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# Mitochondrial phylogeography of a Beringian relict: the endemic freshwater genus of blackfish *Dallia* (Esociformes)

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Mitochondrial genetic variability among populations of the blackfish genus *Dallia* (Esociformes) across Beringia was examined. Levels of divergence and patterns of geographic distribution of mitochondrial DNA lineages were characterized using phylogenetic inference, median-joining haplotype networks, Bayesian skyline plots, mismatch analysis and spatial analysis of molecular variance (SAMOVA) to infer genealogical relationships and to assess patterns of phylogeography among extant mitochondrial lineages in populations of species of *Dallia*. The observed variation includes extensive standing mitochondrial genetic diversity and patterns of distinct spatial segregation corresponding to historical and contemporary barriers with minimal or no mixing of mitochondrial haplotypes between geographic areas. Mitochondrial diversity is highest in the common delta formed by the Yukon and Kuskokwim Rivers where they meet the Bering Sea. Other regions sampled in this study host comparatively low levels of mitochondrial diversity. The observed levels of mitochondrial diversity and the spatial distribution of that diversity are consistent with persistence of mitochondrial lineages in multiple refugia through the last glacial maximum.



Key words: Alaska; Arctic; Beringia; Chukotka; glaciaton; mitochondrial DNA.

## INTRODUCTION

Pleistocene glaciations played a dominant role in shaping the present diversity, distribution and genetic variability of Holarctic biota (Hewitt, 2000) and in particular that of Beringia, a biogeographic region encompassing Alaska, parts of the Yukon Territory, north-eastern Siberia and the Bering land bridge (Pielou, 1991). The Cordilleran and Laurentide ice sheets covered much of North America to the east of Beringia isolating it from the rest of the continent. West of Beringia, the location of ice sheets and timing of their advances and retreats are not as well known. During stages of maximum glacial advance, significant areas across Beringia remained free of ice sheets, potentially serving as refugia for terrestrial and freshwater organisms. The

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degree of genetic variability and its spatial distribution among populations of species currently occupying Beringia can provide insights into the roles played by the land bridge, glacial refugia and glacial cycling on the region's fauna.

Glacial advances brought along more arid conditions, sea-level changes, and the emergence of the Bering land bridge and other shallow coastal areas of Asia and North America. The emergence of the inter-continental land bridge facilitated movement of terrestrial and freshwater organisms between North America and Eurasia. In the end, the land bridge faunal exchange and ice sheet barriers to the east produced a Beringian biota ecologically most similar to Asia (Pielou, 1991). Following the end of the most recent period of glacial advance or Last Glacial Maximum (LGM; 26 500–19 000 years before present (B.P.), Clark *et al.*, 2009), the growing availability of and accessibility to suitable habitat led to post-Pleistocene range expansions in and out of Beringia for most organisms in the region (Hewitt, 2000).

Pleistocene climatic fluctuations had significant effects on the Beringian freshwater fauna (Pielou, 1991). Advancing ice sheets extirpated aquatic organisms by obliterating or severely altering habitats (Pielou, 1991). In addition, changing sea levels during the Pleistocene caused large and rapid fluctuations in the amount and distribution of available aquatic habitat (Lindsey & McPhail, 1986). Knowledge of prevailing habitat and climate characteristics in high-latitude areas during glacial advances is a subject of ongoing research and debate (Elias, 2001; Lozhkin *et al.*, 2011). Generally, it is inferred that during glacial advances, the exposed continental shelf of the Bering land bridge had a mesic climate while surrounding sea ice and glaciated mountains promoted xeric conditions in the remainder of Beringia (Hopkins, 1972). During interglacial periods, the continental shelf was submerged and conditions across much of the remaining terrestrial parts of Beringia were probably similar to those observed today (Hopkins, 1972).

The genus *Dallia* Ban 1880 is unique among strictly freshwater fishes in that, at present, it is restricted to Beringia (Lindsey & McPhail, 1986). As many as three species of *Dallia* have been recognized; however, there is an ongoing disagreement on the validity of two forms restricted to Asia (Mecklenburg *et al.*, 2002; Nelson, 2006). The Alaska blackfish *Dallia pectoralis* Bean 1880 occurs in Asia and North America, with the largest proportion of its range present in the mainland of Alaska, U.S.A. In North America, *D. pectoralis* occurs along the Alaskan coast west from the Colville River along the Arctic coastal plain and to the northern side of the Alaska Peninsula with a gap of undefined extent created by the influence of the Brooks Range as it meets the Chukchi Sea. In interior Alaska, the range of *D. pectoralis* extends along the Yukon River drainage to the vicinity of Fairbanks on the Tanana River and along the upper Kuskokwim River drainage to the north of the Alaska Range. Interestingly, this species also occurs naturally in Bering Sea islands (*e.g.* St Lawrence Island) that were once part of the Bering land bridge (Mecklenburg *et al.*, 2002). In Asia, *D. pectoralis* is found along the Arctic coast of the Chukotka Peninsula to the coastal areas of the Bristol Gulf in far eastern Russia (Balushkin & Chereshnev, 1982; Gudkov, 1998). The Pilkhykay blackfish *Dallia delicatissima* Smitt 1881 is restricted to the northern coast of the Chukotka Peninsula east of the Amguema River (Balushkin & Chereshnev, 1982). The Amguema blackfish *Dallia admirabilis* Chereshnev 1980, which is considered by some as a dwarf form of *D. pectoralis* (Andreev, 2004), is restricted to the Amguema River basin in Chukotka (Chereshnev & Balushkin, 1981).

In this study, standing mitochondrial DNA variation was examined in populations spanning the range of the genus *Dallia* in Alaska and far eastern Siberia. The primary goal was to understand how events associated with glacial cycling shaped the present diversity and distribution of these populations. An *a priori* expectation that extant populations of *Dallia* are characterized by high interpopulation genetic differentiation and spatially patterned distribution of genetic diversity was tested and discussed in the context of the phylogeography of the genus, paleoclimate, paleogeography and patterns documented in other species of Beringian freshwater fishes. Given the unique present distribution of *Dallia* and dearth of strictly freshwater species in Beringia, this study offers unique insights into the biological effects of glacial cycling on the freshwater habitats of the region.

## MATERIALS AND METHODS

The study sample included 188 specimens from 23 localities broadly representing the range of the genus *Dallia*. This sample includes the Alaska mainland, St Lawrence Island, two sites on the Chukotka Peninsula and the location of an introduced population south of the Alaska Range (Table I and Fig. 1). Samples were collected as frozen whole fish, whole fish with fin clips or as fin clips. When available, preserved whole fishes were accessioned at the University of Alaska Museum (UAM). Fin clips were stored at $-20^{\circ}$ C in either a solution of 10% dimethyl sulphoxide, 0·25 M ethylenediaminetetraacetic acid and sodium chloride to saturation (Seutin *et al.*, 1991) or 95% ethanol. All fish handling procedures used in this study adhered to a protocol evaluated and approved by the University of Alaska Fairbanks' Institutional Animal Care and Use Committee in protocol number 09-02. In addition, the study sample included museum collection samples from Asian localities from the collections at University of Washington's Burke Museum of Natural History and Culture (*D. pectoralis*: UW 41670 and UW 41671; *D. admirabilis* UW 41669) and Alaskan collections from the Royal Ontario Museum, Toronto (*D. pectoralis*: TO2653M, TO2654H, TO2655H, TO2656M, TO2657M, TO2658M, TO2659M, TO2660M, TO2689M, TO2690M, TO2691H, TO4305M, TO2692M, TO2693H, TO2696M, TO2697M, TO2699L and TO2700M).

Taxon-specific oligonucleotide primers were designed targeting sections of the mitochondrial cytochrome c oxidase I (*coI*) gene and the control region (CR) guided by a publicly available mitochondrial genome sequence from *D. pectoralis* (GenBank accession number AP004102). Names and sequences of the *coI* primers are Dallia_pectoralis_COI_5444, 5′-GCC ATC TTA CCT GTG GCA ATC AC-3′ and Dallia_pectoralis_COI_5997, 5′-AGT AAA AGG ACT GCT GTA ATC AGC-3′. Names and sequences of the CR primers are Dallia_pectoralis_ControlRegion_16011, 5′-CCT TAC GAC TCG TTA CCC ACC-3′ and Dallia_pectoralis_ControlRegion_16817, 5′-CAA AAC CGA TGC TCT TCT CTG-3′.

Total genomic DNA from preserved tissues was isolated using the reagents and protocols of the Qiagen DNEasy Blood & Tissue Kit (Qiagen, Inc.; www.qiagen.com). Optimal polymerase chain reaction (PCR) conditions for *coI* and CR amplifications were 1X Green GoTaq Flexi Buffer (Promega Corp.; www.promega.com), 0·2 mM dNTPs, 1·5 mM $MgCl_2$, 0·4 μM forward primer, 0·4 μM reverse primer, 0·025 U $\mu l^{-1}$ GoTaq DNA polymerase (Promega Corp.) and 1 μl template (variable DNA concentrations). The thermal cycling profile included the following steps: an initial incubation at $94^{\circ}$ C for 2 min, followed by 35 cycles at $94^{\circ}$ C for 30 s, $52^{\circ}$ C for 30 s and $72^{\circ}$ C for 45 s and a final incubation at $72^{\circ}$ C for 5 min. Unincorporated deoxynucleotide triphosphate (dNTP) and primers in PCRs were eliminated using the ExoSap-IT digestion protocol (Affymetrix; www.affymetrix.com). Purified PCR amplicons were used as templates for Sanger sequencing reactions (ABI Big-Dye v3.1 chemistry; www.appliedbiosystems.com) at the High-Throughput Genomics Center, where amplicon sequences were determined by capillary electrophoresis of Sanger reaction products on ABI 3730XL machines.

Raw sequencer output was reviewed and edited using the chromatogram manipulation features implemented in CodonCode Aligner version 3.0.3 (CodonCode Corporation;

TABLE I. Collection localities of examined samples: corresponding location number from Fig. 1, town or region where the sample was collected, geographic co-ordinates of sample and sample size ($n$) of *Dallia* obtained for this study during 2008–2010, from Alaska, U.S.A. and Chukotka, Russia. Site numbers match those used in Fig. 1

| Location number | Location | Latitude (N) | Longitude (W) | $n$ |
|---|---|---|---|---|
| 1 | Fairbanks | 64·8692 | 147·8254 | 10 |
| 2 | Fairbanks | 64·9117 | 147·8288 | 10 |
| 3 | Wasilla | 61·5374 | 149·2550 | 5 |
| 4 | Kuskokwim Basin | 61·1945 | 156·1535 | 4 |
| 5 | Kuskokwim Basin | 61·0812 | 156·4840 | 9 |
| 6 | Kuskokwim Basin | 61·5597 | 156·9341 | 1 |
| 7 | Kuskokwim Basin | 61·4300 | 158·9114 | 2 |
| 8 | Kuskokwim Basin | 61·5406 | 159·3765 | 1 |
| 9 | Russian Mission | 61·7952 | 161·2443 | 15 |
| 10 | Togiak | 59·0546 | 160·3962 | 12 |
| 11 | Bethel | 60·7904 | 161·7799 | 16 |
| 12 | Galena | 64·7167 | 157·0000 | 12 |
| 13 | Unalakleet | 63·8099 | 160·7590 | 11 |
| 14 | Nome | 64·5061 | 165·4305 | 15 |
| 15 | St Lawrence Island | 63·3451 | 169·4893 | 5 |
| 16 | North Slope | 70·2768 | 156·9182 | 6 |
| 17 | North Slope | 70·1981 | 156·1973 | 2 |
| 18 | North Slope | 70·2528 | 155·5849 | 3 |
| 19 | North Slope | 70·3683 | 155·5697 | 4 |
| 20 | Colville River | 70·3333 | 151·2000 | 6 |
| 21 | Novoe Chaplino | 64·4085 | 172·2590 | 10 |
| 22 | Ievineem River | 65·6808 | 172·5542 | 10 |
| 23 | Amguema Basin | 67·4346 | 178·6985 | 8 |

www.codoncode.com). Edited sequence data were assembled into manually aligned multiple sequence alignments (MSA) using Mesquite version 2.71 (Maddison & Maddison, 2009). To verify the identity of sequenced PCR products, basic local alignment search tool (BLAST; Benson *et al.*, 2005) searches were performed using representative sequences generated in this study as queries against the GenBank database. The MSAs of *coI* and CR sequences were concatenated using PhyUtility version 2.2 (Smith & Dunn, 2008). Unique haplotypes, haplotype diversity, average pair-wise differences ($k$) and per site nucleotide diversity ($\pi$) were determined from the concatenated MSA using routines implemented in DnaSP 5 (Librado & Rozas, 2009).

Genealogical relationships among the sampled haplotypes were estimated under a Bayesian phylogenetic inference framework using the analysis tools implemented in MrBayes 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) using the general time-reversible (GTR) substitution model with among-site rate variation. Model choice was guided by results of likelihood-ratio tests implemented in ModelTest 3.7 (Posada & Crandall, 1998). Bayesian tree inference used the following analysis settings: four chains, 10 million generations sampled every 1000 generations and a burn-in fraction of 25%. Convergence of parameters was examined with Tracer version 1.5.3 (Rambaut & Drummond, 2007). In addition, levels and distribution patterns of mtDNA genetic diversity and their correspondence with geography were examined using a median-joining network of haplotypes (Bandelt *et al.*, 1999) generated with Network 4.516 (www.fluxus-engineering.com).

To assess the influence of putative barriers and connections between sampled locations on the distribution of mtDNA genetic variation, a spatial analysis of molecular variance

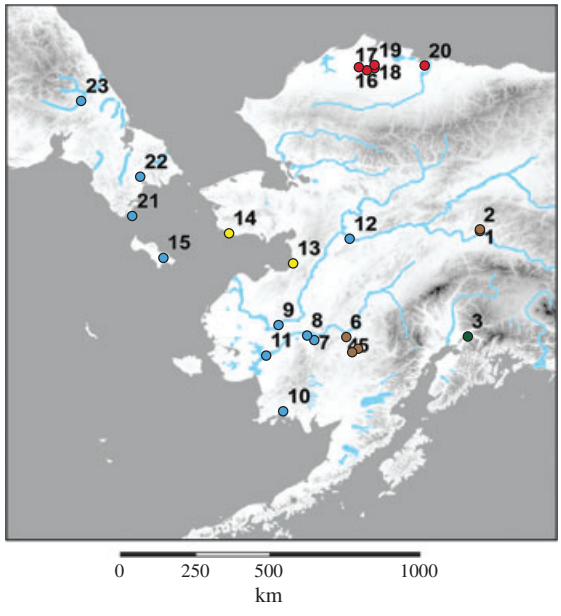


FIG. 1. Sampled localities in Alaska, U.S.A. (sample locations: 1–20) and Chukotka, Russia (sample locations: 21–23). Numbers on the map refer to localities listed in Table I; colour symbols relate to the main phylogeographic regions proposed in this study.

(SAMOVA) was conducted using the programme SAMOVA 1.0 (Dupanloup *et al.*, 2002). SAMOVA 1.0 uses a simulated annealing algorithm to place $N$ populations into $K \geq 2$ groups by maximizing the proportion of total genetic variance due to differences between groups of populations ($F_{CT}$). An analysis series was performed with $K = \{2, \ldots, 6\}$ to create two through six groups of sampled populations with maximized $F_{CT}$. Analysis settings were 100 simulated annealing processes and pair-wise differences to calculate fixation indices. SAMOVA 1.0 assigns $N$ sampled populations to $K$ groups and reports the genetic structure corresponding to $K$, and three fixation indices based on this structure: $F_{CT}$; $F_{SC}$, the proportion of genetic variance due to differences between populations within each group and $F_{ST}$, the genetic variance due to the overall differences between populations not considering group structure. The significance level of the fixation indices is evaluated through 1000 permutations of populations among groups.

Finally, to better understand how glacial cycling, geography and demography shaped the present diversity of the genus *Dallia*, Bayesian skyline plot (BSP) and mismatch analyses to estimate current and past effective population sizes ($N_E$) based on observed genetic variation were employed. BSP analysis as implemented in BEAST 1.5.3 (Drummond *et al.*, 2002, 2005; Rambaut & Drummond, 2007) was performed to estimate the history of change in effective population size implied by the variability among sampled haplotypes. Substitution rates over intraspecific divergence timelines are known to vary widely and to be significantly higher than estimates derived from interspecific divergences. In light of this uncertainty, the effect of assuming substitution rates ranging from lows of 1 and 2% M year$^{-1}$ to a high of 10% M year$^{-1}$ (Burridge *et al.*, 2008; Peterson & Masel, 2009) and generation times of 2 and 3 years (Blackett, 1962; N. Aspinwall, unpubl. data) when converting genetic divergence estimates into inferences of absolute divergence time was assessed. Based on the geographic considerations, the SAMOVA results and Bayesian phylogenetic inference, sampling locations were categorized into Interior Alaska, Unalakleet–Nome, Arctic Coastal Plain and Coastal population groups because BSP analysis assumes panmixia. BSP analyses employed the best-fit mutational model available in BEAST based on the results from ModelTest (Posada & Crandall, 1998). For each population, results from three separate Markov chain Monte–Carlo

(MCMC) searches were pooled and re-sampled with Logcombiner 1.5.3 (Rambaut & Drummond, 2007). Each MCMC search was 100 million steps in length, with sampling for trees and parameters every 500 steps, and a burn-in of 10 million steps (Marko *et al.*, 2010). The pooled data were then examined with Tracer 1.5.3 (Rambaut & Drummond, 2007).

Mismatch analysis served as an alternative method to infer changes in $N_E$ (Rogers & Harpending, 1992). This analysis was conducted with DnaSP 5 (Librado & Rozas, 2009) on the concatenated sequence data. For mismatch distributions, all sampled sequences were examined in a single test as well as in the following sub-sets: Arctic Coastal Plain, Interior Alaska, Unalakleet–Nome and Bering Coast populations. Each sub-set was examined for evidence of changing or constant population size. To estimate the time in years to intrapopulation coalescence ($T$), a combination of the estimate of intrapopulation coalescent time ($\tau$) produced by this analysis, the generation time in years for species of *Dallia* and a mtDNA mutation rate were used. As described above, a generation time of 2–3 years (Blackett, 1962; N. Aspinwall, unpubl. data) and low and high estimates of mutation rate were used to calculate a range of estimates of intrapopulation coalescent time in years.

## RESULTS

The concatenated dataset consists of the 165 individuals from which both gene regions were sequenced (Table II). Sequences from 169 and 170 individuals were determined for *coI* and CR, respectively. All sequences are archived in GenBank (accession numbers: JX961713–JX962051). The MSAs for *coI* and CR sequences are 521 and 718 sites, respectively. The concatenated mtDNA alignment includes 89 variable sites that define 48 unique haplotypes (Table III) for an overall haplotype diversity of 0·957. Over the entire dataset, the average pair-wise difference between sequences ($k$) is 11·19 (or 0·9%) and nucleotide diversity ($\pi$) is 0·00914. The maximum uncorrected pair-wise divergence observed in the sample is 1·79%.

Bayesian phylogenetic inference and median-joining network analyses of the concatenated mitochondrial alignment reveal four distinct mitochondrial lineages that are spatially segregated among sampled locations: Interior Alaska, Unalakleet–Nome, Arctic Coastal Plain and the Bering Coast (Figs 2 and 3). The Interior Alaska lineage is present in fishes sampled from the Kuskokwim River upstream of the Kuskokwim Mountains and the Tanana River (sample locations: 1, 2 and 4–6). Only one individual sampled outside this geographic area carried a haplotype associated with this group (Figs 2 and 3). The Interior Alaska cluster comprises eight haplotypes (Table II and Fig. 2). Six haplotypes found in fishes sampled in Unalakleet and Nome (sample locations: 13 and 14) form a lineage restricted to those localities. In the sample, the Arctic Coastal Plain mtDNA lineage (Table II and Fig. 2; sample locations: 16–20) consists of four haplotypes found only in fishes from North Slope localities. Finally, a diverse group of haplotypes is found in fishes from the Lower Kuskokwim, Togiak, Bethel, Galena, St Lawrence Island and Russia (sample locations: 7–12, 15 and 21–23). This Bering Coast group of haplotypes constitutes a paraphyletic assemblage (Fig. 3). Samples from the introduced population of *D. pectoralis* in south central Alaska (sample location: 3) carry a haplotype that groups with Bering Coast lineages, but that has yet to be sampled from the species' native range.

Results of SAMOVA support spatial genetic structure patterns concordant with the phylogeny and network analyses. With $K = 2$, sampled sites are split between all Interior Alaska localities (sample locations: 1, 2 and 4–6) and all others. Further

TABLE II. Geographic distribution of the 48 mtDNA haplotypes documented in this study of the *Dallia genus* from Alaska, U.S.A. and Chukotka, Russia. Sampling location numbers correspond to those given in Table I and in Fig. 1. Numbers in each cell denote the number of examined individuals from a given sampling site that carry a particular haplotype. Haplotype names correspond to those shown in Fig. 3

| | Sample location | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| BP1 | 5 | | | | | | | | | | | | | | | | | | | | | | |
| BP2 | 4 | | | | | | | | | | | | | | | | | | | | | | |
| GO | | 10 | | | | | | | | | | | | | | | | | | | | | |
| RS | | | 4 | | | | | | | | | | | | | | | | | | | | |
| UKU1 | | | | 1 | 7 | | | | | | | | | | | | | | | | | | |
| UKU2 | | | | 3 | | | | | | | | | | | | | | | | | | | |
| UKU3 | | | | | 1 | | | | | | | | | | | | | | | | | | |
| UKU4 | | | | | 1 | | | | | | | | | | | | | | | | | | |
| UKU5 | | | | | | 1 | | | | | | | | | | | | | | | | | |
| LKU1 | | | | | | | 1 | | | | | | | | | | | | | | | | |
| LKU2 | | | | | | | 1 | | | | | | | | | | | | | | | | |
| LKU3 | | | | | | | | 1 | | | 1 | | | | | | | | | | | | |
| RM1 | | | | | | | | | 9 | | | 9 | | | | | | | | | | | |
| RM2 | | | | | | | | | 2 | | | | | | | | | | | | | | |
| RM3 | | | | | | | | | 1 | | | | | | | | | | | | | | |
| RM4 | | | | | | | | | 1 | | | | | | | | | | | | | | |
| RM5 | | | | | | | | | 1 | | | | | | | | | | | | | | |
| RM6 | | | | | | | | | 1 | | | | | | | | | | | | | | |
| TO1 | | | | | | | | | | 6 | | | | | | | | | | | | | |
| TO2 | | | | | | | | | | 3 | | | | | | | | | | | | | |
| TO3 | | | | | | | | | | 3 | | | | | | | | | | | | | |
| BE1 | | | | | | | | | | | 4 | | | | | | | | | | | | |
| BE2 | | | | | | | | | | | 3 | | | | | | | | | | | | |
| BE3 | | | | | | | | | | | 2 | | | | | | | | | | | | |
| BE4 | | | | | | | | | | | 2 | | | | | | | | | | | | |
| BE5 | | | | | | | | | | | 1 | | | | | | | | | | | | |
| BE6 | | | | | | | | | | | 1 | | | | | | | | | | | | |
| BE7 | | | | | | | | | | | 1 | | | | | | | | | | | | |
| GA1 | | | | | | | | | | | | 1 | | | | | | | | | | | |
| GA2 | | | | | | | | | | | | 1 | | | | | | | | | | | |
| GA3 | | | | | | | | | | | | 1 | | | | | | | | | | | |
| UN1 | | | | | | | | | | | | | 7 | | | | | | | | | | |
| UN2 | | | | | | | | | | | | | 3 | | | | | | | | | | |
| UN3 | | | | | | | | | | | | | 1 | | | | | | | | | | |
| NO1 | | | | | | | | | | | | | | 8 | | | | | | | | | |
| NO2 | | | | | | | | | | | | | | 5 | | | | | | | | | |
| NO3 | | | | | | | | | | | | | | 2 | | | | | | | | | |
| SL1 | | | | | | | | | | | | | | | 2 | | | | | | | | |
| SL2 | | | | | | | | | | | | | | | 2 | | | | | | | | |
| SL3 | | | | | | | | | | | | | | | 1 | | | | | | | | |
| NS1 | | | | | | | | | | | | | | | | 4 | 2 | 2 | 4 | 7 | | | |
| NS2 | | | | | | | | | | | | | | | | 1 | | | | | | | |
| NS3 | | | | | | | | | | | | | | | | 1 | | | | | | | |
| NS4 | | | | | | | | | | | | | | | | | | 1 | | | | | |
| NC1 | | | | | | | | | | | | | | | | | | | | | 6 | | |
| IR1 | | | | | | | | | | | | | | | | | | | | | | 6 | 3 |
| IR2 | | | | | | | | | | | | | | | | | | | | | | 1 | |
| AM1 | | | | | | | | | | | | | | | | | | | | | | | 1 |

TABLE III. Fixation indices from spatial analysis of molecular variance (SAMOVA) with $K = \{1,\ldots,6\}$. $F_{CT}$ is the proportion of total genetic variance due to differences between groups, $F_{SC}$ is the proportion of total genetic variance due to differences between populations within each group and $F_{ST}$ is the proportion of total genetic variance due to differences between populations. All calculations are from 164 individuals, 1230 base pair cytochrome c oxidase I and control region (CR) mitochondrial DNA alignment of *Dallia* from 22 naturally occurring populations across Alaska, U.S.A. and Chukotka, Russia

| | *K* | | | | |
|---|---|---|---|---|---|
| Index | 2 | 3 | 4 | 5 | 6 |
| $F_{CT}$ | 0·48 | 0·56 | 0·63 | 0·68 | 0·71 |
| $F_{SC}$ | 0·8 | 0·74 | 0·67 | 0·57 | 0·52 |
| $F_{ST}$ | 0·9 | 0·88 | 0·88 | 0·86 | 0·86 |

apportioning of molecular variance ($K = 3$) produces the following divisions: the Interior group described above, a group comprising Unalakleet and Nome (sample locations: 13 and 14) and a group for all remaining sampled sites. From this last set, a group composed of all the Arctic Coastal Plain (sample locations: 16–20) samples is delineated under $K = 4$. With another increment of the number of expected groups ($K = 5$), samples spanning the historical Bering land bridge are divided into two groups that largely correspond with the division between Alaska mainland locations and those in Asia and Bering Sea; one sample from the Alaska mainland, however,

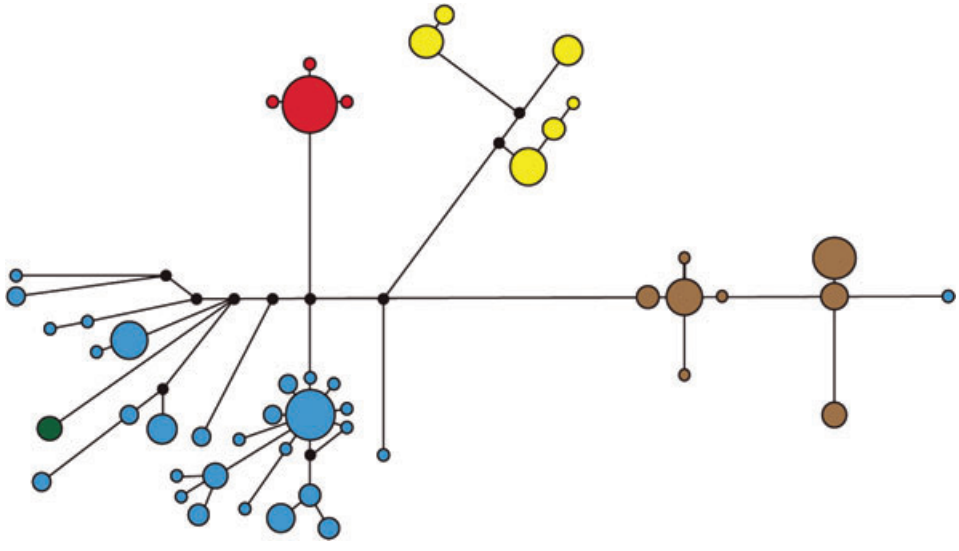

FIG. 2. Median-joining network based on concatenated mitochondrial DNA sequences from 168 individuals from the *Dallia genus* from Alaska, U.S.A. and Chukotka, Russia. Branches are proportional to mutational steps. Sampling localities on map and haplotype symbols on network are colour coded following the main phylogeographic regions proposed in this study: ●, Interior Alaska; ●, Unalakleet–Nome; ●, Bering Coast; ●, Arctic Coastal Plain; ●, Introduced.

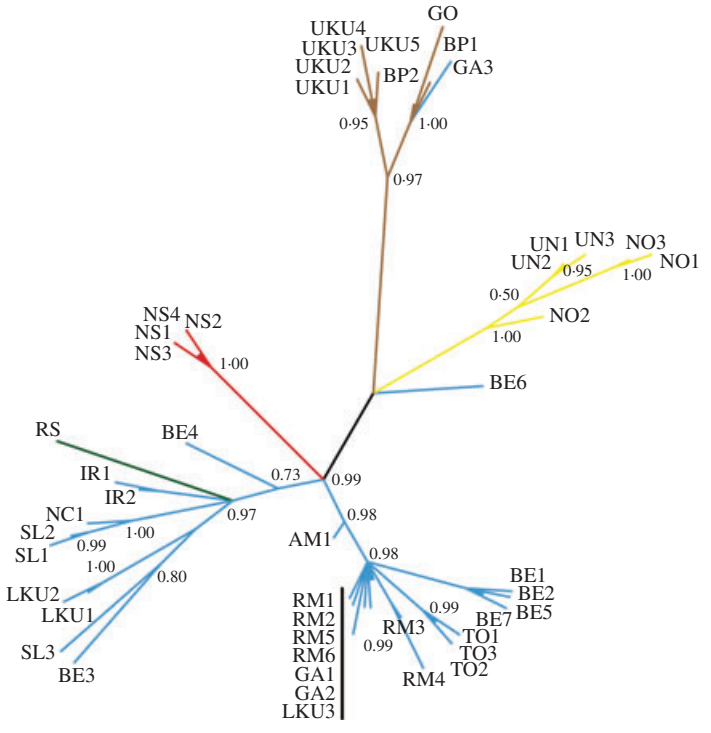


FIG. 3. Bayesian tree of concatenated mitochondrial DNA sequences from 168 *Dallia* from Alaska, U.S.A. and Chukotka, Russia. Tree was constructed under the general time-reversible (GTR) model with a gamma distributed rate variation in four categories (Γ). Bayesian posterior probability values for each clade are included on the tree. Haplotype naming follows Table II. The four major mitochondrial groups and introduced population from Wasilla, Alaska are identified. Branch colours relate the main phylogeographic regions proposed in this study.

(sample location: 8) groups with the Asian samples. At $K = 6$, the Interior group first delineated at $K = 2$ is split into Yukon and Kuskokwim components, all other groupings are as in $K = 5$. Fixation indices for the different clustering levels are given in Table III.

Together, the results of phylogenetic and molecular variance analyses support the delineation of four distinct mitochondrial phylogeographic units among extant populations of *Dallia*. Those phylogeographic units are (1) Interior Alaska (sample locations: 1, 2 and 4–6) including populations found in the Yukon River drainage upstream of Galena and the Kuskokwim River drainage upstream of the Kuskokwim Mountains; (2) Unalakleet–Nome (sample locations: 13 and 14) found in Norton Sound coastal drainages; (3) Arctic Coastal Plain (sample locations: 16–20) encompassing the range of *D. pectoralis* to the north of the Brooks Range and (4) Bering Coast (all remaining sample locations), including populations from areas on or surrounding the Bering land bridge (*e.g.* Chukotka and western Coastal Alaska) and islands on the Bering Sea. The introduced population in south central Alaska probably originated from a population belonging to the Bering Coast phylogeographic unit.

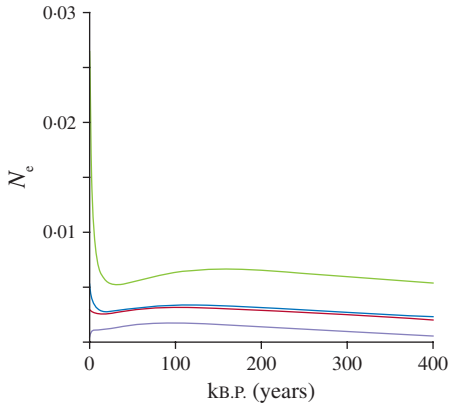


FIG. 4. Bayesian skyline plot for the Coastal mitochondrial lineage using concatenated mtDNA from 79 *Dallia* from Alaska, U.S.A. and Chukotka, Russia. The *x*-axis (thousands of years before present, kB.P.) represents time to the lower bound of the 95% highest posterior density interval, the *y*-axis is effective population size ($N_E$). Upper (——) and lower (——) estimates of $N_E$ are included, with mean (——) and median (——) values of $N_E$ calculated for a generation time of 3 years.

BSP analysis of the Bering Coast phylogeographic unit suggests a population bottleneck ending *c*. 20 000 B.P., followed by a rapid increase in effective population size (Fig. 4). Sequence data from other phylogeographic units proved insufficiently variable to yield informative BSPs. Estimated intrapopulation coalescent time for all mtDNA variants sampled ranges from 59 000 to 896 000 B.P. (Table IV) for high and low estimates of mutation rate, respectively. The Interior Alaska and Unalakleet–Nome phylogeographic units have similar intrapopulation coalescent times. The estimate of intrapopulation coalescent time is most recent in the Arctic Coastal Plain phylogeographic unit and is in the range between 15 000 and 22 000 years. The oldest intrapopulation coalescent time estimate in the analysis is for the Bering Coastal phylogeographic cluster and it ranges between 27 000 and 408 000 years.

## DISCUSSION

### *DALLIA* AND BERINGIAN PHYLOGEOGRAPHY

The analyses support two main conclusions: (1) several divergent, spatially segregated mtDNA lineages are found in modern populations of *Dallia* spp. and (2) the present distribution of these mtDNA lineages is best explained by the influence of geographic features and historical events, such as episodic land connections between Asia and North America. Specifically, there is evidence of standing mtDNA diversity with origins that predate the most recent glacial advances and with patterns of spatial distribution indicative of broad connectivity among populations that spanned the Bering land bridge. The observed levels of mtDNA divergence and non-random and heterogeneous distribution of observed mtDNA lineages are incongruent with an alternative hypothesis in which all modern populations are the product of post-LGM

TABLE IV. Divergence age estimates from mismatch analyses. $\tau$, estimator of coalescent time and $T$, corresponding estimates of timing of expansion $T$ in thousands of years before present (kb.p.) for 2 and 3 year generation times and for substitution rates of 10, 2 and 1% per million years. Location numbers correspond to those listed in Table I and Fig. 1

| | | | $T$ (kb.p.) for 2 years per generation | | |
|---|---|---|---|---|---|
| Group | Location | $\tau$ | 10% | 2% | 1% |
| All | 1–19 | 7·35 | 59·78 | 298·90 | 597·80 |
| Interior Alaska | 1–2, 4–6 | 1·73 | 14·06 | 70·28 | 140·57 |
| Unalakleet–Nome | 12–13 | 1·75 | 14·26 | 71·30 | 142·60 |
| Arctic Coastal Plain | 15–18 | 0·18 | 1·48 | 7·40 | 14·80 |
| Bering Coast | 7–10, 14 | 3·35 | 27·24 | 136·22 | 272·44 |
| | | | $T$ (kb.p.) for 3 years per generation | | |
| Group | Location | $\tau$ | 10% | 2% | 1% |
| All | 1–19 | 7·35 | 89·67 | 448·35 | 896·71 |
| Interior Alaska | 1–2, 4–6 | 1·73 | 21·09 | 105·43 | 210·85 |
| Unalakleet–Nome | 12–13 | 1·75 | 21·39 | 106·95 | 213·90 |
| Arctic Coastal Plain | 15–18 | 0·18 | 2·22 | 11·10 | 22·20 |
| Bering Coast | 7–10, 14 | 3·35 | 40·87 | 204·33 | 408·66 |

expansion into the present range. These findings provide a new perspective on the roles that glacial ice sheets and the Bering land bridge played in the evolution of aquatic faunas in Beringia.

The mtDNA sequence variability reported shows phylogeographic patterns that are concordant with major features of the landscape. With few exceptions, haplotypes and haplotype clusters are found only at a particular sample location or within a defined geographic region (*e.g.* the coastal plains north of the Brooks Range). Observed within-species mtDNA divergence is high when compared to other Beringian freshwater fishes (Bernatchez & Wilson, 1998). For example, comparable mitochondrial phylogeography studies on *Lota lota* (L. 1758) and *Salvelinus* spp. document individual haplotypes with distributions covering areas much greater than haplotypes observed within the genus *Dallia* (Brunner *et al.*, 2001; Van Houdt *et al.*, 2005; Elmer *et al.*, 2008; Alekseyev *et al.*, 2009). Reported values of intraspecific mtDNA divergences among *Coregonus* spp. and *Thymallus arcticus* (Pallas 1776) are lower than those observed in *Dallia* over comparable spatial scales (Bernatchez & Dodson, 1990, 1991; Lu *et al.*, 2001; Turgeon & Bernatchez, 2001; Stamford & Taylor, 2004; Harris & Taylor, 2010). The high mtDNA intraspecific diversity in *Dallia* spp. may be the product of large effective population size, lack of migration between populations and, most importantly, survival across Pleistocene climatic oscillations of multiple populations in Beringia.

Evidence for absence or very limited rates of migration across biogeographic barriers is best exemplified by the distribution of the mtDNA haplotypes among fishes sampled from the Kuskokwim River drainage (sample locations: 4–8). Haplotypes recovered from fish upstream of the Kuskokwim Mountains (sample locations: 4–6) belong to the Interior Alaska phylogeographic unit and those in fishes sampled from

locations downstream of that feature (sample locations: 7 and 8) belong to the Bering Coast phylogeographic unit. Given the sample sizes for both of these phylogeographic units, the pattern shows that there is extremely low to no effective female migration between coastal and upper portions of the Kuskokwim River. No known aspect of the biology of this taxon suggests that the case would be different for males. Furthermore, initial microsatellite data support the high degree of population isolation between Interior Alaska and the Bering Coast populations (Campbell *et al.* 2013) as found in mitochondrial data in this study. The closest Bering Coast and Interior Alaska populations sampled in this study are both on the Kuskokwim Basin and are separated by <300 river km; however, the most closely related sampled populations to Interior Kuskokwim fishes are >1000 river km away and require inferring a historical connection between the Yukon and upper Kuskokwim Basins. The boundary between Bering Coast and Interior Alaska mtDNA regions on the Kuskokwim River is marked by a dramatic change in the morphology of that river as it cuts across the mountain range. In this stretch of the river, the extensive backwater habitats present elsewhere along its course are completely absent. A similar phylogeographic pattern has been documented among populations of *Oncorhynchus kisutch* (Walbaum 1792), suggesting a role for historical factors in addition to dispersal limitations (Olsen *et al.*, 2011).

The median-joining network of mtDNA haplotypes shows several distinct clusters separated by varying degrees of divergence. The observed pattern contrasts with the null expectation of a single common haplotype class comprising the majority of the sample and numerous and less frequent closely related haplotypes, which would be the pattern most consistent with post-LGM expansion from a single refugium. The observed pattern is evidence of population genetic structure persisting across glaciation cycles. The BSP analyses suggest that the Bering Coast population underwent a recent bottleneck. Given limitations of BSP analyses, however, inferences of effective population size prior to the bottleneck are not reliable. Mismatch analyses support previous changes in effective population size for populations of *Dallia* and a coalescence time among all haplotypes at a minimum of 59 000 B.P.

The analyses provide strong indication that populations of *Dallia* survived in subrefugia within Beringia throughout the Wisconsinan glaciation (110 000–10 000 B.P.). Assuming a mitochondrial divergence rate of 0·5–1% per million years (Nabholz *et al.*, 2008), the various mtDNA lineages present in extant populations of *Dallia* evolved within the last 2 million years. Observed mtDNA diversity indicates that several region-specific mitochondrial lineages have deep intrapopulation coalescent times preceding the LGM (26 500–19 000 B.P.; Clark *et al.*, 2009), which is difficult to reconcile with the scenario of a post-LGM expansion from small relict populations. Among populations examined, the mitochondrial haplotype lineage that defines and is restricted to the Arctic Coastal Plain phylogeographic unit has a comparatively recent intrapopulation coalescent time. The Arctic Coastal Plain population size change can be modelled under an exponential growth curve with an initial $\Theta$ of 0·00 and a final $\Theta$ of 1000·00 starting between 15 and 22 000 B.P. At the end of the Wisconsinan glaciation, populations of *Dallia* may have been able to access the coastal plains of the Arctic Alaska through coastal drainages surrounding the hypothesized north-flowing Chukchi Sea River. The Chukchi Sea River drainage included northern Alaska, St Lawrence Island, the northern Chukotka Peninsula to the Amguema River in the west, the Seward Peninsula and Kotzebue Sound (Lindsey & McPhail, 1986).

Expanded coastal plains surrounding the Bering land bridge may have provided contiguous low-gradient habitat along what is now the north-western coast of Alaska, allowing the Brooks Range barrier to be circumvented. Congruent with this scenario, the Arctic Coastal Plain mtDNA lineage is nested within the Bering Coast haplotype group, including those found in St Lawrence Island.

The survival of *D. pectoralis* and its congeners through extreme climatic fluctuations and associated glacial cycling may have been aided by high tolerance to both summer and winter hypoxia. Air breathing throughout the ice-free months allows these species to survive in waters that other Beringian fishes cannot (Blackett, 1962; Crawford, 1974). *Dallia pectoralis* has been observed during ice-free months in waters with dissolved oxygen levels as low as $2{\cdot}3\,\mathrm{mg\,l^{-1}}$ at $7{\cdot}8^{\circ}$ C (Ostdiek & Roland, 1959). *Dallia pectoralis* also use muskrat *Ondatra zibethicus* ice holes to gain access to air in the winter (Armstrong, 1994). Based on their widespread occurrence in shallow seasonally ice-covered shallow ponds (Gudkov, 1998), it is probable that members of *Dallia* can survive near-freezing, hypoxic conditions without access to atmospheric air for extended periods of time; however, the mechanisms responsible for this remarkable tolerance are not yet known (Ultsch, 1989).

## IMPLICATIONS FOR FUTURE TAXONOMIC RESEARCH

The observations presented here are restricted to mitochondrial DNA variation, so they offer no definitive test of currently accepted taxonomic boundaries within the genus. Observed patterns of mtDNA diversity, however, combined with previous analyses of variation strengthens the case for a need to re-evaluate those boundaries. Specifically, this study did not yield evidence of distinct mitochondrial lineages in populations from Asia. The mtDNA haplotypes found in samples from three Asian localities, including regions where putative Asian species occur, are nested within an assemblage of the Bering Coast locations largely composed of *D. pectoralis*.

Another observation with potential taxonomic relevance is the absence of shared mtDNA genetic variability north and south of the Brooks Range. This pattern points to an impermeable barrier for genetic exchange between these segments of the distribution of *D. pectoralis*. On the other hand, although no shared haplotypes between the Arctic Coastal Plain and other phylogeographic units were observed in this study, the mean and range of observed uncorrected mtDNA sequence divergence between haplotypes from the Arctic Coastal Plain and all other mtDNA variants documented in this study (1%, 0·5–1·6%) fall well within the range of divergence observed in within-species comparisons in other fish groups (0·3%, 0–7·4% in freshwater fishes of Canada; Hubert *et al.*, 2008). Chromosome number differences between populations of *D. pectoralis* to the north and south of the Brooks Range in Alaska provide additional evidence that this geographic barrier has promoted the development of distinct gene pools. The karyotypes of fish sampled from the Colville River on the Arctic Coastal Plain of Alaska is fixed at $2n = 74$ while those from fish sampled in the Yukon River show a wide range of variation with $2n$ ranging from 70 to 82 (Beamish *et al.*, 1971; Crossman & Rab, 1996). Intriguingly, the modal chromosome number for the 150 Yukon fish karyotypes examined is 77, which suggests ongoing instability in chromosome segregation in this population. Evaluating the taxonomic significance of this incipient level of mtDNA differentiation and dramatic karyotypic differences will require more extensive documentation of reproductive

isolation among phylogeographic units and of levels of phenotypic and genotypic differentiation.

The analysis of mtDNA variability in populations of *Dallia* spp. helps to characterize freshwater phylogeographic patterns within Beringia and offers support for the idea that multiple freshwater refugia were available and occupied during the Pleistocene glaciations. The largest of these putative refugia spanned across the Bering land bridge and was home to ancestral populations with modern descendants in both Asia and North America. Establishing whether the land bridge supported highly connected hydrographic networks conducive to freshwater fish migration will help refine understanding of the effects of Pleistocene climatic oscillations on the intercontinental exchange of aquatic species. A broader examination of genomic variability in present populations of *Dallia* spp. would yield relevant insights into this regard.

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