineage across the Canadian Arctic Archipelago. We also provided evidence of post-glacial secondary contact and hybridization between two glacial lineages in the eastern North American Arctic.

Implications of comparisons with a previous mtDNA analysis

Brunner *et al.* (2001) constitutes the only global phylogeography study of Arctic char to date. Our study, however, uncovered several inconsistencies that we confirmed were due to sequencing errors. A major consequence of these sequencing errors was a re-evaluation of the amount of genetic diversity present in the North American Arctic; our samples were dominated by the widespread haplotype ARC19, which was initially described in Alekseyev *et al.* (2009) and Power *et al.* (2009), but which was not identified in Brunner *et al.* (2001). The sequencing errors, however, do not change significantly the geographical distribution of the major glacial lineages described by Brunner *et al.* (2001). Indeed, all samples resequenced for the present study were assigned to the same glacial lineage as in Brunner *et al.* (2001) and changes to our interpretations of the geographical distribution of haplotypes in Beringia (see next section) are only due to increased geographical sampling.

Distribution of two highly divergent mitochondrial lineages

We documented the presence of two highly divergent mtDNA lineages in the North American Arctic. This suggested that a vicariance event separated populations of Arctic char, which we estimated to have occurred 716,000–1,432,000 years ago. These estimates should obviously be interpreted with caution, but they support a minimum divergence time that pre-dates the onset of the last glaciation (*c.* 250,000 years ago; Dyke *et al.*, 2003). They also rely on a molecular clock rate that is in the upper range of those reported in fishes (Burridge *et al.*, 2008), and therefore probably provide a lower time boundary (i.e. the divergence could be older). It should also be noted that the Bering lineage was not reciprocally monophyletic when all samples were analysed, further indicating that caution should be used when interpreting divergence dates. Reciprocal monophyly of Bering haplotypes was recovered when Dolly Varden samples were excluded, but the date of divergence between mtDNA lineages was still estimated as prior to the onset of the last glaciation (5.83% sequence divergence compared to 7.16% with Dolly Varden included).

Increased sampling in Beringia revealed differences in geographical distribution of mtDNA haplotypes compared to Brunner *et al.* (2001), implying that a reinterpretation of the history of these lineages in the region is necessary. The major difference was that Brunner *et al.* (2001) did not identify Bering lineage haplotypes in northern Beringia (Fig. 1), whereas we found this lineage to be widespread in samples of Dolly Varden in northern Alaska and in samples of Arctic char from Russia (Fig. 3). This would suggest that Bering lineage char survived the last glaciation throughout Beringia and were not restricted to southern Beringia as previously suggested.

The distribution of the Arctic lineage uncovered here, however, was consistent with that reported by Brunner *et al.* (2001). The Arctic lineage dominated from the Yukon in the west to Greenland and Québec in the east. The distribution of the Arctic lineage also overlaps with Bering lineage haplotypes, being found in samples of Arctic char from northern Alaska and the Chukotka Peninsula, as well as in two rivers of the Brooks Range. It is interesting to note that, except for the high prevalence of haplotype ARC19 in Horizon Lake Arctic char in Alaska, the Arctic lineage haplotypes found in these areas of overlap differ from elsewhere in the Arctic. For instance, the only location where Arctic lineage haplotypes were identified from Chukotka (Lake Elgygytgyn) contained unique haplotypes (ARC32 and ARC33) not found elsewhere. Similarly, one of the two locations where Arctic and Bering lineage haplotypes co-existed in Dolly Varden populations of the Brooks Range of Alaska (Anaktuvik) contained a unique haplotype (ARC34; Ayers, 2010). Clearly, further sampling in the topographically complex region of northern Beringia would be valuable to determine whether Arctic lineage haplotypes found in the region are isolated from those found west of the Yukon.

Evidence for recolonization of the Canadian Arctic from a small source population

Low genetic diversity in the Arctic lineage implied recolonization of the Canadian Arctic Archipelago from a small source population; estimates of nucleotide and haplotype diversity were an order of magnitude lower in the Arctic

lineage than in the Bering lineage. Furthermore, the star-shaped phylogeny of the Arctic lineage, with one common haplotype (ARC19, shared by 95.3% of the individuals sequenced) found in high frequency in all populations, and numerous rare, geographically restricted haplotypes, were consistent with a recent population expansion (Slatkin & Hudson, 1991; Excoffier *et al.*, 2009). The only other geographically widespread haplotypes (ARC20 and ARC22) were both restricted to the eastern Arctic, supporting the hypothesis of a population expansion from west to east.

The historical demographic analyses suggested a recent population expansion. In the Arctic lineage, negative values of the statistics Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997) were consistent with a recent population expansion (Excoffier *et al.*, 2009), while values for the Bering lineage were not. Note that this contrasted with evidence from Dolly Varden suggesting population expansion in Beringia (Oleinik *et al.*, 2013). The mismatch distribution analysis was also consistent with the hypothesis of a recent population expansion in the Arctic lineage – but not in the Bering lineage. Unfortunately, the information content of the mtDNA data set did not allow for more sophisticated analyses such as Bayesian skyline plots to confirm these interpretations (Drummond *et al.*, 2005).

One major alternative explanation for the lack of variation in mtDNA is that it reflects positive selection for a mitochondrial gene (or genes) linked to the control region haplotypes used to define the Arctic lineage. Selection on mitochondrial genes is common and can severely bias historical interpretations based on the assumption of neutrality (Ballard & Rand, 2005). Furthermore, Arctic char mtDNA has been shown to introgress to fixation (mitochondrial capture) into other related species (lake trout, *S. namaycush* and brook trout, *S. fontinalis*; Bernatchez *et al.*, 1995; Glémet *et al.*, 1998; Wilson & Bernatchez, 1998), perhaps suggesting selection on mitochondrial genes. Concordance between the patterns of genetic variation at nine microsatellite markers, however, makes this alternative explanation unlikely. The STRUCTURE analysis suggested that, apart from populations that showed evidence for introgression from Atlantic lineage Arctic char, most populations in the Canadian Arctic clustered together. One exception was the two Yukon sampling locations, which formed their own cluster. It seems unlikely that the Yukon populations, which had dramatically lower genetic diversity, survived in a separate refugium given that microsatellite allele sizes at all loci were within the range observed in other locations. Low haplotype diversity in the Canadian Arctic could also be the result of allele 'surfing' at the expanding edge of the post-glacial range expansion (Excoffier *et al.*, 2009), but according to this hypothesis haplotype diversity should be higher at the centre of the range expansion. In summary, we argue that the most likely explanation for the lack of diversity in the Arctic lineage is the result of recolonization from a small source population.

Refugial origin of the Arctic lineage

Evidence for the presence of a high Arctic refugium in the Arctic Archipelago now exists for a variety of taxa (birds: Holder *et al.*, 1999; mammals: Fedorov & Stenseth, 2002; plants: Tremblay & Schoen, 1999). Our data are partly consistent with recolonization from a high Arctic refugium, but the presence of Arctic lineage haplotypes in northern Beringia from Alaska and Russia is also consistent with the alternative hypothesis of survival in Beringia. This hypothesis implies that two highly divergent lineages would have survived in proximity for several thousands of years without gene flow, the topographically complex region having allowed isolation in allopatry despite geographical proximity. Such a pattern of 'refugia-within-refugia' has been documented in Europe from the Iberian Peninsula (Gómez & Lunt, 2007), and in Beringia itself (Shafer *et al.*, 2010). The presence of the common ARC19 haplotype in Lake Horizon in Alaska supports the hypothesis of recolonization of the Arctic Archipelago from Beringia. All other instances of the presence of Arctic haplotypes in Beringia, however, largely differ in haplotype composition from those found elsewhere (discussed earlier), which would not be expected if Beringia was the source of recolonization. In addition, we might expect that if Arctic char survived in Beringia, a large ice-free area, it may have survived as a larger population size and harboured more genetic diversity than displayed by our data. Finally, recolonization would have had to occur via anadromous (i.e. searun) individuals, but anadromy is not documented from any Arctic char populations west of the Mackenzie Delta (Reist *et al.*, 1997). In summary, survival of the Arctic lineage in Beringia during the LGM appears likely, but whether these populations were the source for the recolonization of the Arctic Archipelago remains unclear. More data, from increased sampling in northern Beringia and the use of higher resolution markers, will be required to better understand the refugial origin of this lineage.

Secondary contact between glacial lineages

Many studies have shown that genetic diversity declines as distance from putative glacial refugia increases (Turgeon & Bernatchez, 2001; Stamford & Taylor, 2004; Harris & Taylor, 2010; Shafer *et al.*, 2011). In our study, we did not observe a decline of genetic diversity with distance from putative refugia, perhaps because of secondary contact between glacial lineages in the eastern Arctic. A STRUCTURE analysis confirmed that anadromous populations from the eastern Arctic shared part of their nuclear genome with 'Atlantic lineage' Arctic char from Labrador and suggested that secondary contact could have contributed to increased genetic diversity. This was mostly observed in anadromous, but not in landlocked, populations, indicating that this introgression happened following the initial recolonization or that it is due to ongoing gene flow.

Secondary contact left no trace in the mitochondrial DNA, and Arctic populations with 'Atlantic' microsatellite alleles (e.g. south-east Baffin Island populations) remained fixed for the Arctic lineage mtDNA. In contrast, the Atlantic and Arctic lineages co-existed in the northern Labrador sampling locations. This mixing of mitochondrial haplotypes further supports the hypothesis of introgression. This finding is also consistent with the findings of Wilson *et al.* (1996), who documented the presence of two mitochondrial lineages in Labrador Arctic char. Few nuclear alleles from an Arctic lineage background, however, were detected in the Labrador sampling site. This could constitute an example of asymmetric cyto-nuclear or mito-nuclear discordance, a phenomenon commonly observed in other taxa (reviewed in Toews & Brelsford, 2012).

CONCLUSIONS

Our data contribute to a growing number of studies suggesting the importance of multiple glacial refugia north of the ice sheets (Provan & Bennett, 2008; Stewart *et al.*, 2010). The evidence presented is most consistent with the conclusion that Arctic char currently inhabiting the Canadian high Arctic originated from a small refugial population situated in the Arctic Archipelago itself or isolated within Beringia. Additional genetic data of increased geographical scope (most notably from the Brooks Range of Alaska and from eastern Siberia) and with higher resolution markers would be useful to strengthen this conclusion. We also provided evidence for a secondary contact zone between Arctic and Atlantic lineage Arctic char in the eastern Canadian Arctic. Increased genetic diversity in these admixed populations could have implications for adaptation to a changing Arctic (Reist *et al.*, 2006).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Sampling sites information.
Appendix S2 Details of the mtDNA analyses.
Appendix S3 Details of the microsatellite analyses.

DATA ACCESSIBILITY

All mtDNA sequences were deposited to GenBank (see Appendix S1 for accession numbers). Microsatellite raw genotypes (in GENEPOP format) and STRUCTURE data and parameter files were deposited to DRYAD and can be accessed at the following link: http://dx.doi.org/10.5061/dryad.doi:10.5061/dryad.35bn3.

BIOSKETCH

Jean-Sébastien Moore is a post-doctoral researcher working on northern fishes at Laval University. This work was part of his doctoral thesis at the University of British Columbia on patterns and consequences of dispersal in Arctic char from the Canadian Arctic. He is generally interested in molecular ecology and evolutionary biology, and their applications in conservation and management of wild fish populations.

Author contributions: J.-S.M. devised the study, conducted most analyses and wrote the manuscript. R.B. did most of the mtDNA sequencing and provided help with the analyses. J.D.R. and E.B.T. provided supervision, and helped design the study and writing the article. They are all interested in applying genetic techniques to the study of the diversity of native northern fishes.

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