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Pleistocene glaciations and contemporary genetic diversity in a Beringian fish, the broad whitefish, *Coregonus nasus* (Pallas): inferences from microsatellite DNA variation

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broad whitefish;
Coregonus nasus;
phylogeography;
Pleistocene glaciations;
post-glacial dispersal.

Abstract

The contemporary distribution of genetic variation within and among high latitude populations cannot be fully understood without taking into consideration how species responded to the impacts of Pleistocene glaciations. Broad whitefish, *Coregonus nasus*, a species endemic to northwest North America and the Arctic coast of Russia, was undoubtedly impacted by such events because its geographic distribution suggests that it survived solely within the Beringian refuge from where it dispersed post-glacially to achieve its current range. We used microsatellite DNA to investigate the role of glaciations in promoting intraspecific genetic variation in broad whitefish (N = 14 localities, 664 fish) throughout their North American range and in one Russian sample. Broad whitefish exhibited relatively high intrapopulation variation (average of 11.7 alleles per locus, average H_E = 0.61) and moderate levels of interpopulation divergence (overall F_{ST} = 0.10). The main regions assayed in our study (Russia, Alaska, Mackenzie River and Travaillant Lake systems) were genetically differentiated from each other and there were declines in genetic diversity with distance from putative refugia. Additionally, Mackenzie River system populations showed less developed and more variable patterns of isolation-by-distance than populations occupying former Alaskan portions of Beringia. Finally, our data suggest that broad whitefish dispersed from Beringia using coastal environments and opportunistically via headwater stream connections that once existed between Yukon and Mackenzie River drainages. Our results illustrate the importance of history (e.g. glaciation) and contemporary dispersal ecology in shaping the current genetic population structure of Arctic faunas.

Introduction

Investigating the geographic factors and circumstances that promote genetic variation between and within populations is fundamental for understanding how species evolve and adapt to environmental change (Allendorf & Luikart, 2007; Trénel *et al.*, 2008). The contemporary structuring of genetic diversity, however, cannot be fully understood without knowledge of how organisms have been impacted by historical events. For instance, Pleistocene glaciations have played important roles in shaping the current distributions and patterns of genetic diversity in many biotas in a diversity of habitats (e.g. Hewitt, 2000; Schönswetter *et al.*, 2002; Fraser *et al.*, 2009). In particular, freshwater fishes are excellent subjects with which to study the effects of glaciations owing to their restriction within glacial refugia peripheral to the ice sheets and their reliance on aquatic habitats for post-glacial dispersal. The most recent glaciation (referred to as the Wisconsinan in North America), in particular, had a profound impact on Nearctic and Palearctic freshwater fishes (Lindsay & McPhail, 1986; Rempel & Smith, 1998; Bernatchez & Wilson, 1998).

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At least six glacial refugia are recognized for North American fishes (McPhail & Lindsey, 1970). During interglacial periods organisms were able to disperse from refugia and colonize novel environments in a variety of ways (e.g. Rempel & Smith, 1997; Wilson & Hebert, 1998). For instance, deglaciation produced enormous volumes of meltwater that created temporary proglacial lakes and spillways and lowered the salinity of coastal waters (Lindsay & McPhail, 1986; Pielou, 1991; Wilson & Hebert, 1998). As such, what are now distinct drainages were sometimes connected to each other temporarily providing opportunities for fish to disperse into newly deglaciated regions (e.g. Rempel & Smith, 1997; Costello *et al.*, 2003). One of these refugia, known as Beringia, encompassed unglaciated portions of northeast Siberia, including parts of the Kamtchatka Peninsula, and the Yukon River Valley and adjacent areas in Alaska and northwestern Canada. Despite the rapid development of phylogeographic research, species inhabiting Arctic/Antarctic regions are relatively under-studied and Weider & Hobæk (2000) cited studies of Beringian species as a high priority in their review of Arctic phylogeography.

Isolation in Beringia had important impacts on contemporary distributions and population structure in many Nearctic and Palaearctic fish species (e.g. Lindsay & McPhail, 1986; Politov *et al.*, 2004). For fishes in areas previously covered by glaciers, several *a priori* predictions can be made regarding how glaciation impacted genetic diversity and intraspecific phylogeographic structure. First, post-glacial range expansions should create clines of decreasing genetic diversity with increasing distance from refugia (McAllister *et al.*, 1986; Bernatchez & Wilson, 1998; Beebee & Rowe, 2000; Castric & Bernatchez, 2003; Stamford & Taylor, 2004; Muller *et al.*, 2008). Founder effects and bottlenecks associated with post-glacial dispersal also may have caused reductions in genetic diversity in contemporary populations of fishes (Hewitt, 1996; Bernatchez & Wilson, 1998; Taylor *et al.*, 2003). Detecting such clines in genetic diversity can often provide insight into the location of glacial refugia and routes of colonization (e.g. Costello *et al.*, 2003; Mellers, 2006; Konopinski *et al.*, 2007).

Second, isolation-by-distance (IBD, Wright, 1943) should be stronger in populations in the heart of putative refugia, compared to those at the periphery of the range (Hutchison & Templeton, 1999). Under the latter scenario, populations may not have had sufficient time since colonization to reach equilibrium between gene flow and drift (Slatkin, 1993; Turgeon & Bernatchez, 2001a) and therefore no IBD is expected. Although this result has been reported in several cases there are also many exceptions and further model systems need to be examined to assess the generality of IBD expectations (Crispo & Hendry, 2005). Finally, the extent of intraspecific phylogeographic structure should be a reflection of the extent to which particular species were isolated in separate refugia (see Avise *et al.*, 1987; Bernatchez & Wilson, 1998; Petit *et al.*, 2003).

The broad whitefish, *Coregonus nasus*, is an Arctic species that probably survived only in Beringia during the Wisconsinan glaciation (Lindsay & McPhail, 1986; Scott & Crossman, 1998). In North America, this species is distributed through virtually all of former Beringia, and has dispersed post-glacially east to the Perry River, Northwest Territories, and currently occupies several river systems in western Arctic Canada such as the Mackenzie River (McPhail & Lindsey, 1970; Scott & Crossman, 1998). Post-glacial dispersal of freshwater fish from Beringia to the Mackenzie River was probably limited to two main routes (Fig. 1): dispersal solely through freshwater and dispersal through marine habitats along the Alaskan north coast, which was also largely unglaciated (Lindsay & McPhail, 1986). Under the first scenario – the 'interior hypothesis' – meltwater from retreating glaciers formed large proglacial lakes (e.g. Lakes Bonnet Plume and Old Crow) and advancing ice sheets blocked and diverted numerous rivers. Consequently, some of these rivers that were originally tributary to the Yukon River reversed their flow into the Mackenzie River providing temporary connections between these now isolated drainages (e.g. via the Peel and Porcupine river diversions, Bodaly & Lindsey, 1977; Lindsay & McPhail, 1986; Pielou, 1991). In fact, Bodaly & Lindsey (1977) presented morphological, distribution and allozyme evidence of affinities between Mackenzie River and upper Yukon River populations of six fresh-

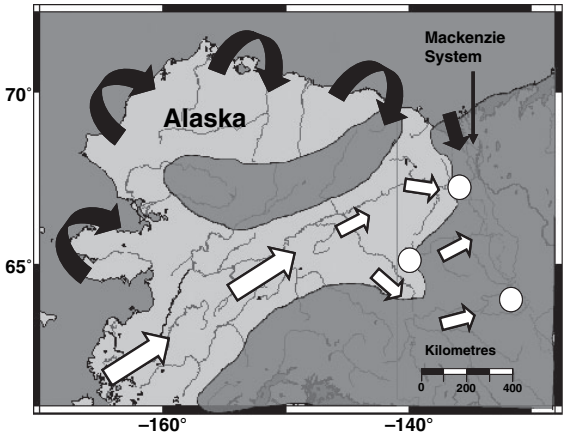

Fig. 1 Map showing the two potential dispersal routes from Beringian North America to the Mackenzie River system. Black arrows show dispersal via coastal marine environments and white arrows represent inland freshwater dispersal. The white circles indicate areas where there were, at one time, connections between the Yukon and Mackenzie River systems that allowed for faunal exchange. The dark grey-shaded regions indicate areas of ice cover during the height of the most recent Wisconsinan glaciation (modified from Lindsay & McPhail, 1986).

water fish species that appeared to result from such post-glacial watershed exchanges.

Alternatively, under the 'coastal hypothesis', broad whitefish dispersed into the Mackenzie River system utilizing coastal habitats of the Arctic Ocean as glacial ice retreated (Wilson *et al.*, 1996; Wilson & Hebert, 1998). Broad whitefish are well adapted to brackish water environments as evidenced by the anadromous life history observed in most populations (Tallman & Reist, 1997 and references therein) and therefore dispersal via marine environments along the Alaskan Arctic coast until reaching the Mackenzie River system is plausible.

Microsatellites are typically highly polymorphic and thus are an excellent tool for inferring dispersal routes from refugia because post-glacial dispersal took place within only the past 10 000–12 000 years after the retreat of Wisconsinan glaciers (Lindsay & McPhail, 1986; Angers & Bernatchez, 1998; Koskinen *et al.*, 2002). We used microsatellite DNA to test several predictions about the potential influences of historical processes on spatial genetic variation within and between contemporary populations of North American broad whitefish. Specifically, if broad whitefish had been isolated in multiple refugia in addition to Beringia, then we expected to resolve major genetic distinctions (distinct clusters, unique alleles) among whitefish located in distinct areas, or admixtures of divergent groups in single regions that could result from secondary contact between glacial lineages (Turgeon & Bernatchez, 2001a,b; Petit *et al.*, 2003). Next, we predicted that the highest genetic diversity would be observed in populations occupying drainages covering areas of the former Beringian refuge (i.e. populations from Alaska) and genetic diversity would decrease in populations at the periphery of the range farther away from the refuge (i.e. populations in the Mackenzie River system). We also expected to see differences in patterns of IBD between samples collected from areas covering putative refugia and those at the periphery of the range that have colonized the area more recently. Finally, we use molecular data to test alternative hypotheses of how broad whitefish colonized the Mackenzie River system: via headwater connections between the Peel and Porcupine rivers (of the Mackenzie and Yukon river systems, respectively) or via coastal environments along the North Slope of Alaska.

Materials and methods

Fish collection and DNA extraction

A total sample of 664 adult broad whitefish was obtained from 14 locations from in 2004 and 2005 (Table 1, Fig. 2). Tissues consisted of fin or muscle preserved in 95% ethanol. DNA was isolated from approximately 5 mg of tissue using following Qiagen (Valencia, CA, USA) DNA extraction kit protocols.

Table 1 Broad whitefish sampling locations and sample sizes (*N*). Numerals following location names refer to locations on Fig. 2.

Region/sampling location	Latitude	Longitude	*N*
Mackenzie River System			
Peel River (1)	67°15	134°53	189
Arctic Red River (2)	66°58′	133°16′	35
Point Separation (3)	67°35′	134°04′	23
Fort Good Hope (4)	66°39′	129°25′	34
Campbell Lake (5)	68°12′	133°27′	29
Travaillant Lake System			
Travaillant River South (6)	67°36′	131°51′	69
Travaillant River North (7)	67°45′	131°51′	65
Alaska			
Yukon River (8)	65°11′	151°58′	50
Yukon River–Rampart Rapids (9)	65°34′	151°06′	30
Tanana River (10)	64°45′	149°56′	12
Teshekpuk Lake (11)	70°45′	153°57′	32
Selawik River (12)	66°57′	160°21′	30
Whitefish Lake (13)	61°24′	160°01′	30
Russia			
Pechora River (14)	66°53′	53°47′	36

Microsatellite amplification and scoring

Microsatellite loci used in this study were Cocl-Lav4, Cocl-Lav6, Cocl-Lav8, Cocl-Lav10, Cocl-Lav18, Cocl-Lav27 (Rogers *et al.*, 2004) and Ots103 (Small *et al.*, 1998). Polymerase chain reaction (PCR) protocols were as described in Rogers *et al.* (2004) for the Cocl-Lav primers and Small *et al.* (1998) for Ots103, with slight modifications. Briefly, each PCR was performed in a 10-μL volume with 1-μL of genomic DNA, 2 μM of the fluorescently labelled forward primer and 5 μM of the unlabelled reverse primer, 10 mM dNTP, 1 μL reaction buffer (New England Biolabs) and 0.1 U of *Taq* polymerase (New England Biolabs, Ipswich, MA, USA).

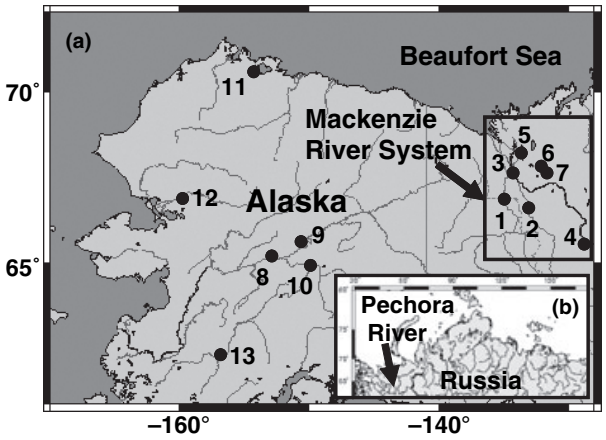

Fig. 2 Map showing sampling locations for throughout Alaska (a), the Mackenzie River system (b) and the Pechora River, Russia (c). Numbers correspond to populations outlined in Table 1.

Fluorescently labelled PCR products were visualized on a Beckman-Coulter CEQ 8000 automated genotyper where alleles were scored by eye using the Beckman-Coulter 400 bp size standard.

Genetic analysis

Basic descriptive statistics of microsatellite variation, including the number of alleles (N_A), expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated using TFPGA ver. 1.3 (Miller, 1997). Because the observed number of alleles per locus (N_A) depends on sample size, allelic richness (A_R) was calculated using FSTAT ver. 2.9.3.2. (Goudet, 2002) to account for different sample sizes. Tests for deviations from Hardy–Weinberg expectations were performed in GENEPOP ver. 3.4 (Raymond & Rousset, 2003) for each locus–population combination using an exact test in which two-tailed *P*-values were estimated using a Markov chain method of Guo & Thompson (1992). Tests for genotypic linkage disequilibrium for locus pairs within populations were also conducted in GENEPOP.

We tested for population differentiation between all pairs of populations over all loci combined using log-likelihood (G)-based exact tests (Goudet *et al.*, 1996) with default values in GENEPOP. The significant level for these tests was adjusted using the sequential Bonferroni procedure with an initial alpha level of 0.05. Population structure was estimated with *F*-statistics (F_{ST}) to measure genetic differentiation between populations. Specifically, we used Weir & Cockerham's (1984) θ to estimate F_{ST} using FSTAT where resampling procedures using jackknife and bootstrap methods over loci were used to generate *P*-values and 95% confidence intervals.

Hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) was conducted using ARLEQUIN version 3.1 (Excoffier *et al.*, 2005), to determine how the genetic variation was partitioned geographically. The percentage of the total genetic variation within populations (V_c), among populations within groups (V_b) and by differences among groups (V_a) was calculated under a variety of geographical groupings hypotheses to assess the presence of major geographic divisions that may reflect isolation in distinct refugia.

We tested for differences in extents and geographic patterns of genetic variation between groups of contemporary populations of broad whitefish. First, we used FSTAT's permutation procedures to test for differences in allelic richness (A_R) and expected multilocus heterozygosity (H_E), between groups of populations suspected to have different histories: those from Alaska occupying drainages covering areas of the former Beringian refuge and more recently founded populations from the Mackenzie River and the Pechora River. We predicted that populations further away from the Beringian refugium would have lower levels of genetic variation than those found in and around central Alaska. Additionally, North American populations were organized by fluvial distance from the putative Beringian refuge (at the confluence of the Koyukuk and Yukon rivers, see Stamford & Taylor, 2004) and this variable was regressed against measures of genetic variation (H_E and A_R) using JMPIN (version 3.2.1) to test for trends in genetic variation and distance from the refugium.

Second, the IBD model predicts that genetic distance between populations increases as the geographic distance increases because of reduced gene flow at greater distances (Slatkin, 1993). Patterns of IBD are revealed by a direct relationship between estimates of genetic differentiation (e.g. F_{ST}) and geographical distances when populations are at equilibrium under genetic drift and migration. We predicted that IBD would be more pronounced in populations inhabiting former refugia (coastal and central Alaska) as they have presumably had a longer time period to establish equilibrium between drift and migration compared to post-glacial populations (Mackenzie River populations, Hutchison & Templeton, 1999). We tested for the strengths of IBD using the Mantel test option in FSTAT by assessing the significance of correlations between geographical distance (i.e. fluvial distance between sampling locations) and genetic distance (F_{ST}). Geographic distances between sampling locations within the study area was determined using the Geographic Information System (GIS) program, ARCVIEW (version 3.14, ESRI). Isolation-by-distance was tested over all sampling locations, then separately for all locations in Alaska and all locations in the Mackenzie River drainage.

Third, we also expected that signatures of demographic bottlenecks would be more apparent in populations from more recently colonized areas (i.e. the Mackenzie River and Pechora River). We used the program BOTTLENECK (Cornuet & Luikart, 1997) to test this prediction. The program uses coalescent simulations to generate gene diversities for each population and locus that are expected from the observed number of alleles given the sample sizes and assuming mutation-drift equilibrium. The calculated average expected gene diversity is then compared to that observed gene diversity to assess whether there is gene diversity excess or deficit at each locus. Populations that have undergone recent bottlenecks will show gene diversities that are excessive relative to that expected given the observed number of alleles because allele number declines more rapidly than heterozygosity during bottlenecks. Both the SMM and the two-phase (TPM) model were assumed in making calculations using Wilcoxon signed-rank tests (Luikart & Cornuet, 1998) with 1000 iterations. Under the TPM of mutation, 95% single-step mutations (12% variance of multi-step mutations) were used as suggested by Piry *et al.* (1999).

We used a series of tests to assess the 'coastal' vs. 'inland' post-glacial recolonization hypotheses. First, we assessed general patterns of population similarity using

two clustering programs. We identified population groups exhibiting major genetic discontinuities by visualizing genetic differentiation among samples using a factorial correspondence analysis (FCA) performed with GENETIX 4.05.02 (Belkhir *et al.*, 2004). Second, the Bayesian model-based clustering algorithm implemented under the STRUCTURE software (Pritchard *et al.*, 2000) was used to assess the level of population subdivision without any *a priori* designation of populations. This analysis uses a likelihood approach to find the most likely number of K populations in the total data set that minimizes departures from Hardy–Weinburg equilibrium and linkage equilibrium. The STRUCTURE analysis was conducted assuming an admixture model with a burn-in period of 10 000 followed by 10 000 MCMC replications (longer runs did not alter the results). We assessed the likelihood of different values of K from one to six (cf. Evanno *et al.*, 2005) across 20 independent runs to check for variability of obtained log-likelihood values (Pritchard *et al.*, 2000). We chose the K with the highest average log-likelihood score as representing the most likely number of populations; we did not employ the *ad hoc* delta K method of Evanno *et al.* (2005) as our average F_{ST} values were lower (i.e. generally less than 0.1 – see below) that appear appropriate for optimal use of this method (Waples & Gaggiotti, 2006). Under both the GENETIX and STRUCTURE clustering procedures, we expected Mackenzie River populations to be most similar to the Teshekpuk Lake broad whitefish under the coastal hypothesis and to central Alaskan fish under the interior recolonization hypothesis.

Lastly, we inferred past (long term) and contemporary levels of migration between the Mackenzie River area and Alaskan samples grouped into either 'coastal' (Teshekpuk Lake, Selawik River) or 'interior' assemblages (all other Alaskan populations). We estimated contemporary migration levels using BAYESASS (vers 1.3, Wilson & Rannala, 2003). Identification of immigrants or the offspring of immigrants is facilitated by identifying individuals that display genotypic disequilibrium relative to the population on which they were sampled. We ran 3×10^6 iterations with a burnin over the first 10^6 iterations and a sampling frequency of 2000 iterations. Next, we estimated long term migration rates using MIGRATE'S (vers. 3.03, Beerli, 2008) maximum-likelihood approach employing 10 short chains and three long chains, and a burn-in period of 10 000 trees for each chain, and an adaptive, four temperature heating scheme. Under the 'coastal' hypothesis, we expected both contemporary and long-term migration levels to be highest between the Mackenzie River and coastal Alaskan populations. Under the 'interior' hypothesis, we expected long-term migration level estimates to be highest between the Mackenzie River and 'interior' Alaskan populations. By contrast, and because there is no current connection between the upper Yukon River and the Mackenzie River and because intervening coastal populations are much closer to the Mackenzie River than areas sampled within the Yukon River, we expected that contemporary migration levels to be higher between the Mackenzie River and coastal populations. Given the intensive computations involved in MIGRATE we reduced the dataset by sub-sampling a random 50 individuals from each of the four geographic regions where total sample sizes permitted (i.e. Mackenzie River drainage, coastal Alaskan populations, Yukon River populations). This allowed us to re-run the MIGRATE analysis several times to check for consistency of results. The results using the subsets of data were similar to the results obtained over fewer runs that were conducted on the full dataset. MIGRATE also allows one to restrict migration parameters to hypothetical values to test these values against maximum-likelihood estimates using the likelihood ratio test (Beerli, 2008). We tested whether the maximum likelihood migration values obtained from the full dataset were significantly better than hypothesized scenarios of zero historical migration between the Mackenzie River and coastal Alaska, interior Alaska, or a combination of both coastal and interior exchange with the Mackenzie River. The analyses in MIGRATE assumes a Fisher–Wright population model with each population constant in size over time and with constant population exchange rates over time. The results of the analyses are also most robust when all known populations have been sampled (Beerli, 2004). All of these ideals are likely violated by our data to some extent and our interpretation of the results of these analyses are restricted to assessing *a priori* predictions about relative migration levels under the alternative post-glacial colonization hypotheses.

Results

Intrapopulation genetic variation

All seven loci were polymorphic in this study, with allele numbers ranging from five (Cocl-Lav27) to 18 (Cocl-Lav8), with an average of 11.7 alleles per locus (Table S1). Expected heterozygosity (H_E) ranged from 0.45 (Cocl-Lav27) to 0.70 (Ots103) and the overall mean allelic richness was 3.5 (based on a minimum sample size of nine diploid individuals), and varied from 2.6 in the Pechora River, Russia sample to 4.4 in the Yukon River at the Rampart Rapids, Alaska. The mean H_E over all sampling locations was 0.58, and ranged from 0.42 in the Pechora River, Russia, to 0.64 in the Yukon River at the Rampart Rapids. Following Bonferroni corrections for simultaneous multiple tests (new alpha = 0.00052), conformation to Hardy–Weinberg equilibrium was not rejected in any of the 98 exact tests and there were no instances of significant genotypic linkage disequilibrium in any of 924 tests.

When sampling locations were grouped into those from areas that served as putative refugia during the last Wisconsinan glaciation (i.e. samples from Alaska) and

those from previously glaciated areas at the periphery of the current range (i.e. the Mackenzie River system and Pechora River), significant differences were observed between allelic richness and expected heterozygosity (permutation tests, all $P < 0.01$). As predicted, samples from Alaska had significantly higher average A_R (3.9 compared to 3.4) and average H_E heterozygosity (0.63 compared to 0.53) when compared with nonrefugial populations. Additionally, broad whitefish genetic diversity declined with fluvial geographical distance from the lower Yukon River in Alaska (Fig. 3); there was a significant negative correlation between geographical distance from the lower Yukon River and both A_R ($r = -0.78$, $P = 0.0019$) and H_E ($r = -0.79$, $P = 0.0012$).

We found no evidence that any of the populations assayed in this study have been subjected to a recent bottleneck under both the stepwise and TPM of mutation (Table 2). All populations exhibited some loci with higher than expected heterozygosity given the observed number of alleles; for no population, however, was there a significant ($P < 0.05$) deviation from equilibrium (non-bottlenecked) expectations.

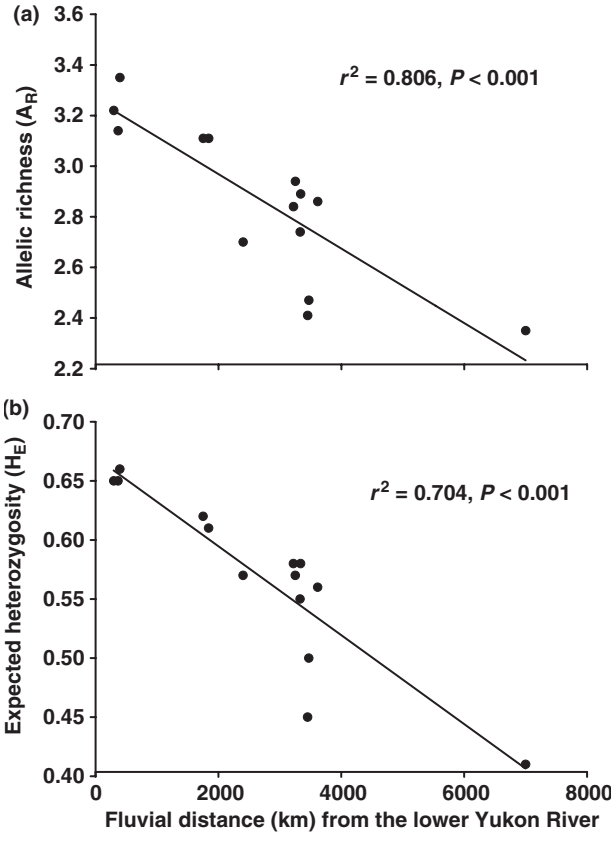

Fig. 3 Change in microsatellite genetic diversity within broad whitefish populations vs. their geographical distance from the lower Yukon River, at the confluence with the Koyukuk River. (a) Allelic richness (A_R) vs. geographical distance (km); (b) Expected heterozygosity (H_E) vs. geographical distance (km).

Interpopulation divergence and genetic structure

Log-likelihood (G)-based exact tests of population differentiation suggested that the two main regions included in this study (i.e. Mackenzie River system populations and Alaska populations) are significantly differentiated from each other ($P < 0.05$). All North American populations differed significantly from the Pechora River population. Alaskan populations were differentiated from all other populations, with the exception of the two Yukon River samples and the sample from the Tanana River which was only significantly differentiated from the Travaillant Lake (Mackenzie River system) populations.

Overall, F_{ST} (θ) ranged from 0.034 (Cocl-Lav8) to 0.206 (Cocl-Lav6) and the overall level of population subdivision based on pairwise estimates was moderate ($\theta = 0.10$, 95% CI 0.059–0.138) among all populations. Among populations, pairwise θ values ranged from 0.0 (between two Yukon River populations) to 0.45 (between the Pechora River in Russia and a sample from the Travaillant Lake system). Most differences in θ were statistically significant (83 of 105 comparisons, $P < 0.01$). There were, however, several comparisons that were not significant and these usually consisted of populations within the same river or lake system (Table S2).

We found evidence of large genetic differences among various populations assembled into groups largely based on the topologies of the STRUCTURE and FCA analysis

Table 2 Wilcoxon sign-rank tests for heterozygosity excess in 14 populations of *Coregonus nasus* under a stepwise (SMM) two-phase model (TPM) of mutation.

	SMM		TPM	
Population	He/Hd	*P*	He/Hd	*P*
Peel River	1/6	1.00	1/6	0.99
Arctic Red River	2/5	0.98	2/5	0.97
Point Separation	3/4	0.81	4/3	0.77
Fort Goodhope	1/6	0.99	1/6	0.98
Campbell Lake	3/4	0.95	3/4	0.95
Travaillant Lk South	1/6	0.99	0/7	0.99
Travaillant Lk North	1/6	0.95	3/4	0.77
Yukon River	2/5	0.81	5/2	0.47
Yukon River-Ramparts	3/4	0.82	3/4	0.71
Whitefish Lake	3/4	0.77	3/4	0.71
Teshekpuk Lake	1/6	0.97	1/6	0.96
Tanana River	3/4	0.71	3/4	0.66
Selawik River	4/3	0.53	4/3	0.34
Pechora River	3/4	0.95	3/4	0.77

He/Hd represents the ratio of the number of loci with a heterozygosity excess to the number with a heterozygosity deficiency where *P* the statistical significance of any deviation from equilibrium (nonbottleneck) expectations (≤ 1 : 1).

Table 3 Results of analysis of molecular variance showing the grouping hypotheses tested in this study.

Grouping hypothesis	V_a	V_b	V_c
1. North America vs. Russia	30.6***	3.3***	66.1***
2. Mackenzie System (Including Travaillant Lake) vs. Alaska	3.8***	2.7***	93.4***
3. Mackenzie System (Including Travaillant Lake) vs. Alaska vs. Russia	13.4***	3.2***	83.4***
4. Mackenzie System vs. Travaillant System vs. Alaska vs. Russia	9.8***	1.8***	88.6***
5. Mackenzie System vs. Travaillant Lake System	3.1***	0.9***	96.0***

Variation among groups (V_a), variation among populations within groups (V_b) and variation within populations (V_c).
***$P < 0.001$.

(Table 3, see below). When all North American samples were pooled and compared against the sample from Russia, 30.6% of the variation in allele frequencies was attributable to this grouping ($P < 0.001$). When Alaskan samples and the Russian sample were combined and compared to all samples from the Mackenzie River system only 3.8% of the variation in allele frequencies was attributable to this grouping ($P < 0.001$), but when samples were grouped into Russian vs. Alaskan vs. Mackenzie River system, 13.8% variance was explained by this grouping ($P < 0.001$). There was only one Russian population in our survey so we could not calculate variation among populations within regions (V_b) for Russia. The large difference between the Russian and North America samples, however, was driven largely by major differences in frequencies of shared alleles rather than by the presence of unique allele; there were only three alleles out of total of 82 resolved (across two of the seven loci) that were found at frequencies of ≥ 5% in the Russian sample that were not found in the North American samples.

The FCA revealed a strong geographical pattern of genetic variation suggesting the distinct genetic composition of Alaskan and Mackenzie River system groups of populations (Fig. 4a). In the FCA, three broad groupings of broad whitefish were resolved in addition to the Pechora River which was highly divergent from North American samples. Within North America, groupings were largely influenced by the geographic location of the collection site. One group (A) consisted of a clustering of broad whitefish collected from Alaska, except for the Teshekpuk Lake population. A second group (B) con-

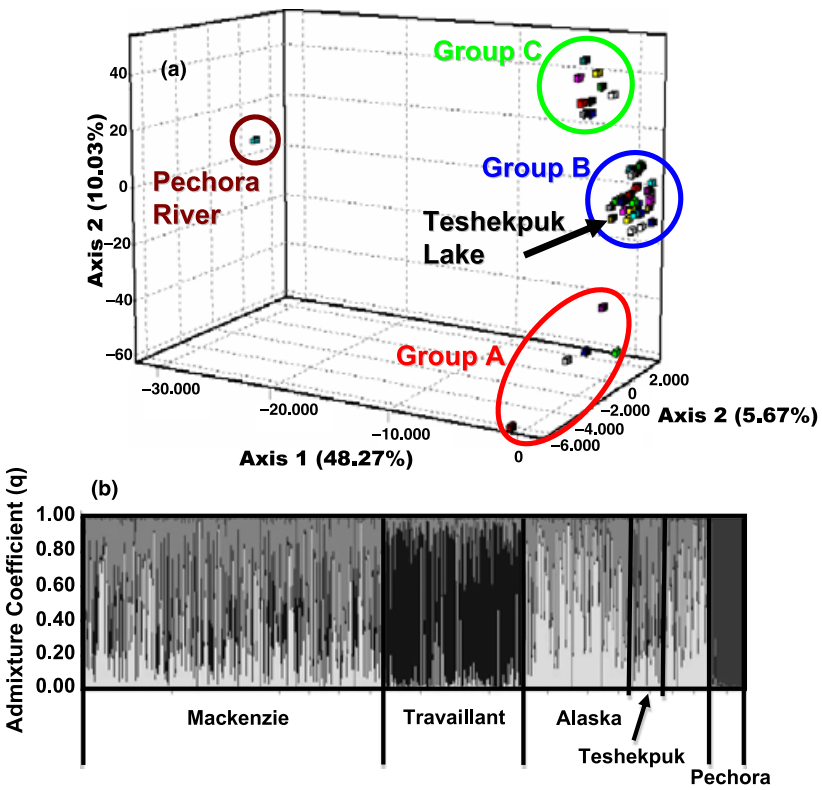

Fig. 4 Factorial correspondence analysis (a) of all samples from Alaska (Group A), all samples from the Mackenzie River system excluding those from Travaillant Lake (Group B) and all samples solely from the Travaillant Lake system (Group C), and (b) results of the STRUCTURE analysis highlighting the same groupings. Note the clustering of the Teshekpuk Lake population from Alaska with the Mackenzie River populations in both analyses.

sisted of all samples collected from the Mackenzie River system (i.e. Mackenzie River proper, Peel and Arctic Red rivers), but excluding Travaillant Lake. Interestingly, this Mackenzie River system grouping also contained the Teshekpuk Lake population which is found north of the Brooks Mountain Range on Alaska's North Slope. The third group (C), a very tight clustering, consisted of all samples collected in the Travaillant Lake system. Results of the STRUCTURE analysis (Fig. 4b) were strikingly similar to that of the FCA. The most likely number of clusters (K) resolved in our study was four (log likelihood = −9940.3) and these groupings, in general, corresponded to geographic location; fish from the Mackenzie River, Travaillant Lake, and Teshekpuk Lake on Alaska's North Slope were characterized by genetic groups 1, 2 and 3, but in different proportions; Travaillant Lake fish were dominated by group 2 (Fig. 4b). Fish from other, interior, Alaskan populations were largely characterized by genetic group 1 and 3, and Pechora River fish were dominated by the fourth genetic group (Fig. 4b).

Isolation-by-distance

Across the study area, the extent of genetic differentiation between populations was strongly related to geographic distance ($r = 0.86$, $P < 0.0001$, Fig. 5a). As predicted, however, within this overall pattern there were some notable deviations in patterns of IBD between regions within the study area. For example, patterns of IBD across all North American populations revealed a significant relationship between F_{ST} and geographic distance ($r = 0.43$, $P < 0.0001$, Fig. 5b). Alternatively, when samples from Alaska (the Beringian refuge area) were tested for patterns of IBD, the relationship was positive, but nonsignificant ($r = 0.40$; $P = 0.14$, Fig. 5c). Within the Mackenzie River system itself, however, the pattern of IBD was much weaker and also nonsignificant ($r = 0.062$, $P = 0.79$, Fig. 5d).

Migration levels and post-glacial dispersal routes

Our estimates of current and historical migration among populations provided evidence for post-glacial recolonization of the Mackenzie River both by coastal and interior routes. Our Bayesian estimates of contemporary migration rates varied from $m = 0.0$–0.303 and were typically asymmetrical. The highest levels of inferred migration were from the coastal Alaskan populations to the Mackenzie River populations and to the interior (Yukon River) assemblage of populations (Fig. 6). These were the only contemporary migration rate estimates whose 95% confidence intervals did not include 0.

Estimates of long-term migration (scaled by mutation rate) also tended to be asymmetric and varied from a low of < 1.0 from all localities and the Pechora River to highs of 2.5–2.8 between coastal Alaska and the Mackenzie River (Table 4). In general, the levels of inferred exchange between the Mackenzie River and either coastal or interior (Yukon River) Alaska were similar and the confidence intervals overlapped (Table 4). When, however, the analyses were run using only the Peel River (a western tributary of the Mackenzie River), inferred historical exchange between interior Alaska and the Peel River was consistently higher than between coastal Alaska and the Peel River (Table 4). By contrast, when all Mackenzie River populations except the Peel River were included in the analysis, inferred historical

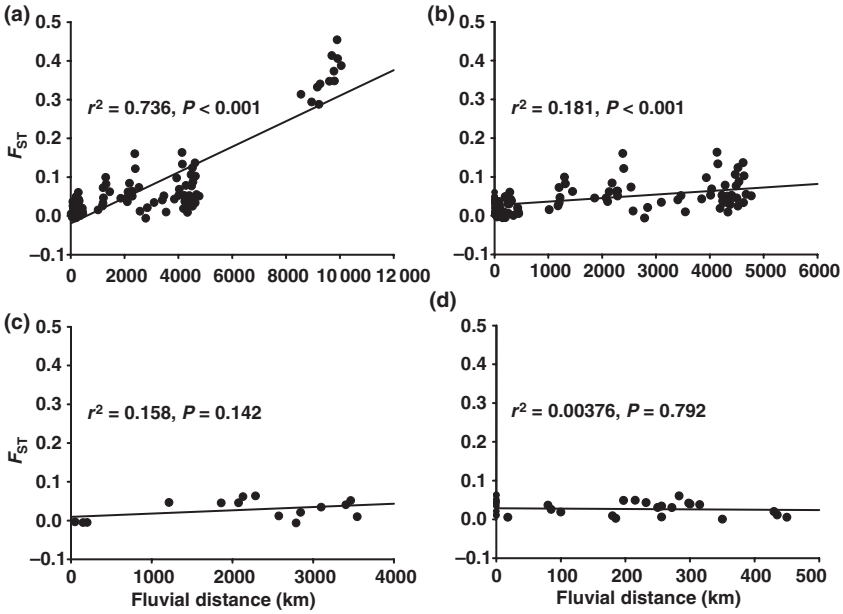

Fig. 5 Isolation-by-distance patterns over all populations (a), across all North American populations (b), only those from former Beringia (c) and those from the Mackenzie River system (d). F_{ST} as defined by Weir & Cockerham (1984).

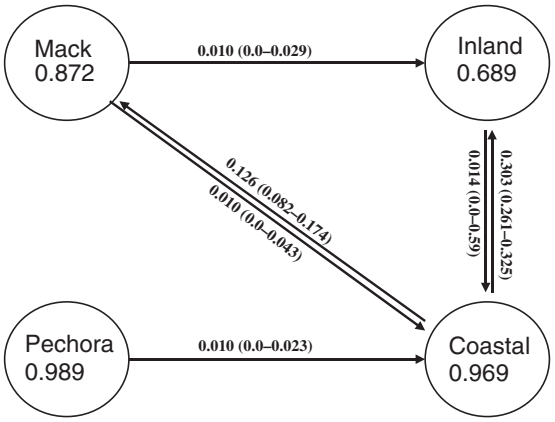

Fig. 6 Inferred directions of gene flow between sample areas based on contemporary (BAYEASS) migration estimates for North American and Russian broad whitefish (see Fig. 1). Only migration rates (*m*, the proportion of the population that migrated in the direction show by the arrow per generation) estimates greater than 0.01 are shown (with confidence intervals). Arrows represent direction of gene flow. 'MACK' refers to localities 1–7, 'INLAND' to localities 8–10, 13, 'COASTAL' to localities 11–12 and 'PECHORA' to locality 14 (Table 1).

migration was again higher between the coastal Alaskan populations and the Mackenzie River (Table 4). Using the full dataset, the maximum-likelihood estimates of *M* were all significantly better than alternative models that enforced no historical migration between coastal and interior Alaska and the Mackenzie River (likelihood ratio tests, d.f. 2–10, all $P < 0.001$).

Discussion

Genetic differentiation within and between populations of *C. nasus*

Our study represents one of relatively few large scale examinations of genetic diversity conducted on a freshwater fish with a primarily Arctic distribution. We found higher levels of genetic diversity within populations of *C. nasus* located in or close to putative refugia (i.e. Alaskan samples) compared to those at the periphery of their current range (i.e. Mackenzie River system and Pechora River samples). This is consistent with empirical evidence provided by several other studies examining genetic diversity in post-glacial populations of north temperate fishes of North America (e.g. Castric & Bernatchez, 2003; Stamford & Taylor, 2004). Furthermore, we showed that genetic diversity (H_E) declined significantly with distance from the lower Yukon River in Alaska. A decrease in genetic diversity with distance from putative refugia has also been shown in other studies of north temperate vertebrates (Merilä *et al.*, 1996; Bernatchez & Wilson, 1998; Castric & Bernatchez, 2003; Costello *et al.*, 2003; Stamford & Taylor, 2004). The observations suggest that founder events and population bottlenecks associated with post-glacial recolonization from Beringia have likely played the most important role in shaping the patterns of extant variation in North American broad whitefish. Through chance founding events during colonization, populations surviving on the periphery of the colonization front would likely be composed of smaller, more isolated populations with reduced genetic variability compared to source populations that survived in putative glacial refugia. This phenomenon is quite common in newly founded or bottlenecked populations (Nei *et al.*, 1975; Castric & Bernatchez, 2003; Costello *et al.*, 2003; England *et al.*, 2003). The decrease in genetic variation with distance from refugia may be particularly pronounced in Mackenzie River populations because, except for only two other much smaller rivers (the Anderson River and Coppermine rivers), the Mackenzie River represents the eastern periphery of North American broad whitefish range (Scott & Crossman, 1998). Furthermore, the founding populations in the Mackenzie River may have been quite small because of 'leading edge' colonization where the effects of drift would have been more pronounced in these populations. These putative historical effects assume that there are no contemporary factors that have generated consistently lower effective population sizes (EPS) in all areas sampled outside the proposed refugium. Unfortunately, there are no good data on EPS in extant populations, but certainly within the Mackenzie River system spawning populations are thought to number in the millions of adults (Thera, 1998 – see below).

The FCA and STRUCTURE results revealed four population genetic clusters that are a general reflection of the geographic distribution of the populations: Russia, Alaska, the Mackenzie River system and the Travaillant Lake system. A substantial genetic discontinuity between North American populations and the Pechora River population from Russia was indicated by all analyses. This is not surprising because of the wide separation of populations sampled (~3470 km). Sampling more geographically intermediate populations would undoubtedly reduce the level of genetic distinction between the Russian and North American samples, yet there is probably some real distinction between populations from the different continents given their physical separation and their probable use of distinct glacial refugia. In addition, Lindsay & McPhail (1986) and Politov *et al.* (2004) also found that *Coregonus* spp. on opposite sides of the Bering Strait were highly divergent from each other. By contrast, others have found that populations of fishes from Alaskan portions of Beringia were more closely related to those from Siberia and not to their North American counterparts that survived in distinct North American refugia (Brunner *et al.*, 2001; Van Houdt *et al.*, 2003; Stamford & Taylor, 2004). A major implication of these results is that Beringia appears to be a zone

Table 4 Results of MIGRATE analysis to infer historical levels of gene flow between sample localities.

	IA > MR	CA > MR	PR > MR	MR > IA	CA > IA	PR > IA	MR ≥ CA	IA > CA	PR > CA	MR > PR	IA > PR	CA > PR
All Mackenzie River												
Mean	2.11	2.55	0.49	2.33	2.22	1.22	2.87	2.08	0.64	0.58	0.43	0.60
L95%	0.92	1.37	0.00	1.15	1.03	0.05	1.69	0.90	0.00	0.00	0.00	0.00
U95%	3.30	3.74	1.68	3.52	3.40	2.40	4.10	3.27	1.83	1.77	1.61	1.78
	IA > PeR	CA > PeR	PR > PeR	PeR > IA	CA > IA	PR > IA	PeR > CA	IA > CA	PR > CA	PeR > PR	IA > PR	CA > PR
Peel River only												
Mean	3.50	2.10	0.40	2.61	2.10	0.92	1.40	2.01	0.42	0.33	0.34	0.32
L95%	0.92	1.37	0.00	1.15	1.03	0.05	1.69	0.90	0.00	0.00	0.00	0.00
U95%	3.30	3.74	1.68	3.52	3.40	2.40	4.10	3.27	1.83	1.77	1.61	1.78
	IA > MR	CA > MR	PR > MR	MR > IA	CA > IA	PR > IA	MR > CA	IA > CA	PR > CA	MR > PR	IA > PR	CA > PR
Mackenzie River except Peel River												
Mean	1.73	2.68	0.71	3.80	2.74	0.94	2.12	2.52	0.67	0.25	0.64	0.38
L95%	0.74	1.69	0.00	2.80	1.75	0.00	1.13	1.53	0.00	0.00	0.00	0.00
U95%	2.72	3.67	1.70	4.79	3.73	1.92	3.11	3.51	1.65	1.24	1.63	1.37

Values shown are M and represent estimated migration parameter scaled by mutation rate and are inferred from variation at seven microsatellite DNA loci. Each value is the average from five MIGRATE analyses and each is accompanied by upper and lower 95% confidence values. 'All Mackenzie River' refers to analyses run with all localities within the Mackenzie River system used (Peel River, Arctic Red River, Campbell Lake, Fort Good Hope, Point Separation and Travaillant Lake). 'Peel River only' refers to analyses run using only samples from the Peel River. Boldface contrasts indicate those under the 'Interior dispersal hypothesis'; those underlined indicate those under the 'Coastal dispersal hypothesis'.

IA, Interior Alaska; MR, Mackenzie River; CA, Coastal Alaska; PR, Pechora River; PeR, Peel River. The migration parameter (M) is presented as migration from one population into (>) the other.

of secondary contact between Nearctic fish and those of a Eurasian origin (e.g. Bernatchez & Dodson, 1994).

Within North America, Alaskan populations were almost always significantly differentiated from Mackenzie River system populations. Much of the current genetic structuring of North American broad whitefish can again be attributed to historical events (see below), but restricted contemporary gene flow between these regions is probably also an important factor influencing the levels of genetic differentiation between Alaskan and Mackenzie River populations. Given the anadromous life history of most broad whitefish populations (Reist & Chang-Kue, 1997), the movement of individuals between the Mackenzie River system and proximate systems in Alaska (i.e. Sagnavirnoktok River System), or *vice versa*, is quite plausible. Such movement could explain the clustering of Teshekpuk Lake populations with those from the Mackenzie River system rather than with other Alaskan populations. Future tagging studies (e.g. Floy or telemetry) coupled with microsatellite analyses on intervening populations could provide more insight into the occurrence of broad whitefish coastal migrations between Alaskan and Mackenzie river systems, especially along the North Slope of Alaska.

Range-wide, a strong pattern of IBD was detected in our study area although there were some notable regional departures in patterns of IBD within different regions. Our prediction that populations in the heart of their species range, or those in areas that previously served as glacial refugia (i.e. Beringian populations) should exhibit strong IBD because they have been established longer allowing enough time for migration-drift equilibrium to be established was not supported, at least statistically. Crispo & Hendry's (2005) meta-analysis of IBD found only weak evidence for an association between time since colonization and IBD. These authors suggested that other factors, such as dispersal ability, geographic barriers to dispersal, proximity to putative refugia and EPS are important to consider when disentangling the effects of gene flow, drift and geographic distances between populations. Although it appears Beringian populations are closer to equilibrium between gene flow and drift in comparison to those from the Mackenzie River system (i.e. $r = 0.40$ vs. 0.062 in the systems, respectively), the lack of statistically significant IBD in the former group may be a result of one of these aforementioned factors. For example, in the Yukon River system where barriers to dispersal are limited, broad whitefish populations migrate upwards of 1700 km on route to spawning locations (R. Brown, U.S. Fish and Wildlife Service, Anchorage, AK, personal communication) possibly implicating high dispersal ability in the lack of IBD (Crispo & Hendry, 2005) in some Beringian populations. Furthermore, the number of populations that we sampled within the former Beringia in Alaska was relatively small ($N = 6$) and a larger sample would increase the power to detect subtle IBD.

Consistent with many other studies incorporating peripheral populations (Hutchison & Templeton, 1999; Holder *et al.*, 2000; Rafínski & Babik, 2000) our data suggest that Mackenzie River populations have not existed long enough for regional equilibrium between gene flow and drift to be reached. Turgeon & Bernatchez (2001a) also resolved a nonsignificant pattern of IBD between populations of another Mackenzie River coregonine, the lake cisco (*C. artedi*). Our data, therefore, may suggest the emergence of 'nascent' population structure in Mackenzie River broad whitefish, consistent with its recent post-glacial recolonization to the area in which there is still a strong signal of historical gene flow augmented by contemporary gene flow that is sufficient to overwhelm contemporary drift (e.g. scenario II of Hutchison & Templeton, 1999), especially if contemporary EPS are large. Effective population sizes in this system are unknown, but crude census sizes on spawning grounds are thought to be as high as 7 300 000 (Thera, 1998). The relatively small range (scatter) of F_{ST} values within the Mackenzie River system is also suggestive of the overwhelming effects of local gene flow within this system and geographic scale. The nascent population structure in the Mackenzie River system was also evidenced by significantly lower H_E, and A_R compared to that in Alaska likely due to the founder effects associated with the recent colonization of the area.

Historical impacts on microsatellite variation – zoogeographic inferences

Although the utility of using microsatellites to assess phylogeography and post-glacial dispersal history has only come to light relatively recently, there are now numerous studies that have shown these markers can be informative for such analyses (e.g. Angers & Bernatchez, 1998; Koskinen *et al.*, 2002; Costello *et al.*, 2003). The microsatellite loci used in our study have provided some insight into the phylogeography and post-glacial dispersal history of broad whitefish in North America. Evidence for the single North American refugial origin (i.e. Beringia) for broad whitefish is suggested by the current broad whitefish distribution, by the clustering together of all North American samples, low interpopulation divergence among North American samples (e.g. low F_{ST} values), low variation among regions in North America revealed by the AMOVA and, for the most part, the lack of unique alleles within North America populations from eastern and western portions of their range. If more pronounced differentiation was evident, especially over short geographic distances, or as indicated by the presence of multiple unique alleles, this may have indicated survival in different refugia (Bernatchez & Wilson, 1998; Turgeon & Bernatchez, 2001a; Koskinen *et al.*, 2002), but this was not the case in our study. Glacial refugia and other sources of dispersal can also be identified by elevated levels of genetic diversity (e.g. Mellers, 2006; Excoffier & Ray, 2008; Latch *et al.*, 2009) and our samples from Alaska had higher levels of genetic diversity than more eastern populations within the Mackenzie River system which is consistent with central Alaska serving a the Beringian origin of contemporary populations located in the Mackenzie River. Although our data provide strong support for a single North American glacial refuge for broad whitefish, we cannot reject the possibility of a second refugium in Eurasia. Indeed, there was a large distinction between the single western Russian sample and all others, but only a denser, spatially balanced sampling of Eurasian populations can test this idea more robustly (Guillot & Santos, 2009).

Two hypotheses have been proposed regarding post-glacial dispersal of broad whitefish from Beringia to the Mackenzie River system and our results suggest recolonization of the Mackenzie River system that involves at least some contribution from the interior post-glacial route. Although the FCA and STRUCTURE results both showed that there are close affinities between Mackenzie River populations and Teshekpuk Lake on the North Slope of Alaska and there was a strong signal of contemporary migration between these areas, the results from MIGRATE suggested a strong historical signal of migration between interior Alaska and the Peel River, a western tributary of the Mackenzie River, as predicted by the 'interior' hypothesis of headwater exchanges between these areas (see below). By contrast, broad whitefish from other portions of the Mackenzie River system showed stronger contemporary and historical signals of gene flow with coastal regions. Consequently, our analyses suggest that the Mackenzie River system was invaded post-glacially by broad whitefish from two sources: interior regions of Alaska (upper Yukon River) and from coastal areas.

The idea that the Mackenzie River system was post-glacially invaded from two directions is consistent with its very large size, the geological history of the area, and the contemporary biology of *C. nasus*. The Mackenzie River is the longest river in Canada and the single largest drainage basin ($\sim$1.8 million km^2) and thus has the potential to present a large 'biogeographic target' for post-glacially dispersing fishes. In addition, meltwater from glaciers retreating during deglaciation formed large proglacial lakes (e.g. Lakes Bonnet Plume, Davis and Old Crow) and advancing ice sheets blocked and diverted the Porcupine River (through McDougall Pass), previously (and currently) a tributary to the Yukon River, such that its flow reversed into the upper Mackenzie River and provided temporary connections between these drainages (Bodaly & Lindsey, 1977; Lindsay & McPhail, 1986; Pielou, 1991). Bodaly & Lindsey (1977) presented morphological, distribution and allozyme evidence of affinities between Mackenzie River and upper Yukon rivers populations of six freshwater fish species that appeared to result from such post-glacial watershed exchanges via the Peel and Porcupine rivers. Because

broad whitefish occur up to the headwaters of the Yukon River (Scott & Crossman, 1998), and presumably did so historically in this unglaciated portion of Beringia, these headwater connections could have provided ephemeral opportunities for post-glacial recolonization of the Peel River basin.

In addition, a stepping stone-like dispersal from Beringia eastward in marine waters of lowered salinity along the Arctic coast has also been suggested for lake trout (*Salvelinus namaycush*, Wilson & Hebert, 1998), Arctic char (*S. alpinus*, Wilson *et al.*, 1996), and likely applies to a number of coregonines species based on current distributions (e.g. Politov *et al.*, 2004). For example, Arctic cisco (*C. autumnalis*) from the Colville River system on the North Slope of Alaska are genetically indistinguishable from Mackenzie River system populations (J. L. Nielsen, Alaska Science Center, US Geological Survey, Anchorage, AK, personal communication). The Colville River is one of the first major river systems to the west of the Mackenzie River system on the North Slope of Alaska and our data, along with the Arctic cisco data, argue that these populations have not been isolated in separate river systems for long periods of time or that gene flow between these systems is still quite high. Furthermore, coastal dispersal, rather than via inland routes, seems likely for broad whitefish given the higher degree of anadromy, compared to potamodromy (migrations within a wholly freshwater existence), in this species (Reist & Chang-Kue, 1997).

The contribution of the Beringian refuge fish to the colonization of the Mackenzie River system varies considerably among fish species. For example, lake whitefish that survived in Beringia did not disperse to the Mackenzie River system, and it appears that this area was recolonized almost exclusively by Mississippian refuge lake whitefish (Bernatchez & Dodson, 1991; Rempel & Smith, 1997). By contrast, other species have been able to successfully disperse from Beringia. Arctic grayling for example, have dispersed far south and east from Beringia following the retreat of the glaciers (Stamford & Taylor, 2004). The majority of the current geographic range of lake trout in North America, approximately two-thirds, is thought to result from recolonization from the Beringian refuge (Wilson & Hebert, 1998). The current distribution of broad whitefish argues for a Beringian refuge exclusively and that there has been limited dispersal from this refuge, in comparison to other species such as lake trout. This may be due to specific environmental limitations on eastward dispersal in broad whitefish as the relatively warmer western Arctic yields to cooler conditions in the central and eastern Arctic. Additionally, a waterfall on the Mackenzie River that existed from approximately 11 500 to 6100 ya (Lindsay & McPhail, 1986) or specific thermal preferences (McPhail & Lindsey, 1970) may have prevented extensive upstream dispersal in the Mackenzie River system by *C. nasus*.

Conclusions

Our study provides the first comprehensive evaluation of how historical processes have influenced genetic variation within North American populations of broad whitefish and one of the few examples of such processes for Arctic basin freshwater fishes. Our results provide strong evidence that North American broad whitefish represent a species of a single glacial lineage surviving solely within Beringia. Subsequent to Pleistocene glacial events, it appears that broad whitefish recolonized newly available habitat in the Mackenzie River in an eastward direction from Beringia, largely via marine environments, but also opportunistically via headwater connections between interior portions of the upper Yukon and western tributaries of the Mackenzie River. Furthermore, the results of our study also illustrate how post-glacial range expansion has influenced genetic diversity within and among populations of this species, especially those at the periphery of their range. Founding events, and subsequent drift, associated with a stepping-stone like pattern of dispersal from glacial refugia, have contributed to the extant genetic variation observed in contemporary populations of broad whitefish. Populations of *C. nasus* nearer the periphery of the range appear to be farther from equilibrium between genetic drift and gene flow, which is consistent with expectations for populations in recently colonized areas. More generally, our data contribute to a better understanding of the historical processes that can influence current patterns of biodiversity in Arctic faunas which, in general, have received comparatively less attention than those in temperate or tropical freshwaters.

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References

Allendorf, F.W. & Luikart, G. 2007. *Conservation and the Genetics of Populations*. Blackwell Publishing, Oxford, UK.

Angers, B. & Bernatchez, L. 1998. Combined use if SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. *Mol. Biol. Evol.* **15**: 143–159.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. & Saunders, N.C. 1987. Intraspecific phylogeography, the mitochondrial DNA bridge between population genetics and systematics. *Annu. Rev. Ecol. Syst.* **18**: 489–522.

Beebee, T.J.C. & Rowe, G. 2000. Microsatellite analysis of *Bufo calamita* Laurenti populations, consequences of dispersal from a Pleistocene refugium. *Biol. J. Linn. Soc.* **69**: 367–381.

Beerli, P. 2004. Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Mol. Ecol.* **13**: 827–836.

Beerli, P. 2008. *Migrate-N*, version 3.0.3. [www document]. URL http://popgen.scs.fsu.edu/Migrate-n.html

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. 2004. *Genetix 4.05, logiciel sous windows TM pour la génétique des populations, version 4.05*. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Bernatchez, L. & Dodson, J.J. 1991. Phylogeographic structure in mitochondrial DNA of the Lake Whitefish (*Coregonus clupeaformis*) in North America and its relationships to Pleistocene glaciations. *Evolution* **45**: 1016–1035.

Bernatchez, L. & Dodson, J.J. 1994. Phylogenetic relationships among Palearctic and Nearctic whitefish (*Coregonus* sp.) populations as revealed by mitochondrial DNA variation. *Can. J. Fish. Aquat. Sci.* **51**: 240–251.

Bernatchez, L. & Wilson, C.C. 1998. Comparative phylogeography of nearctic and palearctic freshwater fishes. *Mol. Ecol.* **7**: 431–452.

Bodaly, R.A. & Lindsey, C.C. 1977. Pleistocene watershed exchanges and the fish fauna of the Peel River Basin, Yukon Territory. *J. Fish. Res. Board Can.* **34**: 388–395.

Brunner, P.C., Douglas, M.R., Osinov, A., Wilson, C.C. & Bernatchez, L. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. *Evolution* **55**: 573–586.

Castric, V. & Bernatchez, L. 2003. The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). *Genetics* **163**: 983–996.

Cornuet, J.M. & Luikart, G. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–2014.

Costello, A.B., Down, T.E., Pollard, S.M., Pacas, C.J. & Taylor, E.B. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species, and examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus*, (Pisces, Salmonidae). *Evolution* **57**: 328–344.

Crispo, E. & Hendry, A.P. 2005. Does time since colonization influence isolation by distance? A meta-analysis. *Conserv. Genet.* **66**: 665–682.

England, P.R., Osler, G.H., Woodworth, L.M., Montgomery, M.E., Briscoe, D.A. & Frankham, R. 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. *Conserv. Genet.* **4**: 595–604.

Evanno, G., Regnault, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**: 2611–2620.

Excoffier, L. & Ray, N. 2008. Surfing during population expansions promotes genetic revolutions and structuration. *Trends Ecol. Evol.* **23**: 347–351.

Excoffier, L., Smouse, P. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes, application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Excoffier, L., Laval, G. & Schneider, S. 2005. Arlequin ver. 3.0, an integrated software package for population genetics data analysis. *Evol. Bioinform.* **1**: 47–50.

Fraser, C.I., Spencer, H.G. & Waters, J.M. 2009. Glacial oceanographic contrasts explain phylogeography of Australian bull kelp. *Mol. Ecol.* **18**: 2287–2296.

Goudet, J. 2002. *FSTAT, A Program to Estimate and Test Gene Diversities and Fixation Indices, version 2.9.3.2*. [www document]. URL http://www.unil.ch/izea/softwares/fstat.html.

Goudet, J., Raymond, M., Demeeus, T. & Rousset, F. 1996. Testing differentiation in diploid populations. *Genetics* **144**: 1933–1940.

Guillot, G. & Santos, F. 2009. A computer program to simulate multilocus genotype data with spatially autocorrelated allele frequencies. *Mol. Ecol. Resour.* **9**: 1112–1120.

Guo, S.W. & Thompson, E.A. 1991. Monte Carlo EM for the estimation of multiple random effects models on large pedigrees. *IMA J. Math. Appl. Med. Biol.* **8**: 171–189.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58**: 247–276.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary icea ages. *Nature* **405**: 907–913.

Holder, K., Montgomerie, R. & Friesen, V.L. 2000. Glacial vicariance and historical biogeography of rock ptarmigan (*Lagopus mutus*) in the Bering region. *Mol. Ecol.* **9**: 1265–1278.

Hutchison, D.W. & Templeton, A.R. 1999. Correlation between pairwise genetic and geographic distance measures, inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**: 1898–1914.

Konopinski, M.K., Amirowicz, A. & Kukula, K. 2007. Probable direction of the postglacial colonization on the northern slopes on the Carpathian Ridge by *Barbus carpathicus* (Teleostei, Cyprinidae) evidenced by cline of genetic variation. *J. Fish Biol.* **70**: 406–415.

Koskinen, N.T., Nilsson, J., Veselov, A., Potutkin, A.G., Ranta, E. & Primmer, C.R. 2002. Microsatellite data resolve phylogeographic patterns in European grayling, *Thymallus thymallus,* Salmonidae. *Heredity* **88**: 391–401.

Latch, E.K., Heffelfinger, J.R., Fike, J.A. & Rhodes, O.E. Jr. 2009. Species-wide phylogeography of North American mule deer (*Odocoileus hemionus*): cryptic glacial refugia and postglacial recolonization. *Mol. Ecol.* **18**: 1730–1745.

Lindsay, C.C. & McPhail, J.D. 1986. Zoogeography of fishes of the Yukon and Mackenzie basins. In: *The Zoogeography of North American Freshwater Fishes* (C.H. Hocutt & E.O. Wiley, eds), pp. 639–674. Wiley, New York.

Luikart, G. & Cornuet, J.-M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol.* **12**: 228–237.

McAllister, D.E., Platania, S.P., Schueler, F.W., Baldwin, M.E. & Lee, D.S. 1986. Ichthyofaunal patterns on a geographic grid. In: *The Zoogeography of North American Freshwater Fishes* (C.H. Hocutt & E.O. Wiley, eds), pp. 17–51. Wiley, New York.

McPhail, J.D. & Lindsey, C.C. 1970. *Freshwater Fishes of Northwestern Canada and Alaska*. Fisheries Research Board of Canada, Ottawa. Bulletin No. 173.

Mellers, P. 2006. Going east: new genetic and archaeological perspectives on the modern human colonization of Eurasia. *Science*, **313**: 796–800.

Merilä, J., Björklund, M. & Baker, A.J. 1996. Genetic population structure and the gradual northward decline of genetic variability in the greenfinch (*Carduelis chloris*). *Evolution* **50**: 2548–2557.

Miller, M.P. 1997. *Tools for Population Genetic Analysis*. Version 1.3. Department of Biological Sciences, Northern Arizona University, Flagstaff.

Muller, M.H., Leppala, J. & Savolainen, O. 2008. Genome-wide effects of postglacial colonization in *Arabidopsis lyrata*. *Heredity* **100**: 47–58.

Nei, M., Maruyama, T. & Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution* **29**: 1–10.

Petit, R.J., Aguinagalde, I., de Beauliew, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palmé, A., Martín, J.P., Rendell, S. & Vendramin, G.C. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* **300**: 1563–1565.

Pielou, E.C. 1991. *After the Ice Age. The Return of Life to Glaciated North America*. University of Chicago press, Chicago, IL.

Piry, S., Luikart, G. & Cornuet, J.-M. 1999. BOTTLENECK: a computer program for detecting recent reductions in effective population size using allele frequency data. *J. Hered.* **90**: 502–503.

Politov, D.V., Bickham, J.W. & Patton, J.C. 2004. Molecular phylogeography of Palearctic and Nearctic ciscoes. *Ann. Zool. Fenn.* **41**: 13–23.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Rafínski, J. & Babik, W. 2000. Genetic differentiation among northern and southern populations of the moor frog *Rana arvalis* in central Europe. *Heredity* **84**: 610–618.

Raymond, M. & Rousset, F. 2003. GENEPOP 3.4., an updated version of GENEPOP V.1.2 (1995), population genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249.

Reist, J.D. & Chang-Kue, K.T.J. 1997. The life history and habitat usage of broad whitefish in the lower Mackenzie River basin. In: *The Proceedings of the Broad Whitefish Workshop, the Biology, Traditional Knowledge and Scientific Management of Broad Whitefish (Coregonus nasus (Pallus)) in the lower Mackenzie River* (R.F. Tallman & J.D. Reist, eds), pp. 63–74. *Can. Tech. Rep. Fish. Aquat. Sci.* No. 2193: 219.

Rempel, L.L. & Smith, D.G. 1997. Postglacial fish dispersal of fish from the Mississippi refuge to the Mackenzie River basin. *Can. J. Fish. Aquat. Sci.* **55**: 893–899.

Rogers, S.M., Marchand, M.-H. & Bernatchez, L. 2004. Isolation, characterization and cross-salmonid amplification of 31 microsatellite loci in the lake whitefish (*Coregonus clupeaformis*, Mitchill). *Mol. Ecol. Notes* **4**: 89–92.

Schönswetter, P., Tribsch, A., Barfuss, M. & Niklfeld, H. 2002. Several Pleistocene refugia detected in the high alpine plant Phyteuma globulariifolium Sternb & Hoppe (Campanulaceae) in the European Alps. *Mol. Ecol.* **11**: 2637–2647.

Scott, W.B. & Crossman, E.J. 1998. *Freshwater Fishes of Canada. Fish. Res. Bd. Canada Bull. No. 184.* Reprinted by Galt House Publications, Burlington, ON.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**: 264–279.

Small, M.P., Beacham, T.D., Withler, R.E. & Nelson, R.J. 1998. Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia. *Mol. Ecol.* **7**: 141–155.

Stamford, M.D. & Taylor, E.B. 2004. Phylogeographic lineages of Arctic grayling (*Thymallus arcticus*) in North America, divergence, origins and affinities with Eurasian *Thymallus*. *Mol. Ecol.* **13**: 1533–1549.

Tallman, R.F. & Reist, J.D. (eds) 1997. The Proceedings of the Broad Whitefish Workshop, the Biology, Traditional Knowledge and Scientific Management of Broad Whitefish (*Coregonus nasus* (Pallus)) in the lower Mackenzie River. *Can. Tech. Rep. Fish. Aquat. Sci.* **2193**: 219.

Taylor, E.B., Stamford, M.D. & Baxter, J.S. 2003. Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range, evolutionary inferences and conservation implications. *Mol. Ecol.* **12**: 2609–2622.

Thera, T. 1998. A quantitative life-cycle model to identify research priorities and test management strategies for the Mackenzie River broad whitefish (Coregonus nasus Pallus). PhD Thesis, University of Manitoba, Winnipeg.

Trénel, P., Hansen, M.M., Normand, S. & Borchsenius, F. 2008. Landscape genetics, historical isolation and cross-Andean gene flow in the wax palm, *Ceroxylon echinulatum* (Arecaceae). *Mol. Ecol.* **17**: 3528–3540.

Turgeon, J. & Bernatchez, L. 2001a. Clinal variation at microsatellite loci reveals historical secondary intergradation between glacial races of *Coregonus artedi* (Teleostei, Coregoninae). *Evolution* **11**: 2274–2286.

Turgeon, J. & Bernatchez, L. 2001b. MtDNA phylogeography of lake cisco (*Coregonus artedi*), evidence supporting extensive secondary contacts between two glacial races. *Mol. Ecol.* **10**: 987–1001.

Van Houdt, J.K., Hellemans, B. & Volckaert, F.A.M. 2003. Phylogenetic relationships among Palearctic and Nearctic burbot (*Lota lota*), Pleistocene extinctions and recolonization. *Mol. Phylogenet. Evol.* **29**: 599–612.

Waples, R.S. & Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.* **15**: 1419–1439.

Weider, L.J. & Hobæk, A. 2000. Phylogeography and Arctic biodiversity: a review. *Ann. Zool. Fenn.* **37**: 217–231.

Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Wilson, C.C. & Hebert, P.D.N. 1998. Phylogeography and postglacial dispersal of lake trout, *Salvelinus namaycush*, in North America. *Can. J. Fish. Aquat. Sci.* **55**: 1010–1024.

Wilson, G.A. & Rannala, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**: 1177–1191.

Wilson, C.C., Hebert, P.D.N., Reist, J.D. & Dempson, J.B. 1996. Phylogeography and postglacial dispersal of Arctic charr *Salvelinus alpinus* in North America. *Mol. Ecol.* **5**: 187–198.

Wright, S. 1943. Isolation by distance. *Genetics* **28**: 114–138.

Supporting information

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

Table S1 Basic descriptive statistics for seven microsatellite loci in the 14 populations studied.

Table S2 Results of log-likelihood exact tests for genetic differentiation among pairs of populations (above diagonal) showing nonsignificant (NS) and significant (*) at the 5% nominal level following Bonferroni corrections and pairwise F_{ST} (θ) comparisons among all pairs of populations (below diagonal).

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