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Evidence for genetic distinction among sympatric ecotypes of Arctic char (*Salvelinus alpinus*) in south-western Alaskan lakes

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Abstract – Resource polymorphism may play an important role in the process of speciation. The Arctic char (*Salvelinus alpinus*) exhibits great phenotypic and genetic diversity across its range, making it an ideal species for studies of resource polymorphism and divergence. Here, we investigated genetic variation at 11 microsatellite loci among 287 Arctic char from five isolated yet proximate postglacial lakes in south-western Alaska that were previously examined for resource polymorphism. Significant differences in pairwise F_{ST} were detected among all lakes (range from 0.05 to 0.28, all $P < 0.02$). In one lake (Lower Tazimina Lake), we found evidence for two genetic groups of char and for significant differences in the distribution of microsatellite variability among at least two of the three previously described body size morphotypes ('large'-, 'medium'-, and 'small'-bodied char; maximum $F_{ST} = 0.09$; differences in admixture proportions). We also found a significant association between genetic admixture proportions and gill raker counts among body size morphs ($r = -0.73$, $P < 0.001$). Our data represent the first record of genetically distinct sympatric morphs of Arctic char in Alaska and provide further evidence that differences in morphology associated with feeding (gill rakers) and growth trajectories reflect niche diversification and promote genetic divergence in Holarctic populations of Arctic char.

Key words: *Salvelinus alpinus*; genetic variability; intraspecific divergence; resource polymorphism; morphotypes; Salmonidae

Introduction

Resource polymorphism is the means by which alternative phenotypes exploit distinct niches within a given ecosystem (Skúlason & Smith 1995; Arbour et al. 2011). Resource polymorphism may promote reproductive isolation if the traits associated with differential resource use influence mate choice directly, or if hybrids between differentially adapted trophic ecotypes suffer reduced survival in parental niches (e.g., Hatfield & Schluter 1999; Corrigan et al. 2011; see also McPhee et al. 2012). Morphological diversification is often complex and can occur through environmentally driven plasticity, or it can reflect underlying genetic differences. As resource polymorphism has been proposed as a key factor behind the diversification of vertebrates, understanding if resource polymorphism is associated with genetic variation is critical to our understanding of population divergence and speciation (Wimberger 1994; Skúlason & Smith 1995; Smith & Skúlason 1996; Schluter 1998).

Genetic data that accompany ecological measures of differentiation are especially informative, but are often lacking (Klemetsen 2002). In particular, the relationship between the degree of genetic isolation and resource-driven phenotypic segregation is often poorly understood (Gíslason et al. 1999; Loh et al. 2012). Furthermore, resource polymorphisms may signal underlying genetic differentiation within taxa;

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knowledge of these patterns and processes can result in the development of more nuanced conservation strategies by documenting cryptic variation within taxa (Taylor 1999; Foster et al. 2003; Taylor et al. 2011).

The Arctic char [*Salvelinus alpinus* (L., 1758)] is a circumpolar species and the most northerly distributed freshwater fish. The Arctic char has been very successful in colonising postglacial lakes and river systems, and it is often the only salmonid species present in northern freshwater environments (Klemetsen et al. 2003; Reist et al. 2013). Part of this success may be because Arctic char can vary greatly in resource and habitat use, growth patterns, life-history traits and morphology, resulting in a number of specialised forms inhabiting a range of habitats (e.g., Power et al. 2009; Klemetsen 2010). For example, the size and shape of the jaws and number of gill rakers vary among populations of Arctic char, (Skúlason & Smith 1995) and these traits are typically heritable and associated with differences in trophic ecology (Skúlason et al. 1993; Adams & Huntingford 2002a). Because of its highly polymorphic nature and ability to rapidly undergo trophic diversification following colonisation of relatively young ecosystems, the Arctic char is ideal for examining the role of trophic variation in adaptive radiation. Understanding natural genetic and ecological differentiation across sympatric populations can also provide important insights about how species may adapt to particular habitats during the early stages of divergence.

Differentiated populations from sympatric lake systems have long been used as natural laboratories to study questions regarding the ecological and genetic basis of intraspecific divergence within several fish species (e.g., Frost 1965; Bentzen & McPhail 1984; Bernatchez et al. 1999; Langerhans et al. 2007). Postglacial environments containing alternative forms that differ in resource use, or morphotypes, are especially informative as these young systems often have low biodiversity and high resource availability (see review by Taylor 1999). As multiple unoccupied niches may be exploited by colonising fish, resource polymorphism arising from alternative life-history strategies can be maintained (Berg et al. 2010). Understanding the genetic basis of sympatric systems can thus provide important clues relating to the diversification of alternative forms (Gíslason et al. 1999). Sympatric morphotypes, or morphs, of Arctic char have been documented from European countries such as Scotland, Sweden, Norway, Switzerland and Iceland, and to a lesser extent, North America and Russia (Jónsson & Jónsson 2001; Adams & Huntingford 2002b). A well-known example that showcases the remarkable potential for divergence within Arctic char is the four morphs present in Lake Thingvallavatn, Iceland (Skúlason et al. 1989, 1996; Snorrason et al. 1994). Within the lake, two morphs are benthivorous specialists, but differ in size at maturity, one morph is piscivorous, and the fourth is a pelagic zooplanktivore.

Alaska has one of the richest salmonid faunas in the Northern Hemisphere, including Arctic char which are found from the Kenai Peninsula in south-western Alaska, around the Bering Sea drainages north and east to and including the North Slope (McPhail & Lindsey 1970). Despite this extensive distribution, relatively little is known about the extent of polymorphism in Alaskan Arctic char, investigation of which has been hampered by historical taxonomic confusion involving the relationship between Arctic char and Dolly Varden char (*Salvelinus malma*). Distinguishing the two species has proved challenging due to their similar morphology and life history, but their status as distinct species is supported by strong genetic differentiation in sympatry (e.g., Taylor et al. 2008). Woods et al. (2013) recently examined the extent of resource polymorphism in Arctic char from three isolated lakes in south-western Alaska as a function of lake size, species diversity and degree of isolation from a much larger fourth lake postulated to be the source population of Arctic char in the study area. The lakes were chosen because they exhibit many of the physical criteria consistent with the formation of alternative forms of Arctic char (e.g., long-term isolation and few competitor fish species). Indeed, in one of the three isolated lakes (Lower Tazimina Lake), two sympatric morphs of Arctic char were identified: 'large' and 'small', which were morphologically and ecologically distinct from each other (Woods et al. 2013). Large morphs had an estimated asymptotic size of >50 cm and were gold or orange in colouration. Small morphs had an estimated asymptotic size of ≤20 cm and had silver colouration, deeper bodies with light spots and more gill rakers than large morphs. Large morphs consumed more benthic resources, whereas small morphs fed mostly on zooplankton in the limnetic zone. Large morphs generally had steeper size-at-age growth curves, corresponding to higher growth rates, and the small morphs had shallower growth curves and lower growth rates (Woods et al. 2013). It is unclear, however, to what degree the different morphs in Lower Tazimina Lake are genetically distinct from one another.

Interestingly, Woods et al. (2013) also suggested that a third group of fish was present whose growth rate was intermediate to those of the large and small morphs. These 'medium morphs' were orange to silver in colouration, had an estimated asymptotic size about 33 cm and foraged in the benthic regions of the lake like large morphs, but fed on slightly differ-

ent prey items (i.e., relied more on terrestrial insects and less on snails) and had intermediate gill raker counts. The size-at-age growth curve for medium morphs closely resembled the growth curve of the large morph (Woods et al. 2013); however, it is unknown whether medium morphs are derived from admixture between large and small morphs or whether they represent their own distinct population within Lower Tazimina Lake. To better understand the degree of genetic differentiation between sympatric ecological and morphological forms of Arctic char from south-western Alaska, we used microsatellite DNA markers to (i) test whether the forms from Lower Tazimina Lake are genetically distinct from one another; (ii) determine whether morphological and growth curve data coincide with genetic identity; and (iii) place the genetic variation within Lower Tazimina Lake into the context of genetic differentiation among a series of four other postglacial lakes in south-western Alaska that also contain char, but do not appear to exhibit obvious resource polymorphism. The lakes were selected to represent different scales of geographical isolation; four, including Lower Tazimina Lake, are in the same drainage basin. The fifth lake, Lake Aleknagik, is from an adjacent river system and is accessible by fishes from the sea. We sampled these lakes for char to understand the level to which genetic differences among morphs within a lake compare to divergence between lakes that have been isolated since deglaciation (e.g., Caribou Lakes and Summit Lake) and those that may experience some gene flow via anadromous char (Iliamna Lake and Lake Aleknagik), but that are located in separate watersheds.

Material and methods

Fish collection

A total of 287 tissue samples (fin clips and dorsal muscle plugs) of Arctic char were analysed from four lakes in the Kvichak River (Iliamna Lake) system in Bristol Bay, south-western Alaska, and one lake (Lake Aleknagik) in the Nushagak River system of Bristol Bay (Fig. 1). Gene flow is likely limited between three of the lakes, Caribou Lakes, Lower Tazimina Lake and Summit Lake, due to the presence of physical barriers that restrict the upstream migration of Arctic char and other fish species into the lakes. Summit Lake and Lower Tazimina Lake are above large barrier waterfalls, and migration to Caribou Lakes is limited by a series of rapids in the downstream Koksetna River. Caribou Lakes are at the highest elevation (550 m.a.s.l.), whereas Summit and Lower Tazimina lakes are lower (152 and 194 m.a.s.l., respectively). Caribou (1.2 km^2, max

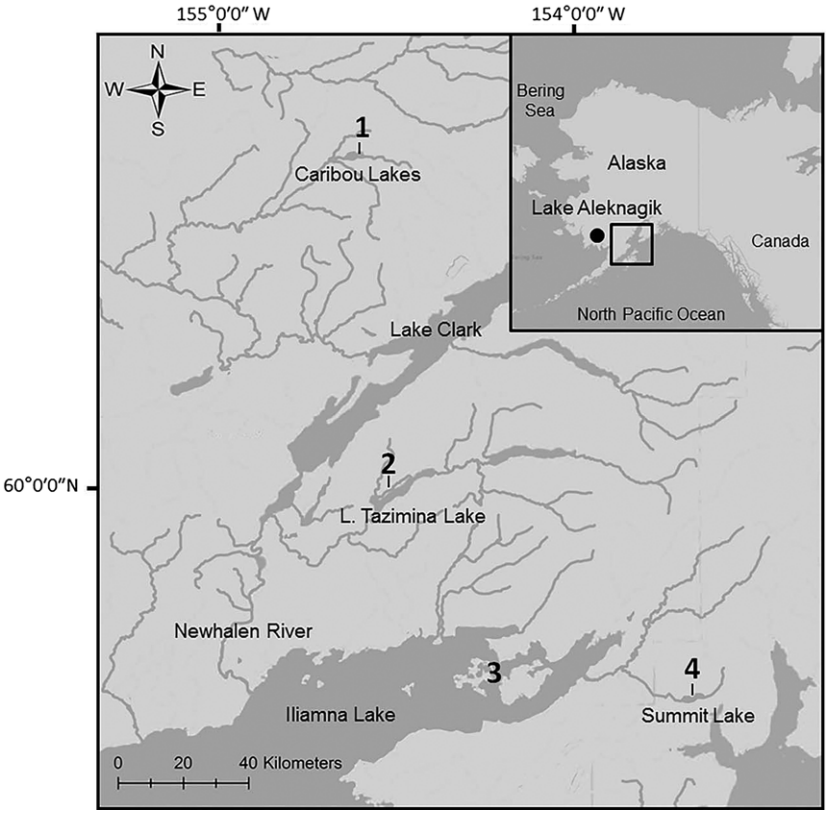

Fig. 1. Collection sites of Arctic char (*Salvelinus alpinus*) in southwestern Alaska. **1** = Caribou Lakes, **2** = Lower Tazimina Lake, **3** = Iliamna Lake, **4** = Summit Lake in the Kvichak River system. The large dot in the inset map indicates Lake Aleknagik in the adjacent Nushagak River system.

depth of 5 m) and Summit lakes (0.6 km^2, max depth of 20 m) are smaller, shallower and more remote than Lower Tazimina Lake, which is larger and deeper (520 km^2, max depth of 60 m). Caribou Lakes, and Lower Tazimina and Summit lakes eventually drain into Iliamna Lake (14 m.a.s.l., 2622 km^2, 301 m max depth), the largest lake in Alaska, which in turn empties into the south-eastern portion of Bristol Bay via the Kvichak River. Iliamna Lake has a much more diverse fish community and is freely accessible to migratory fishes, in contrast to the other three lakes. Fish were also sampled from Lake Aleknagik (12 m.a.s.l., 83 km^2, max depth of 101 m), found in the adjacent Wood River system, which is open to migration from the sea. Sampling was conducted from August–September 2008 to 2011. Fin clips from char were taken from Summit Lake (N = 59), Lower Tazimina Lake (N = 91), Caribou Lakes (N = 25) and Iliamna Lake (N = 42) and stored in 95% ethanol. For Summit Lake, Lower Tazimina Lake and Caribou Lakes, three gill nets with dimensions of 57 m length × 1.6 m depth, 38.1 m × 1.8 m and 30.5 m × 1.8 m were set as bottom nets close to and perpendicular to shore in shallow water (1–7 m deep). An additional sinking gill net and two floating gill nets were set at 17, 9 and 10 m, respectively, in Summit Lake. Mesh sizes ranged from 10 to 102 mm (see Woods et al. 2013 for full details). Iliamna Lake samples were obtained through seining and angling of near-shore habitats that were approximately 1–3 m in depth at the eastern end of the lake (Fig. 1). Exact depth information for individual fish was not recorded for catches originating from bottom set nets, that is, in Summit Lake, Lower Tazimina Lake and Caribou Lakes fish caught at depths between 1 and 17 m in depth could not be distinguished.

Additional samples of Arctic char were collected using a combination of gill nets and stick seines from Lake Aleknagik (N = 70) in July–August 2012 to place the level of divergence and genetic diversity seen within the Kvichak River system in the context of differences from another basin. Lake Aleknagik is located in the Wood River Lakes system, about 85 km west of the Kvichak River system, and also flows into Bristol Bay, but via the Nushagak River. The Wood River Lakes system has a similarly diverse fish community with easy access to the sea by anadromous fishes (Hartman & Burgner 1972).

DNA extractions and microsatellite DNA analyses

We extracted genomic DNA from fin clip samples stored in 95% ethanol using the DNeasy DNA blood and tissue extraction kit (Qiagen Inc., Valencia, CA, USA) following kit protocols. Extracted DNA samples were stored at −20 °C for later use in multiplex polymerase chain reactions (PCR) using the Qiagen multiplex kit (Cat. No. 206145). The DNA was amplified in 10-μl PCRs at 95 °C for 15 min, 94 °C for 30 s, 35 cycles of 1.5 min at an annealing temperature of 55 °C followed by 72 °C for 1 min, and 60 °C for 30 min.

We assayed microsatellite variation using primers labelled with infrared fluorophores and a 3730S 48-capillary DNA Analyzer with GS 500 LIZ or 600 LIZ internal size standards (Applied Biosystems, Carlsbad, CA, USA). Alleles were manually scored using the program GeneMapper (GeneMapper v.3.7; Applied BioSystems). We genotyped fish using 11 microsatellite loci isolated from other salmonid species such as Atlantic salmon, *Salmo salar* (*SSOSL*456; Slettan et al. 1997), bull trout, *Salvelinus confluentus* (*Sco*200, *Sco*215, *Sco*216, *Sco*220; DeHaan & Ardren 2005), Chinook salmon, *Oncorhynchus tshawytscha* (*OtsG*83*b*, *OstG*253*b*; Williamson et al. 2002), Dolly Varden, *S. malma* (*Smm*-17, *Smm*-22, *Smm*-24; Crane et al. 2004) and rainbow trout, *Oncorhynchus mykiss* (*OMM*1105; Rexroad et al. 2002).

To minimise genotyping scoring errors, we used MICRO-CHECKER software (van Oosterhout et al. 2004) to identify instances where alleles failed to amplify owing to mutations in the primer binding sites or failure to detect large alleles ('null alleles' and 'large allele dropout', respectively). Genetic polymorphism was estimated from sample size (N), number of alleles per locus (A), allelic richness (A_R), and observed (H_O) and expected (H_E) heterozygosity. These calculations were performed with FSTAT ver 2.9.3 (Goudet 2001). The following tests were implemented using GENEPOP ver 4.2 (Raymond & Rousset 1995). Tests for departures from Hardy–Weinberg equilibrium (HWE) were performed for each locus–population combination using an exact test in which probability values were determined using a Markov chain method (P). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within a population were also made using a Markov chain method with GENEPOP default values.

Population structure within and among lakes

To assess the level of population structure within and among lakes, we used the Bayesian clustering analysis within STRUCTURE (Pritchard et al. 2000). STRUCTURE uses a Markov chain Monte Carlo (MCMC) to cluster individuals into K randomised and interbreeding groups that minimise departures from HWE and linkage disequilibrium within the groups. We used the admixture model with correlated allele frequencies and a burn-in of 50,000 iterations

proceeded by an additional 450,000 iterations, replicated five times to verify consistency across runs. Because we had prior indications of distinct morphotypes in Lower Tazimina Lake (Woods et al. 2013), we analysed the data in two ways. First, to determine the most likely number of populations (K) across all lakes, we ran simulations of $K = 1$ to $K = 10$ for all samples from all lakes in one analysis. Next, we assessed $K = 1$ to $K = 5$ for each lake separately. For both kinds of analysis, we used STRUCTURE HARVESTER to process the results from multiple runs of STRUCTURE (Earl & vonHoldt 2012) which were visualised using DISTRUCT (Rosenberg 2004).

Pairwise genetic divergence across samples was expressed as F_{ST} and was quantified by calculating θ (Weir & Cockerman 1984). Pairwise values were then tested for significance using GENETIX ver 4.05.2 (Belkhir et al. 2001). A factorial correspondence analysis (FCA) was also conducted on allele frequencies in GENETIX. An FCA is a type of factor analysis that summarises allele frequencies among all samples by finding the linear combination of variables that best describes variation between individual samples and is well-suited analysis for categorical data such as allele frequency counts. All statistical tests of differences between samples were corrected for multiple comparisons following Narum (2006).

Comparisons between genetic and phenotypic data within Lower Tazimina Lake

To assess the distribution of admixture values (Q, the proportion of each fish's genome derived from K genetic groups) estimated by STRUCTURE among the Lower Tazimina Lake morphs, we grouped individual fish into either 'large', 'medium' or 'small' morphs according to their growth curve assignment as outlined by Woods et al. (2013, $N = 74$). We then tested for differences in the mean values of Q among morphs with a one-way ANOVA, with proportional Q values arcsine-transformed, and *post hoc* tests for samples with uneven variances using PAST (vers. 2.17b), a general spreadsheet-based statistical software package (Hammer et al. 2001). The STRUCTURE analysis (see Results) resolved two genetic groups within Lower Tazimina Lake with small-bodied fish tending to have admixture values <0.2, large-bodied fish had admixture values >0.8 and medium-sized fish tended to have intermediate admixture values. To assess whether or not the medium-sized char may represent hybrids between the large- and small-bodied fish, we used all large-bodied fish and all small-bodied fish and the program HYBRIDLAB 1.0 (Nielsen et al. 2006) to generate simulated hybrids ($N = 100$) between these two groups of Arctic char. The upper and lower bounds of Q values for putative hybrids between large- and small-bodied fish were found by running all Lower Tazimina Lake samples and the simulated hybrids in STRUCTURE with 50,000 replications during the presimulation burn-in, proceeded by an additional 450,000 iterations. Simulated hybrids had Q values that were ≤ 0.82 and ≥ 0.19. We then tested for a significant association between morph (large-, medium-, or small-bodied char) and the STRUCTURE-defined genetic groupings as described above (parental = >0.82 or <0.19; hybrid = ≤ 0.82 and ≥ 0.19) by using a 3×3 contingency table in PAST. Finally, Woods et al. (2013) also reported gill raker counts taken on the first arch (ranged from 20 to 32). We assessed the association between morphology and genetic identity by testing for a Pearson's correlation between individual gill raker count and the admixture proportions (Q) from the STRUCTURE analysis using the Hmisc package and the cor function in *R* (version 3.0.2, R Development Core Team 2011).

Results

Microsatellite variation among and within lakes

MICRO-CHECKER found no evidence of a failure to resolve alleles owing to 'large allele dropout' or otherwise nonamplifying ('null') alleles in any of the samples. All loci were polymorphic, except for *Sco*215 in Summit Lake which was monomorphic (Table S1). When examining samples within each lake (i.e., before partitioning by morph or by genetic subgroups identified by STRUCTURE), eight of 54 tests for deviations from HWE (four samples $\times$ 11 loci plus one sample $\times$ 10 loci) were significant at $P \leq 0.0171$, and three of the eight deviations were in the Lower Tazimina Lake sample owing to deficits of heterozygotes (Table S1). Of the tests of linkage disequilibrium per population, 1/45 for Summit Lake, 3/55 for Lower Tazimina Lake, 1/55 for Caribou Lakes, 2/55 for Lake Iliamna and 3/55 for Aleknagik Lake were significant. No locus pairs consistently deviated from linkage equilibrium across lakes, so we retained all loci in subsequent analyses.

The greatest average allelic richness was found in Lower Tazimina Lake (17.7) and Lake Aleknagik (16.4), followed by Iliamna Lake (16.1, Table S1). In Caribou Lakes and Summit Lake fish, allelic richness was lower (7.2 and 7.6, respectively); however, a large proportion of those alleles were unique to those populations. The highest level of gene diversity was observed at the locus *Sco*220 and *Smm*24 (1.0), while the locus *Sco*216 showed the lowest gene diversity (0.32). Across loci and populations, the highest average expected heterozygosity was seen in Lake

Aleknagik fish (0.84) and the lowest heterozygosity was seen in Summit Lake fish (0.68).

Population structure among lakes

Pairwise F_{ST} across all five lakes ranged from 0.050 between Iliamna Lake and Lower Tazimina Lake to 0.283 between Summit Lake and Caribou Lakes ($P < 0.02$, Table 1). In general, F_{ST} was greatest between Caribou Lakes and Summit Lake and all other samples (minimum $F_{ST} = 0.100$, Table 1). The FCA showed a large degree of divergence among populations, especially between Summit Lake and the char from the other four lakes (Figure S1). The FCA also revealed that the Lower Tazimina Lake char were more similar genetically to Iliamna Lake fish when compared across all populations in the Kvichak River system (Figure S1).

Analysis by STRUCTURE indicated that the most likely number of genetic populations was six among the five lakes, as evidenced by a mean log likelihood of −13199.7 versus −13298.7 for $K = 7$ as the next most likely model (Fig. 2). In the $K = 6$ model, each lake formed a distinct genetic group, but two groups were resolved within Lower Tazimina Lake (Fig. 2). When the Lower Tazimina Lake samples were analysed separately, the most likely number of clusters was two (mean log likelihood = −3507.1 vs. −3581.1 for $K = 1$ and −3659.5 for $K = 3$). No distinct clusters were observed within any of the other lakes when they were examined together (Fig. 2) or when the lakes were analysed individually; a $K = 1$ was returned in all cases (S.L. May-McNally, T.P. Quinn, P.J. Woods & E.B. Taylor, unpubl. data).

Comparisons between genetic and phenotypic data within Lower Tazimina Lake

When Lower Tazimina Lake char were grouped by body size (see Woods et al. 2013 for growth curve assignment to body size morph), there were no deviations from HWE (Table S1) and there was only a single deviation from linkage equilibrium in the large morph sample. Further, at 9 of 11 loci, small morphs exhibited a higher A_R per locus than both large and medium morphs as well as similar or higher A_R values relative to the populations from Iliamna Lake and Lake Aleknagik (Table S1).

There was a significant association between admixture values generated by STRUCTURE and body size morph; nine of 13 fish found in the range of Q values of simulated hybrids were of the medium-sized morph, while most large-bodied fish had Q values > 0.82 and most small-bodied fish had Q values < 0.19 ($\chi^2 = 29.6$, $P < 0.05$, d.f. = 4, Table 2). A one-way ANOVA followed by a *post hoc* test revealed significant pairwise differences among the mean Q values of each of the three morphs ($F_{2,71} = 50.9$, $P < 0.001$, all Tukey $P < 0.001$, mean $Q = 0.10$, 0.53 and 0.83 for small-, medium- and large-bodied morphs, respectively). Further, the mean Q value of the simulated hybrids (0.48) was not significantly distinct from that of the medium-sized morphs (Tukey $P = 0.72$), but was significantly distinct from the mean Q values of large- and small-bodied morphs (both $P < 0.001$).

In the FCA projection, large and small morphs showed roughly equivalent divergence from Iliamna Lake fish on the same FCA plot (Figure S2). Genetic distinction in allele frequencies was greatest between the large and small morphs ($F_{ST} = 0.092$, $P < 0.001$, Table 1). The medium and small morphs were also distinct from one another ($F_{ST} = 0.037$, $P < 0.001$), but there was only marginal genetic differentiation detected between the large and medium morphs ($F_{ST} = 0.017$, $P = 0.09$). Generally, Lower Tazimina Lake morphs were most divergent from Caribou and Summit lakes fish (minimum F_{ST} all > 0.108) and least divergent from char in Iliamna Lake (maximum $F_{ST} = 0.080$, Table 1). Pairwise F_{ST} averaged 0.048 among the three morphs in Lower Tazimina Lake and 0.050 between Lower Tazimina Lake morphs and fish from Iliamna Lake (Table 1).

We found a significant negative correlation ($r = -0.73$, $t = -8.67$, d.f. = 72, $P < 0.001$) between genetic admixture value (Q) and gill raker count across individual char (Fig. 3). Fish with low Q

Table 1. Pairwise F_{ST} (θ) estimated by variation across 11 microsatellite DNA loci in Arctic char (*Salvelinus alpinus*) from Caribou Lakes, Summit Lake, Lower Tazimina Lake large (L)-, medium (M)- and small-bodied (S) morphs, Iliamna Lake and Lake Aleknagik. Values accompanied by asterisks are not significantly >0 ($P \leq 0.0185$; Narum 2006).

	Caribou Lakes	Summit Lake	Lower Tazimina Lake-L	Lower Tazimina Lake-M	Lower Tazimina Lake-S	Iliamna Lake
Summit Lake	0.283	–	–	–	–	–
Lower Tazimina Lake-L	0.178	0.153	–	–	–	–
Lower Tazimina Lake-M	0.149	0.132	0.017*	–	–	–
Lower Tazimina Lake-S	0.162	0.117	0.092	0.037	–	–
Iliamna Lake	0.162	0.131	0.060	0.053	0.080	–
Lake Aleknagik	0.100	0.205	0.090	0.062	0.092	0.085

Pairwise comparisons between Caribou, Summit, Iliamna Lake, Lake Aleknagik and the pooled sample from Lower Tazimina Lake were 0.143, 0.108, 0.050 and 0.069, respectively (all $P < 0.0185$).

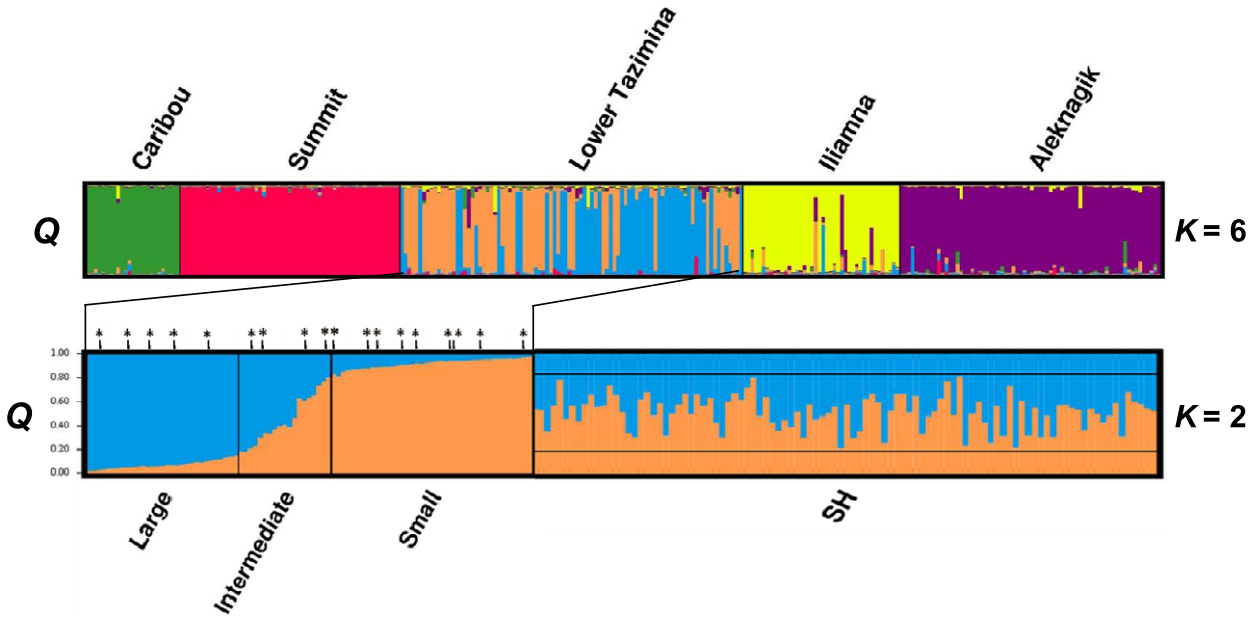

Fig. 2. Distruct plots for STRUCTURE runs of five populations of Arctic char (*Salvelinus alpinus*) from southwestern Alaska: Caribou Lake (N = 25), Summit Lake (N = 59), Lower Tazimina Lake (N = 91), Iliamna Lake (N = 42), Lake Aleknagik (N = 70), and simulated hybrids (SH, N = 100). Each fish is represented by a vertical bar that denotes membership fractions (Q) in K clusters. Lower Tazimina Lake body size morphs (lower panel) were arranged by decreasing Q values and separated according to growth curve assignment (see Woods et al. 2013) into large, medium and small-bodied forms (N = 74). Asterisks denote genetic samples from Lower Tazimina Lake that lack growth curve information, but that were included in the different body size groups according to their Q values. Horizontal solid lines in the SH plot represent the upper and lower boundaries for Q-value defined hybrids.

Table 2. Contingency table analysis of Q value categories and growth curve assignment for small, medium and large morphs of Arctic char (*Salvelinus alpinus*) sampled from Lower Tazimina Lake, south-western Alaska (N = 74).

Q value	Small	Medium	Large
0.0–0.18	28	5	0
0.19–0.82	2	9	2
0.83–1.0	0	9	19

values had higher average gill raker counts than fish with higher Q values reflecting the differentiation, genetically and phenotypically, between the small- and large-bodied morphs, respectively. Medium-sized morphs were characterised by Q values of between 0.19 and 0.82 and had an average gill raker count, of 23.8, compared with 27.9 and 22.8 for the small- and large-bodied morphs, respectively (Fig. 3).

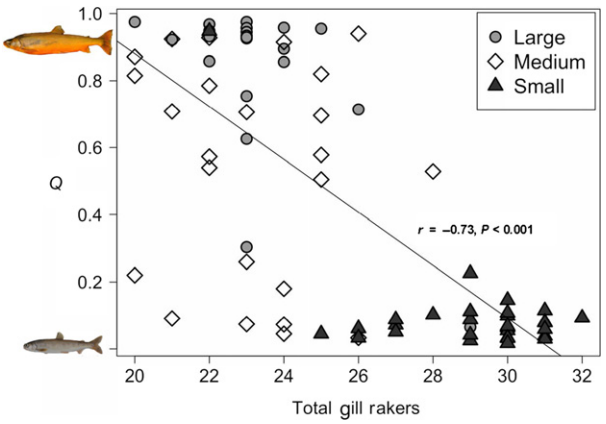

Fig. 3. Pearson's correlation between genetic admixture coefficients (Q) and total gill raker counts for Lower Tazimina Lake, southwestern Alaska, morphs of Arctic char (*Salvelinus alpinus*, N = 74). Fish images on y-axis represent relative body size and colouration differences (images from Woods et al. 2013).

Discussion

The Arctic char is a highly polymorphic species with an impressive ability to undergo rapid trophic diversification in isolated postglacial lakes (reviewed by Klemetsen 2010). The Kvichak River system in southwestern Alaska has phenotypically diverse forms of Arctic char, notably at least two sympatric morphs in Lower Tazimina Lake (Woods et al. 2013). We have provided evidence that this morphological and ecological variability is accompanied by significant genetic differentiation in that at least two of the morphs are strongly divergent from one another and their genetic identity can be predicted to some degree by growth curves and total gill raker counts.

Microsatellite DNA variation across remote freshwater lakes

Our microsatellite data revealed substantial variability in pairwise F_{ST} across Arctic char from five lakes

and are consistent with variability found in other studies involving postglacial lake systems with Arctic char (Bernatchez et al. 1998; Westgaard et al. 2004). The high values of pairwise F_{ST} involving Summit and Caribou lakes are likely attributable to their isolation from Iliamna Lake and Lower Tazimina Lake. Arctic char from both lakes exhibited numerous private alleles as well as dissimilar morphology and Von Bertalanffy growth curves compared with fish from Lower Tazimina Lake and Iliamna Lake (see Woods et al. 2013). Arctic char from Summit Lake and Caribou Lakes may represent populations that were derived from the earliest colonising fish from Kvichak River system. Summit Lake fish, in particular, showed low heterozygosity at multiple loci which may be due to differing demographic histories (e.g., numerous generations of inbreeding and genetic drift) in this very small lake. In addition, the low values for pairwise F_{ST} between Lower Tazimina Lake and Iliamna Lake when contrasted with F_{ST} values involving Summit Lake and Caribou Lakes may indicate a more recent colonisation of Lower Tazimina Lake. Alternatively, because Lower Tazimina Lake and Iliamna Lake (520 and 2622 km^2, respectively) are many times larger than Summit Lake and Caribou Lakes (0.6 and 1.2 km^2, respectively, Woods et al. 2013), they probably have had much larger effective population sizes historically that would constrain neutral divergence between these two large lakes.

The relatively low F_{ST} values between Iliamna Lake and Lake Aleknagik suggest that gene flow has occurred recently between them or is ongoing, although Arctic char in both lakes are generally regarded as nonanadromous populations because they are present in the watersheds throughout the summer when anadromous char are typically in the sea and, at least in Iliamna Lake, diet comparisons inferred from stable isotopes do not support anadromous behaviour of Arctic char (Denton et al. 2010). Anadromy, however, could exist as an alternative, but less common life-history strategy in these systems (e.g., McBride 1980); otolith microchemistry analysis of Arctic char from these lakes would provide a definitive test of this idea. Alternatively, the low F_{ST} between Iliamna Lake and Lake Aleknagik may reflect shared ancestral genetic polymorphism in the face of relatively high historical effective population sizes and recent postglacial founding of these populations.

Genetic differentiation among Lower Tazimina Lake body size morphs

The results of the STRUCTURE analysis in Lower Tazimina Lake were consistent with other studies that have shown that morphologically differentiated forms may also be genetically distinct, especially in systems where profundal and littoral morphs of Arctic char have been identified (e.g., Westgaard et al. 2004; Adams et al. 2007; Gomez-Uchida et al. 2008; Power et al. 2009; Conejeros et al. 2014). The Lower Tazimina Lake morphs shared most of their alleles with fish from Iliamna Lake, which suggests that they were founded by colonists from Iliamna Lake. Moreover, Lower Tazimina Lake morphs were genetically more similar to each other than to fish from one or more other lakes, suggesting a single colonisation event followed by divergence within the lake rather than two or more separate invasions of allopatrically derived forms. It is, however, impossible to discount the possibility that the large and small morphs were derived allopatrically and have become genetically similar by postcolonisation gene flow without a much more extensive survey of Arctic char throughout Alaska. A similar conclusion appears applicable to the sympatric 'pale' and 'dark' morphs on Gander Lake (Gomez-Uchida et al. 2008), and Garduño-Paz et al. (2010) presented genetic evidence that scenarios of allopatric and sympatric divergence of Arctic char may be lake-specific in Scotland.

In most systems with sympatric Arctic char populations, the large-bodied (and usually pelagic) morph is thought to most resemble the original anadromous colonising individuals because anadromous Arctic char are large-bodied and commonly piscivorous (e.g., Dempson et al. 2002). This is likely the case for Lower Tazimina Lake morphs: the large morph is at least partially piscivorous and is more similar in terms of allelic identity, size and morphology to Iliamna Lake fish than is the small morph (Woods et al. 2013). Small morphs from Lower Tazimina Lake showed greater divergence from Iliamna Lake Arctic char and they also had higher allelic richness and more private alleles than the large morphs and Iliamna Lake fish. These observations suggest that following colonisation of Lower Tazimina Lake, body size divergence was accompanied by two trophic shifts: one from piscivory towards greater benthic food consumption in large morphs and another towards foraging on small-sized limnetic prey by small morphs (cf. Woods et al. 2013). While the small morph represents a very distinct gene pool, the large morph population displayed greater admixture and contained more fish that were genetically intermediate between the large and small morphs. Nevertheless, mean admixture values were significantly different among all three morphs, but those of the medium-sized morph and simulated hybrids between large and small-sized morphs were not significantly different from one another. Further, gill raker counts of medium-sized fish were intermediate to those of the large and small-sized morphs and gill raker count typically has a genetic component in fishes (e.g.,

McPhail 1984). These observations are consistent with the idea that the medium-sized morphs are hybrids between the other two morphs of char rather than a third, less isolated population (cf. Snorrason et al. 1994). Also, the F_{ST} value between large and medium morphs was not significant, which suggests that if medium-sized char are hybrids, they backcross preferentially with large-sized char. Despite feeding together in the benthic regions of the lake, large and medium morphs consumed different prey items (Woods et al. 2013). The large morph ate more snails, whereas the medium morph ate more terrestrial insects. Given the detectable genetic divergence of medium-sized morphs from at least the small-sized morph, and polymorphism in prey choice and differential size at maturity, the medium-sized morph could be in the process of diverging into a third distinct population (e.g., Smith et al. 2003; Seehausen 2004).

Biogeography of sympatric morphs

While sympatric morphs of Arctic char have been described from European and Asian portions of their Holarctic range, accounts of sympatric morphs in North America are scant and restricted to the central Arctic or north-eastern North America. The two morphologically distinct 'dark' and 'pale' sympatric forms in Gander Lake, Newfoundland, showed strong genetic segregation (Power et al. 2005; Gomez-Uchida et al. 2008). Divergence between forms was hypothesised to have occurred when the lake became ice-free following the end of the last ice age (*c.* 12,000 years BP). Similarly, Lake Aigneau, in northern Québec, Canada, contains two morphs that are largely segregated in either the pelagic or littoral zones of the lake, and with very little gene flow between forms (F_{ST} = 0.174, Power et al. 2009). Conversely, analysis of 'large' and 'small' sympatric and morphologically distinct morphs from Lake Hazen, Nunavut, in the Canadian High Arctic revealed no evidence of genetic distinction (Arbour et al. 2011). Given that the last glacial retreat only reached the Lake Hazen region *c.* 8000 years BP and glacial ice cover of the lake was likely present up until 4200–3300 years BP, the lack of genetic differentiation may be attributable to a relatively recent colonisation of the lake (Arbour et al. 2011).

While previously documented in other regions of North America, Lower Tazimina Lake morphs represent the first example of sympatric morphotypes of Arctic char found on the west coast of North America. Further, Lower Tazimina Lake morphs exhibit some genetic divergence from one another, but most medium-sized morphs also had intermediate Q values (0.19–0.82) and accounted for approximately 18% of all individuals genotyped. If the medium-sized morphs are indeed hybrids, perhaps the char in Lower Tazimina Lake are currently at an evolutionary intermediate stage of divergence between geologically older lakes such as Gander Lake or Lake Aigneau and younger lakes like Lake Hazen. This hypothesis is consistent with the much stronger degree of divergence found between small-bodied and large-bodied morphs in Lake Aigneau (F_{ST} = 0.174, Power et al. 2009) compared with Lower Tazimina Lake (F_{ST} = 0.092). Lower Tazimina Lake was likely colonised by Arctic char via the Newhalen River sometime after the area became ice-free (*c.* 12,000–15,000 BP, Stilwell & Kaufman 1996; Ramstad et al. 2004), with divergence of char morphs perhaps occurring later than for the Gander Lake morphs, but earlier than for morphs in Lake Hazen. Collectively, Gander Lake, Lake Aigneau, Lower Tazimina Lake and Lake Hazen suggest a gradation of evolutionary change, which may be dependent, in part, on the time of glacial retreat and colonisation (cf. Garduño-Paz et al. 2010).

In addition to differences in divergence time, variation in environmental features of lakes that result in different levels of ecological opportunity probably also influence the levels of phenotypic and genetic divergence in sympatric populations of fishes (e.g., Lu & Bernatchez 1999; Landry et al. 2007; Siwertsson et al. 2010; Ormond et al. 2011). For instance, size-at-age growth curves were an effective means of assigning morphs into discrete populations and identifying intermediate individuals in Lower Tazimina Lake (Woods et al. 2013) which also differ genetically from one another as shown in our study. Further, small morphs had more gill rakers than large or medium morphs, and gill raker counts were strongly associated with genetic admixture (Q) values. Gill rakers, which assist in feeding on small prey items from the water, are generally more numerous in forms with largely limnetic diets in a variety of fishes (e.g., Bentzen & McPhail 1984; Gowell et al. 2012; Roesch et al. 2013). Indeed, Woods et al. (2013) showed that the diet of the small morph was characterised by consumption of more zooplankton and fewer carbon-based prey items derived from benthic resources (fewer snails, terrestrial and aquatic insects and fishes) and occupied a lower trophic position than either the medium or large morph. By contrast, the diets of the medium and large morphs differed only slightly from one another. Morphological adaptations such as gill rakers, diets and growth rates are therefore associated with neutral molecular differentiation, which suggests that genetic changes have accompanied phenotypic shifts as the morphs diverged in Lower Tazimina Lake. Given that resource polymorphism may be driving the isolation of large and small morphs in Lower Tazimina Lake,

an intriguing hypothesis is that variation in physiological and morphological traits has become partitioned across at least two different morphs and enabled optimal exploitation of available trophic resources (cf. Ohlberger et al. 2008; Evans et al. 2012). Further work should investigate whether differences in genes relevant to metabolism are also correlated with genetic identity (e.g., Bernatchez et al. 2010).

Woods et al. (2013) presented evidence for two growth phenotypes in Summit Lake (although a model invoking a single growth form was not substantially less supported). These authors, however, found no evidence of diet differences (from prey occurrence or stable isotope analyses) in Summit Lake char, in contrast to the situation in Lower Tazimina Lake. Similarly, our genetic analysis found no evidence of distinct genetic groups in the char from Summit Lake, so the existence of distinct forms in Summit Lake remains equivocal. In addition, there is no evidence of divergent phenotypes or gene pools within any of the other lakes that we examined (see also Woods et al. 2013). It is possible, however, that more extensive sampling especially with respect to depth could uncover as yet unknown diversity, especially in a large lake such as Iliamna Lake (cf., Finstad & Berg 2004). Alternatively, why some lakes might have divergent forms and others not is an uncertainty common to many instances of postglacial adaptive radiations in fishes and may result from historical or contemporary factors and their interactions (e.g., Bernatchez & Dodson 1990; Lu & Bernatchez 1999; Taylor & McPhail 2000; Landry et al. 2007; Siwertsson et al. 2010; Ormond et al. 2011). Kristjánsson et al. (2011) and Woods et al. (2012) explored characteristics of lakes that might influence morphological variation in Icelandic Arctic char, not including sympatric populations, and concluded that fish community composition was one of the most important factors (see also Ormond et al. 2011 for *Gasterosteus aculeatus*). Fish community composition may be relevant to the potential for sympatric populations, and in our system, diversity ranged from two species (Summit Lake) to at least 10 (Iliamna Lake), but is confounded with many other differences among lakes (Woods et al. 2013). A model that incorporated historical factors (e.g., time since colonisation, opportunities for multiple colonisation) and contemporary aspects of lake physiography and fish community structure across the geographical range of lakes with sympatric char would perhaps shed light on the factors critical to the origin and persistence of sympatric populations.

The North Pacific and Beringia are increasingly being recognised as important wellsprings of biodiversity and have been instrumental in understanding biogeography and speciation in temperate faunas (Cook & Auster 2005; Ilves & Taylor 2008; Taylor et al. 2008). Alaska is home to a diverse and well-established assemblage of Arctic char; however, quantifying the level of biodiversity on the western regions of North America has lagged behind that in the eastern Arctic and Europe owing to taxonomic confusion with Dolly Varden, remoteness of sites and difficulty in obtaining samples. Considering that many Arctic and sub-Arctic species are rapidly becoming at risk due to anthropogenic impacts such as climate change, dispersal of bio-pollutants and the harvesting of natural resources, research into these regions is becoming increasingly critical (Berg et al. 2010; Cook et al. 2013; Reist et al. 2013). Moreover, the study of unique intralacustrine populations of char and other fishes can provide clues about the role of adaptive divergence in speciation. Our findings and those of Woods et al. (2013) suggest that ecological aspects of individuals (gill raker count and associated feeding behaviour) are correlated with genetic identity and support the idea that the availability of divergent habitats and/or ecological opportunities within lakes may help to drive the development of specialised phenotypic traits in Arctic char. Further, the emergence of divergent phenotypes can contribute to reduced gene flow and thus promote the evolution of reproductive isolation between sympatric populations (e.g., Schluter 1996; Adams & Huntingford 2002a; Bernatchez et al. 2010; Klemetsen 2010).

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Supporting Information

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

Table S1. Genetic diversity values for Lower Tazimina Lake morphs (pooled sample and large, medium, and small-size morphs separately) and four populations of Arctic char (*Salvelinus alpinus*) from southwestern Alaska: sample codes; *N*, number of samples; *A*, number of alleles per locus; A_R, Allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; *P* (HW), probability of departure from HWE ($P \leq 0.0185$), and F_{IS}, the inbreeding coefficient.

Figure S1. Factorial correspondence analysis (FCA) of five populations of Arctic char (*Salvelinus alpinus*) from southwestern Alaska.

Figure S2. Factorial correspondence analysis (FCA) of Lower Tazimina Lake, southwestern Alaska, Arctic char (*Salvelinus alpinus*) morphs and Arctic char from Iliamna Lake Populations are denoted by ellipses as follows: Blue = Iliamna Lake; Red = Large and Medium morphs; Green = Small morphs.