The Genomic Consistency of the Loss of Anadromy in an Arctic Fish (*Salvelinus alpinus*)

Sarah J. Salisbury,[1,*] Gregory R. McCracken,[1] Robert Perry,[2] Donald Keefe,[3] Kara K. S. Layton,[4,5,†] Tony Kess,[4] Cameron M. Nugent,[6] Jong S. Leong,[7] Ian R. Bradbury,[1,4,5] Ben F. Koop,[7,8] Moira M. Ferguson,[6] and Daniel E. Ruzzante[1]

1. Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada; 2. Department of Environment, Fish, and Wildlife Division, Government of Yukon, Whitehorse, Yukon, Canada; 3. Department of Fisheries, Forestry, and Agriculture, Forestry and Wildlife Branch, Government of Newfoundland and Labrador, Corner Brook, Newfoundland, Canada; 4. Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland, Canada; 5. Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; 6. Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada; 7. Department of Biology, University of Victoria, Victoria, British Columbia, Canada; 8. Centre for Biomedical Research, University of Victoria, Victoria, British Columbia, Canada

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ABSTRACT: The potentially significant genetic consequences associated with the loss of migratory capacity of diadromous fishes that have become landlocked in freshwater are poorly understood. Consistent selective pressures associated with freshwater residency may drive repeated differentiation both between allopatric landlocked and anadromous populations and within landlocked populations (resulting in sympatric morphs). Alternatively, the strong genetic drift anticipated in isolated landlocked populations could hinder consistent adaptation, limiting genetic parallelism. Understanding the degree of genetic parallelism underlying differentiation has implications for both the predictability of evolution and management practices. We employed an 87k single-nucleotide polymorphism (SNP) array to examine the genetic characteristics of landlocked and anadromous Arctic char (*Salvelinus alpinus*) populations from five drainages within Labrador, Canada. One gene was detected as an outlier between sympatric, size-differentiated morphs in each of two landlocked lakes. While no single locus differentiated all replicate pairs of landlocked and anadromous populations, several SNPs, genes, and paralogs were consistently detected as outliers in at least 70% of these pairwise comparisons. A significant C-score suggested that the amount of shared outlier SNPs across all paired landlocked and anadromous populations was greater than expected by chance. Our results indicate that despite their isolation, selection due to the loss of diadromy may drive consistent genetic responses in landlocked populations.

Keywords: parallelism, allopatry, landlocked, anadromy, incipient speciation, SNPs.

* Corresponding author; email: sarahsalisbury13@gmail.com.

† Present address: School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom.

ORCIDs: Salisbury, https://orcid.org/0000-0001-7637-7742; McCracken, https://orcid.org/0000-0002-1701-1529; Layton, https://orcid.org/0000-0002-4302-3048; Kess, https://orcid.org/0000-0002-1079-3791; Nugent, https://orcid.org/0000-0002-1135-2605; Leong, https://orcid.org/0000-0001-6521-9239; Koop, https://orcid.org/0000-0003-0045-5200; Bradbury, https://orcid.org/0000-0002-8152-4943; Ruzzante, https://orcid.org/0000-0002-8536-8335.

Introduction

The loss of migratory capacity is a fundamental promoter of neutral and adaptive differentiation (Waters et al. 2020). Such a loss is frequently observed in diadromous fishes, whose landlocking in postglacial lakes offers a unique opportunity to study the predictability of evolution (Elmer and Meyer 2011). These populations were formed subsequent to the last glacial maximum (<20,000 years), when anadromous individuals became trapped in freshwater environments (typically lakes) through a variety of mechanisms, including isostatic rebound and physical impoundments (Lee and Bell 1999). Once landlocked, fish maintain a freshwater resident life history, typically exchanging minimal to no gene flow with other populations (e.g., Hindar et al. 1991; Palkovacs et al. 2008; Delgado et al. 2019). This independence makes them ideal natural replicates of evolution (Lee and Bell 1999) that may be compared to assess the consistency of their genetic differentiation in response

to common selective pressures (Elmer et al. 2014; Jacobs et al. 2020; McGee et al. 2020).

The loss of anadromy has predictable selective consequences (Delgado and Ruzzante 2020). For example, landlocked and diadromous populations may reliably differ in their diets (Palkovacs et al. 2008) as well as the predators (Hendry et al. 2004), parasites (Bouillon and Dempson 1989), and fishing pressure (Hendry et al. 2004) they experience. Landlocked populations are released from the selective pressures imposed by saltwater environments, resulting in predictable physiological changes in osmoregulation (Velotta et al. 2014) and swimming capacity (Velotta et al. 2018). Given these consistent environmental and phenotypic differences, one might expect that the same genetic differences repeatedly underlie this adaptation to the loss of anadromy (i.e., genetic parallelism). Parallelism has been observed in threespine stickleback (*Gasterosteus aculeatus*), where loci such as *Eda* (Nelson and Cresko 2018) and *Pitx* (Xie et al. 2019) are known to play a role in the repeated colonization of freshwater from the marine environment. However, the degree of genomic parallelism that underlies the allopatric differentiation of landlocked and diadromous fishes more broadly remains largely unknown (Delgado et al. 2020; Kjærner-Semb et al. 2020).

Divergent selection can also drive morph differentiation within lakes (Lee and Bell 1999; Schultz and McCormick 2012). If divergent selection is consistent within multiple lakes, this can result in repeated morph differentiation (Schluter and Nagel 1995; Schluter 1996). For example, sympatric limnetic and benthic morphs have recurrently evolved in many lacustrine stickleback (Taylor and McPhail 1999) and whitefish (Bernatchez et al. 2010) populations. A growing number of studies have employed genomic data to investigate the degree of genetic parallelism underlying lacustrine radiations (e.g., Elmer et al. 2014; Meier et al. 2018; Jacobs et al. 2020; Härer et al. 2021; Jacobs and Elmer 2021). However, expectations of genetic parallelism underlying repeated morph differentiation in landlocked lakes are generally unknown, particularly in nonmodel fish.

Different levels of genetic parallelism could also be important for driving repeated morph differentiation (Salisbury and Ruzzante 2022). For example, identical alleles/single-nucleotide polymorphisms (SNPs) could contribute to the same phenotypic differentiation. Alternatively, parallelism could occur at the level of the gene, where different SNPs/mutations (but within the same gene) cause morph differentiation in different locations. Repeated morph differentiation could also be due to the employment of different paralogous copies of the same gene. Such genetic parallelism at the level of the paralog is largely unexplored (Nichols et al. 2008; Conte et al. 2012) but may be particularly important for salmonids, given their recent whole genome duplication resulting in numerous homeologs (Macqueen and Johnston 2014).

Many mechanisms could potentially undermine this genetic parallelism. For instance, genetic drift facilitated by a lack of gene flow (Bernatchez et al. 2002), founder effects (Ramstad et al. 2004), or reduced carrying capacities (McCracken et al. 2013) may greatly impede genetic parallelism. Genetic parallelism could also be limited if replicate morphs are subject to different local selective pressures (Campbell and Bernatchez 2004) or are phylogenetically distant, causing a reduction in shared genetic variation (Conte et al. 2012). Alternatively, genetic parallelism will be reduced where multiple genetic pathways can be employed to achieve the same morph differentiation. We were therefore interested in examining the degree of genetic parallelism and the factors limiting it in polymorphic Arctic char (*Salvelinus alpinus*) (1) between replicate landlocked and anadromous populations and (2) between replicate sympatric morphs within landlocked lakes.

Labrador is an ideal location for such work, as it contains numerous landlocked char populations inhabiting the same drainage as anadromous populations (Anderson 1985), thus forming natural paired replicates of allopatric differentiation. This differentiation has occurred recently, as Labrador was deglaciated 9,000 years BP (Bryson et al. 1969; Occhietti et al. 2011). Anadromous populations are fished in Labrador as part of economically and culturally important subsistence, recreational, and commercial fisheries (Andrews and Lear 1956; Scott and Crossman 1973; Dempson et al. 2008) and have been genetically well studied in Labrador (e.g., Bernatchez et al. 1998; Layton et al. 2020, 2021). However, comparatively little is known about landlocked populations. We previously found lower genetic diversity in landlocked than in anadromous populations using microsatellites (Salisbury et al. 2018) and mtDNA (Salisbury et al. 2019). Though neutral genetic differentiation between landlocked and anadromous char populations has previously been assessed (Bernatchez et al. 1998; Kapralova et al. 2011; Salisbury et al. 2018), adaptive genetic differences between landlocked and anadromous populations remain uncharacterized in this species.

Genetically distinguishable sympatric Arctic char morphs have been previously identified in two landlocked lakes in Labrador using neutral microsatellites (Salisbury et al. 2018). Size-differentiated, genetically distinguishable ecotypes of Arctic char have been also observed within nearby lakes in Newfoundland (Kess et al. 2021) and northern Quebec (Power et al. 2009). However, the repeatability of the adaptive genetic differentiation associated with such sympatric morphs within landlocked lakes remains unknown. Our recent work in this region has revealed limited genetic parallelism across size-differentiated sympatric morphs

(consistent with putative resident and anadromous morphs) occurring in three sea-accessible Labrador lakes (Salisbury et al. 2020). Whether the genetic mechanisms driving sympatric morph differentiation in char are the same in landlocked and sea-accessible lakes remains unexplored. In addition, little genetic parallelism has been observed between ecologically differentiated sympatric morphs across Scottish and Russian landlocked populations (Jacobs et al. 2020). However, unlike Scottish and Russian landlocked char populations, which were founded by single lineages (Atlantic or Siberian, respectively; Moore et al. 2015), Labrador landlocked populations demonstrate mtDNA haplotypes consistent with Acadian, Atlantic, and Arctic glacial lineages, reflecting the secondary contact of all three lineages within this region (Salisbury et al. 2019). Individual landlocked populations in Labrador could have therefore been founded by very different ancestral populations (e.g., different glacial lineages).

This potential for differential ancestry among landlocked populations as well as the genetic drift likely experienced by these isolated lakes may have reduced shared genetic variation among landlocked populations, thereby thwarting genetic parallelism between both replicate landlocked and anadromous populations and between replicate sympatric morph differentiation within landlocked lakes. Alternatively, the recent establishment and close geographic proximity of these landlocked populations could have resulted in them sharing genetic variation and selective pressures contributing to genetic parallelism. Therefore, the expected degree of genetic parallelism in response to the loss of anadromy and driving incipient speciation within these Labrador landlocked lakes is unknown.

Such insight into the character and consistency of the adaptive genetic differentiation between allopatric landlocked and anadromous populations and between sympatric morphs within landlocked populations is crucial for both their management and our understanding of the predictability of evolution. We employed a newly designed 87k SNP array (Nugent et al. 2019) to characterize the adaptive differentiation between paired landlocked and anadromous populations from the same drainage area and between sympatric morphs within landlocked populations. Our study takes advantage of natural replicates of landlocked populations across five drainages in Labrador, Canada, to assess for genetic parallelism at the level of the SNP, gene, and paralog. We hypothesize that (1) consistent selective pressures due to the loss of anadromy has resulted in parallel genetic differentiation of landlocked populations and (2) consistent divergent selection within landlocked lakes has resulted in parallel genetic differences between sympatric lacustrine char morphs.

Methods

Sampling

Tissue samples (gill/fin; $N = 342$, table S1; tables S1–S14 are available online) were collected between 2010 and 2017 from landlocked and anadromous Arctic char populations from five drainages in Labrador; these were, from north to south, Saglek Fjord, Hebron Fjord, Okak Region, Anaktalik River, and Voisey Bay (fig. 1). Landlocked populations (code ending with -L) were sampled using variable sized standardized nylon monofilament gillnets (1.27–8.89 cm diagonal) while anadromous populations (code ending with -A) were electrofished. Landlocked specimens were weighed (g), measured for fork length (mm) and assessed for sex and maturity. All samples were immediately stored in 95% ethanol or RNAlater.

For comparative purposes, in a subset of our analyses we also included $N = 178$ individuals reported in Salisbury et al. (2020) from three sea-accessible lakes (according to Anderson 1985; but see Van der Velden et al. 2013) named Ramah (R), Brooklyn (B), and Esker North (E), each of which was found to contain sympatric big (putative anadromous) and small (putative resident) morphs (table S2). These populations were sampled, extracted, and genotyped identically to the anadromous and landlocked populations that are the focus of this study.

Extraction, Sequencing, Genotyping, and Quality Control

DNA was extracted using either a glass milk protocol (modified from Elphinstone et al. 2003), a phenol chloroform protocol (modified from Sambrook and Russell 2006), or a Qiagen DNeasy 96 blood and tissue extraction kit (Qiagen). Samples were quantified using QuantIT PicoGreen (Life Technologies) on the LightCycler 480 II (Roche) and normalized using epMotion 5075 liquid handling robot (Eppendorf) to a volume of 40 μL and a median concentration of 12 ng/μL.

DNA samples were sent to the Clinical Genomic Centre of Mount Sinai Hospital (Toronto) for sequencing using an 87k Affymetrix Axiom Array (Nugent et al. 2019). We employed the "best practices workflow" for a diploid organism in Axiom Analysis Suite (ver. 4.0.1.9; supplemental PDF, available online). After applying Axiom Analysis Suite QC, we retained a total of $N = 307$ individuals (table S1) for further analyses.

A minor allele frequency (MAF) filter of 0.01 was applied using PLINK (ver. 1.9; Chang et al. 2015) when investigating structure within each landlocked population, between each pair of landlocked/anadromous populations, and across all landlocked and anadromous populations. PGDSpider (ver. 2.1.1.5; Lischer and Excoffier 2012) was used to convert between PLINK and Genepop files, and

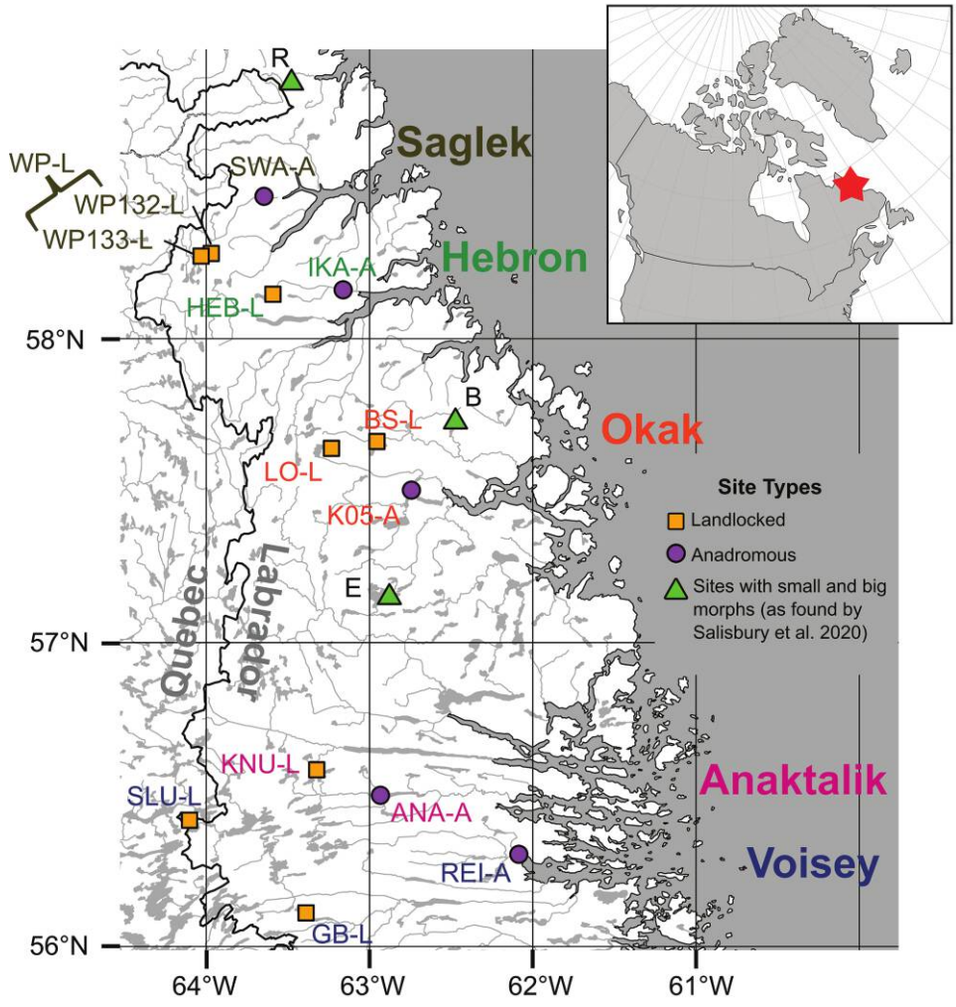

Figure 1: Sampling locations in Labrador, Canada, in five drainages: Saglek Fjord, Hebron Fjord, Okak Region, Anaktalik River, and Voisey Bay. Within each drainage, squares indicate landlocked Arctic char (*Salvelinus alpinus*) populations, and circles indicate anadromous Arctic char populations. Landlocked and anadromous population codes are colored by drainage. Triangles indicate additional putatively sea-accessible lakes identified as containing sympatric small and big morphs by Salisbury et al. (2020). R = Ramah; B = Brooklyn; E = Esker North. Map generated using data from CanVec (Government of Canada).

the R package (R Development Core Team 2013) genepopedit (Stanley et al. 2017) was used to order and arrange Genepop files for downstream analyses.

Genetic Differentiation within Landlocked Populations

Before we could compare landlocked with anadromous populations, it was first necessary to identify any sympatric morphs present within landlocked lakes to prevent genetic substructuring within lakes biasing subsequent comparisons between landlocked and anadromous populations. Therefore, landlocked populations were assessed for $K = 1-5$ with ADMIXTURE (ver. 1.3; Alexander et al. 2009) using 10 cross-validations. Saglek Fjord landlocked samples (WP132 and WP133) were analyzed together because of their close proximity and previously noted genetic similarity (Salisbury et al. 2018). Voisey Bay landlocked samples (SLU-L and GB-L) were analyzed together because of their low sample sizes. For landlocked populations where the best (lowest average cross-validation error) $K > 1$, individuals were assigned to genetic groups based on ADMIXTURE Q values. Genetic substructuring was confirmed using (1) the R package pcadapt (ver. 4.1.0; Luu et al. 2017) testing $K = 1$–20 ($K = 1$–10, where the number of samples is <20) with the default Mahalanobis distance, and (2) the snmf function in the R package LEA (Frichot and François 2015) testing $K = 1$–5 using 10 repetitions. Before assessing the effects of genetic group

assignment and maturity on fork length (mm), we conducted Levene tests of the equality of variances. Where the variance of fork length differed among groups, we conducted Welch's ANOVAs and Games-Howell post hoc tests; otherwise, we conducted ANOVAs and post hoc pairwise *t*-tests using a Bonferroni correction ($\alpha = 0.05$).

Overall Population Structure

To investigate the relationships among all populations and confirm that landlocked populations were most genetically similar to those anadromous populations within the same drainage, we performed a pcadapt analysis (testing $K = 1$–30). We included all populations as well as the sympatric small and big morphs identified in three lakes (Ramah, Brooklyn, Esker North) in Labrador by Salisbury et al. (2020) for a total of $N = 485$ samples. Weir and Cockerham (1984) F_{ST}'s were also estimated between all populations using the package hierfstat (Goudet 2005). To assess whether regions of high linkage disequilibrium (LD) were potentially influencing the genetic structure of all populations, we used an LD pruning method as suggested by Lotterhos (2019). Specifically, using only those SNPs mapped to one of the 39 char linkage groups, we applied an MAF filter of 0.01 before using the –indep-pairwise function in PLINK to scan the genome in windows of 50 SNPs, shifting 5 SNPs at a time, with an r^2 threshold of 0.5.

Genetic Differentiation between Landlocked and Anadromous Populations

Each sympatric morph within a single landlocked population was subsequently separately compared with the anadromous population within the same drainage. The genetic structure between paired landlocked and anadromous populations was assessed using (1) pcadapt and (2) the snmf function in the R package LEA using parameters identical to the tests within landlocked lakes as outlined above.

Outlier Detection

Outlier SNPs were detected using two methods. First, pcadapt using default parameters and an MAF of 0.01 was used to detect outlier SNPs based on their correlation with the first principal component (PC) axis after *P* values were corrected using the false discovery rate (FDR; Storey and Tibshirani 2003) with the R package qvalue (ver. 2.14.1; Storey et al. 2015). Second, using Weir and Cockerham (1984) F_{ST}'s estimated from PLINK, SNPs with an $F_{ST} > 3$ SD above the mean F_{ST} were considered outliers. We compared SNPs to the *Salvelinus* genome (NCBI; https://www.ncbi.nlm.nih.gov/genome/86400) using BEDOPS (Neph et al. 2012) and identified the closest coding sequence (CDS) within 5 kbp of each SNP. By design, many SNPs on the chip were located within the CDS of genes; however, given the observed LD decay between paired comparisons (fig. S1, S2; figs. S1–S19 are available online), associating SNPs with the closest CDS within 5 kbp is likely a conservative yet reasonable approach.

Allelic frequencies of outlier SNPs were visualized using the R package ComplexHeatmap (Gu et al. 2016). Allelic trends for outlier SNPs were defined as parallel if the direction of the difference in major allele frequency (positive or negative) was the same across all the paired comparisons for which the SNP was detected as an outlier.

Gene ontology (GO) enrichment analyses for biological processes (BP) were conducted using the R package TopGO, employing the protein GO annotation file generated for char (Christensen et al. 2018). GO term significance was assessed with a Fisher's exact test using the weight01 algorithm, and *P* values were corrected using the FDR ($\alpha = 0.05$).

Assessing Genetic Parallelism at the Level of the SNP, Gene, and Paralog

For landlocked populations with multiple sympatric morphs, the outliers detected by comparing each morph separately with a common downstream anadromous population were pooled over all morph comparisons. This reflects the fact that each sympatric morph does not represent an independent replicate of landlocking given the potential for gene flow between sympatric morphs. We calculated C-scores (Yeaman et al. 2018) using the R package dgconstraint to investigate whether the amount of shared outlier SNPs detected across replicate pairs of (1) landlocked versus anadromous populations and (2) sympatric morph pairs within landlocked lakes was statistically significant. We applied a binary assignment to outliers and nonoutliers (1 or 0, respectively) and employed a hypergeometric approach when comparing two paired comparisons but a χ^2 approach using 100,000 permutations when comparing more than two paired comparisons (for more details, see the supplemental PDF).

We were also interested in investigating the importance of paralogs to both local adaptation and parallel morph differentiation. Given our use of an SNP chip, we were unable to identify paralogs by direct sequence comparison. Instead, we took the same conservative approach to identifying paralogs as employed by Salisbury et al. (2020), identifying outlier SNPs annotated with identical protein names but different protein codes (XP_) as putative paralogs. While this does not allow for the comprehensive identification of paralogs within our data (e.g., our approach

prevents detection of paralogs with nonidentical names and does not allow assessment of the timing of duplication) and relies upon the accuracy of the reference genome, it nevertheless provides some insight into whether paralogous copies of the same gene may contribute to local adaptation or incipient divergence. Parallelism at the level of paralog was inferred when different paralogous copies of a given gene differentiated different replicates.

Results

Genetic Differentiation within Landlocked Populations

Population structure analyses revealed evidence of genetic substructuring (consistent with sympatric morphs) within only two of the seven landlocked locations sampled in this study. After filtering using an MAF of 0.01 (resulting in 6,404–16,702 SNPs per landlocked lake; table S3), ADMIXTURE analyses supported within-lake genetic substructuring (where the best $K > 1$) in only WP-L and LO-L. In each of these two locations, $K = 2$ genetic groups were detected (fig. 2*a*, 2*d*; table S4). This was confirmed with pcadapt and snmf structure analyses (fig. S3–S6). The two genetic groups detected within WP-L were apparent within each of the two neighboring lakes associated with this location (WP132-L and WP133-L), suggesting recent gene flow between the lakes despite intervening falls (Anderson 1985). Six WP-L individuals had intermediate Q values ($0.4 < Q < 0.6$), suggesting that they were hybrids between the two purebred genetic groups. These samples were removed before conducting all outlier detection analyses to more easily detect signatures of divergent selection. Pairwise mean F_{ST} values between these ADMIXTURE-defined groups was 0.12 within WP-L (calculated excluding putative hybrids) and 0.12 within LO-L. There was no evidence of sympatric differentiation within any of the other landlocked lakes (see the supplemental PDF; table S4; figs. S7, S8).

Size Differences between Sympatric Genetic Groups

The genetic groups detected within WP-L and LO-L differed by fork length. In WP-L, significant differences in length were detected (Levene test, $F_{5,49} = 8.76$, $P < .001$; one-way Welch's ANOVA, $F_{5,7.37} = 22.44$, $P < .001$; based on $N = 55$ individuals after removing three individuals with unknown maturity status), and post hoc Games-Howell tests revealed significant differences in length between genetic groups (fig. 2*b*; table 1; for differences by sex, see fig. S9). The ADMIXTURE-assigned hybrids were larger than either of the purebred big or small morphs ($\bar{x}_{\text{hybrids}} = 385$ mm [$N = 6$]; $\bar{x}_{\text{purebred big}} = 329$ mm [$N = 24$]; $\bar{x}_{\text{purebred small}} = 145$ mm [$N = 28$]; fig. 2*b*; table 1). In LO-L ($N = 29$), there were also significant differences in length (Levene test, $F_{3,25} = 4.75$, $P < .01$; one-way Welch's ANOVA, $F_{3,4.57} = 68.60$, $P < .001$), with post hoc Games-Howell tests again suggesting differences between genetic groups (fig. 2*e*; table 1; for differences by sex, see fig. S10)). Within both WP-L and LO-L, each genetic group comprised mature and immature individuals of both sexes (table 1; for information from other landlocked locations, see table S5). We refer to each genetic group within WP-L and LO-L as small and big morphs hereafter.

Overall Population Structure

Having identified two landlocked lakes with sympatric morphs, we were interested in assessing the overall genetic structure of landlocked and anadromous populations in our study system as well as the genetic relationships of these big and small morphs with those big and small morphs detected in Ramah, Brooklyn, and Esker North from Salisbury et al. (2020). After applying an MAF of 0.01 across all samples ($N = 485$), we retained $N = 21{,}201$ SNPs. Results from pcadapt were nearly identical after applying LD pruning; we report only the results using the full set of 21,201 SNPs (but see fig. S11). The first two PCs separated all anadromous populations from all landlocked populations (fig. 3). Landlocked populations demonstrated strong genetic distinctiveness, in contrast to the anadromous populations. Interestingly, big morphs from Ramah grouped with anadromous populations, unlike big and small morphs from Brooklyn and Esker North, which grouped separately from anadromous populations, like those big and small morphs in WP-L and LO-L. Weighted pairwise F_{ST} estimates confirmed these results and generally demonstrated that landlocked populations were most genetically similar to the anadromous population within their drainage (fig. S12). We next compared landlocked and anadromous populations within drainages to minimize detection of genetic differences potentially due to population structure and local adaptive differences across drainages.

Genetic Differentiation between Landlocked and Anadromous Populations

Consistent with our overall principal component analysis, paired landlocked and anadromous populations demonstrated significant genetic differences. Between 17,321 and 22,540 SNPs passed filtering for each landlocked versus anadromous population comparison (tables 2, S6). Mean F_{ST} values between paired landlocked and anadromous populations from the same drainage ranged from 0.10 to 0.26 (table 2). Significant genetic distances between

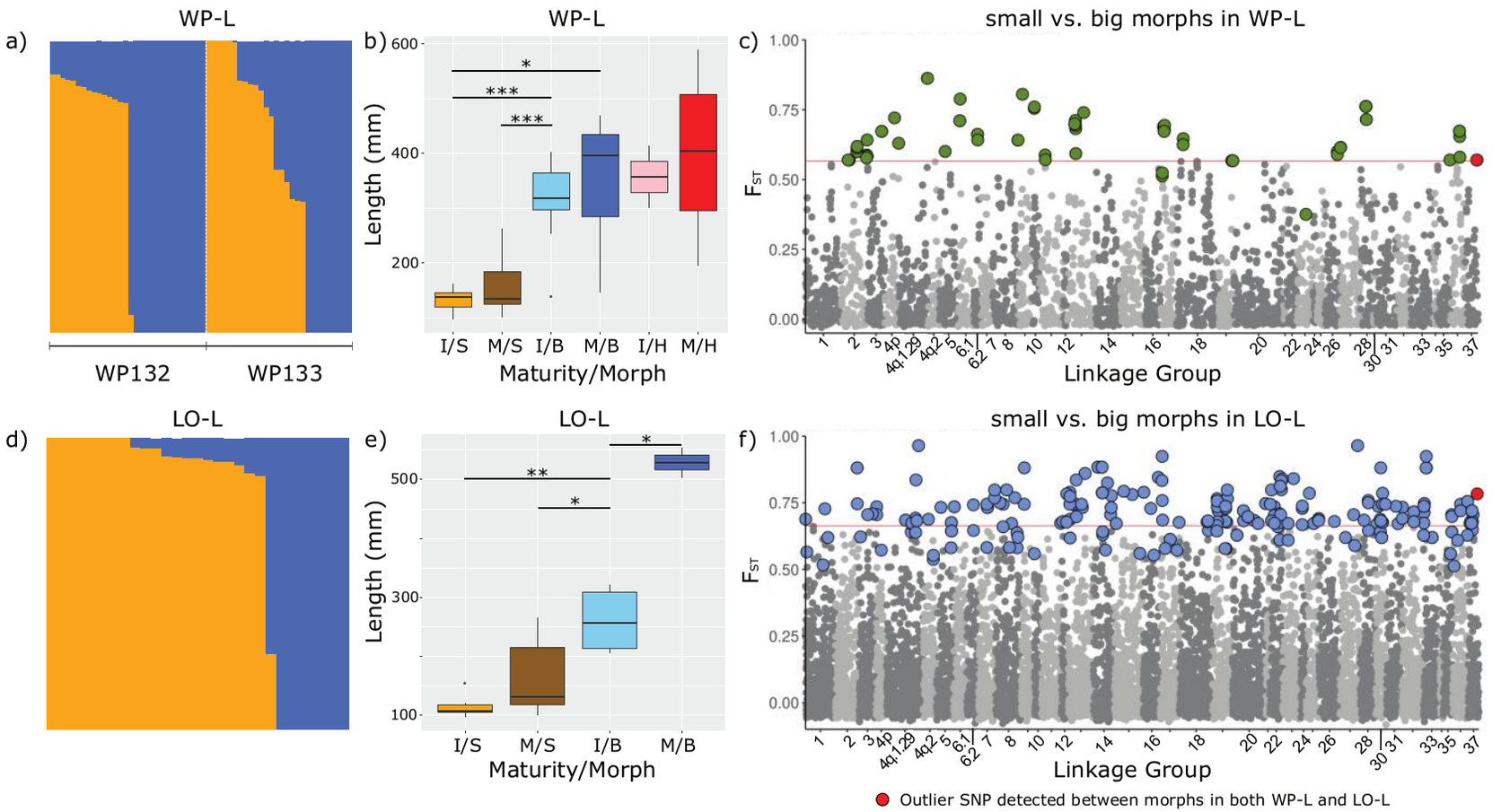

Figure 2: *a*, *d*, ADMIXTURE plots of $K = 2$ for WP-L (*a*) and LO-L (*d*). Orange bars indicate small morph individuals, blue bars indicate big morph individuals. *b*, *e*, Boxplots demonstrating length of fish by maturity (I = immature; M = mature) and morph type (S = small; B = big; H = hybrid) in WP-L ($N = 55$; *b*) and LO-L ($N = 29$; *e*). Asterisks indicate significant post hoc Games-Howell tests ($^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$). *c*, *f*, Manhattan plots demonstrating F_{ST} values of outlier loci detected between small and big morphs (excluding hybrids) in WP-L (*c*) and LO-L (*f*). Red lines indicate 3 standard deviations above the mean F_{ST}, and detected outliers are highlighted.

Table 1: Number of small and big morph Arctic char samples detected within landlocked locations WP-L and LO-L

			Immature		Mature		Lineage	
Location, morph	*N*	Mean (median) length (mm)	Males	Females	Males	Females	Atlantic	Arctic
WP-L:								
Small	28	145 (136)	6	4	7	9	15	0
Big	24	329 (352)	8	8	3	4	14	0
Hybrid	6	385 (372)	1	1	3	1	4	0
LO-L:								
Small	21	145 (125)	2	4	8	7	0	16
Big	8	328 (303)	3	3	1	1	0	7

Note: Arctic and Atlantic lineage haplotypes are from Salisbury et al. (2019). The sum of all immature/mature males/females may not equal the sample number (*N*), as some samples had unknown maturity status.

paired landlocked and anadromous populations were confirmed by pcadapt and snmf population structure analyses (figs. S13–S16). As expected given this strong genetic differentiation, a large number of outlier SNPs were detected between paired landlocked and anadromous populations (370–2,296 SNPs; fig. 4; table 2; file S1; files S1–S3 are provided in a zip file, available online). Outlier SNPs were detected across the genome in 35 or more linkage groups in all comparisons (table 2; see table S7 for SNPs detected by each method and table S8 for polymorphic SNPs overlapping across comparisons).

Genetic Parallelism across Landlocked versus Anadromous Comparisons

A total of 6,357 SNPs were detected as outliers in at least one of the seven landlocked versus anadromous population comparisons. None were detected in all seven comparisons,

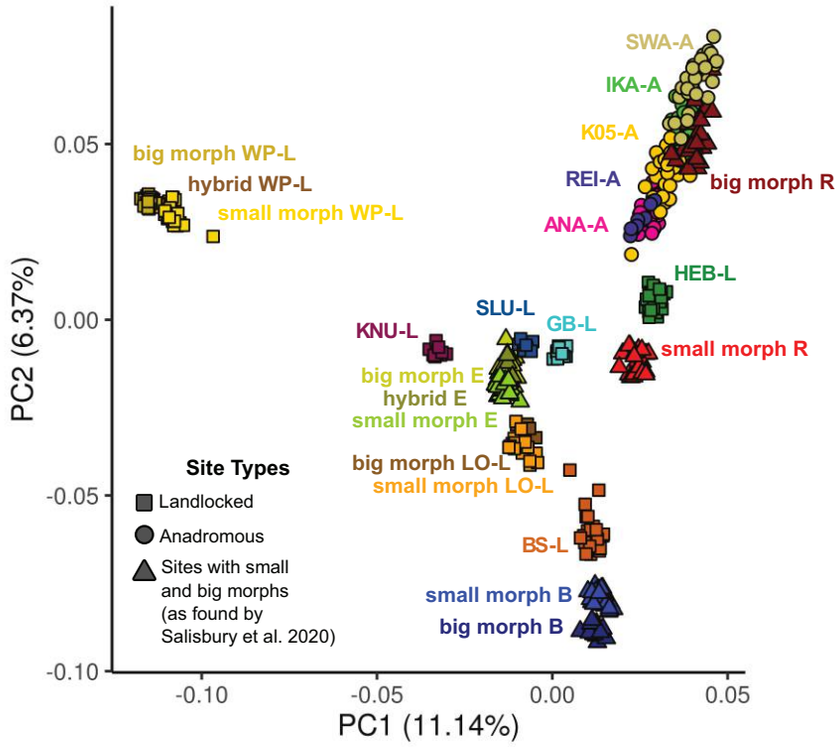

Figure 3: Principal component analysis based on $N = 21{,}201$ SNPs of landlocked (-L; squares) and anadromous (-A; circles) populations as well as small and big morphs from three putatively sea-accessible lakes (R = Ramah; B = Brooklyn; E = Esker North; triangles) detected by Salisbury et al. (2020). Note that small and big morphs were also detected in landlocked populations WP-L and LO-L (see fig. 2).

Table 2: All landlocked and anadromous population comparisons

Landlocked vs. anadromous population comparison	Drainage	Landlocked population compared	*N*	Anadromous population compared	*N*	SNPs	Mean pairwise F_{ST}	Outlier SNPs	Pooled outlier SNPs over all sympatric morph comparisons	Linkage groups with outlier SNPs
1	Saglek	Small morph WP-L	28	SWA-A	30	20,393	.242348	1,616	1,994	39
	Saglek	Big morph WP-L	24	SWA-A	30	20,361	.256677	1,366		39
2	Hebron	HEB-L	30	IKA-A	25	19,613	.191969	548		39
3	Okak	BS-L	29	K05-A	29	20,334	.12467	370		35
4	Okak	Small morph LO-L	21	K05-A	29	22,540	.100295	465	2,454	38
	Okak	Big morph LO-L	8	K05-A	29	22,185	.130439	2,250		39
5	Anaktalik	KNU-L	16	ANA-A	30	19,994	.187325	2,296		39
6	Voisey	SLU-L	10	REI-A	9	17,385	.155127	546		37
7	Voisey	GB-L	12	REI-A	9	17,321	.17172	479		38

Note: In those landlocked populations with multiple sympatric morphs, each was compared independently with the downstream anadromous population to avoid within-lake population structure biasing outlier detection. Outliers detected using each paired comparison with a sympatric morph were then pooled for a single landlocked lake, as each sympatric morph does not represent an independent replicate of landlocking, given the potential for gene flow between sympatric morphs since landlocking.

one SNP was detected as an outlier in six comparisons, 29 were detected in five comparisons, 76 in four, 356 in three, 1,269 in two, and 4,626 in only one comparison. This degree of overlap in outlier SNPs across all landlocked versus anadromous population comparisons was statistically significant (C-score$_{\chi^2}$ = 26.34, $P < 10^{-5}$).

We limit our discussion to only those 30 outlier SNPs that were detected in five or more of the seven landlocked versus anadromous population comparisons. Of these 30 SNPs, 28 showed evidence of parallel allelic trends (fig. 5) and these 30 SNPs corresponded to 21 genes (table 3). An additional two genes contained outlier SNPs in five or more comparisons, but the specific outlier SNP differed between comparisons. Therefore, a total of 23 outlier genes were detected in five or more landlocked versus anadromous populations. No significant BP GO terms were enriched among these 23 genes after adjusting *P* values using the FDR (table S9).

Parallelism at the level of the paralog was detected across replicate landlocked versus anadromous comparisons. For $N = 16$ genes, at least five of the seven landlocked versus anadromous comparisons demonstrated outlier SNPs in different paralogous copies of the same gene. However, none were detected in all seven landlocked versus anadromous comparisons (table S10; see file S2 for all paralogs detected in multiple landlocked versus anadromous comparisons).

Genetic Parallelism of Sympatric Divergence within Landlocked Lakes

A total of $N = 108$ outlier SNPs across 22 linkage groups were detected between sympatric morphs in WP-L, while $N = 400$ outlier SNPs across 37 linkage groups were detected between sympatric morphs in LO-L (fig. 2*c*, 2*f*; file S3). The number of SNPs detected by each method is reported by lake (table S11). While 6,183 SNPs were polymorphic in both WP-L and LO-L, only a single outlier SNP (AX-181980220) was detected in common between morphs in both WP-L and LO-L (table S12). It was located within the VPS10 domain-containing receptor SorCS2 gene and demonstrated parallel allelic trends (fig. S17; table S13). This gene is associated with neural development (Rezgaoui et al. 2001), was also identified as an outlier between sympatric big and small morphs in Esker North (Salisbury et al. 2020), and genetically differentiates fluvial coaster and adfluvial, resident brook trout (*Salvelinus fontinalis*; Elias et al. 2018) as well as resident black Kokanee salmon and river-spawning sockeye salmon (*Oncorhynchus nerka*; Veale and Russello 2017). We speculate that this gene may be important in contributing to incipient speciation in salmonids. However, the overlap of a single outlier SNP between sympatric morphs was not statistically significant (C-score$_{hyper}$ = 0.38, $P = .50$), and no paralogous copies of the same gene were found to differentiate sympatric morphs across replicate landlocked lakes.

Discussion

Our results demonstrate a small but consistent degree of genetic parallelism underlying geographically paired landlocked and anadromous populations. Less evidence was found, however, for parallel genetic differences between genetically distinguishable, size-differentiated, sympatric morphs observed in the two landlocked locations. Drift, local adaptation, or differential colonization histories may have contributed to this overall lack of genetic parallelism.

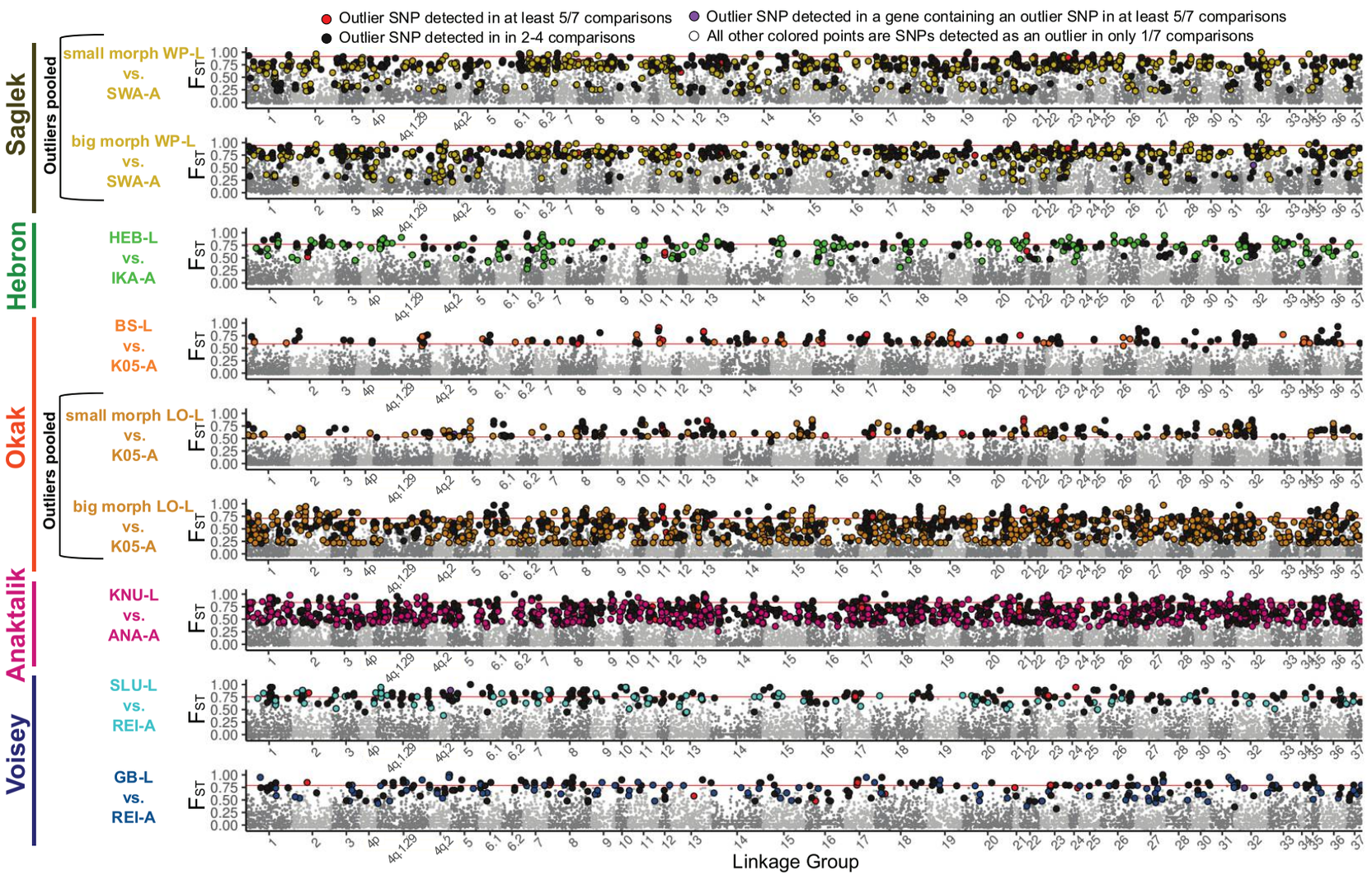

Figure 4: Manhattan plots demonstrating F_{ST} values of outlier loci detected between landlocked versus anadromous populations. Red lines indicate 3 SDs above the mean F_{ST}. Note that mean pairwise F_{ST} was calculated separately for each of the two genetic subgroups (corresponding to small and big morphs) within LO and WP. However, outlier SNPs detected between either morph and the corresponding anadromous population were pooled when identifying SNPs detected in multiple landlocked versus anadromous comparisons. Therefore, we identified outlier SNPs detected in two to four (black circles) and in five or more (red circles) of seven landlocked versus anadromous populations: (1) WP-L versus SWA-A (either small morph WP-L vs. SWA-A or big morph WP-L vs. SWA-A), (2) HEB-L versus IKA-A, (3) BS-L versus K05-A, (4) LO-L versus K05-A (either small morph LO-L vs. K05-A or big morph LO-L vs. K05-A), (5) KNU-L versus ANA-A, (6) SLU-L versus REI-A, and (7) GB-L versus REI-A.

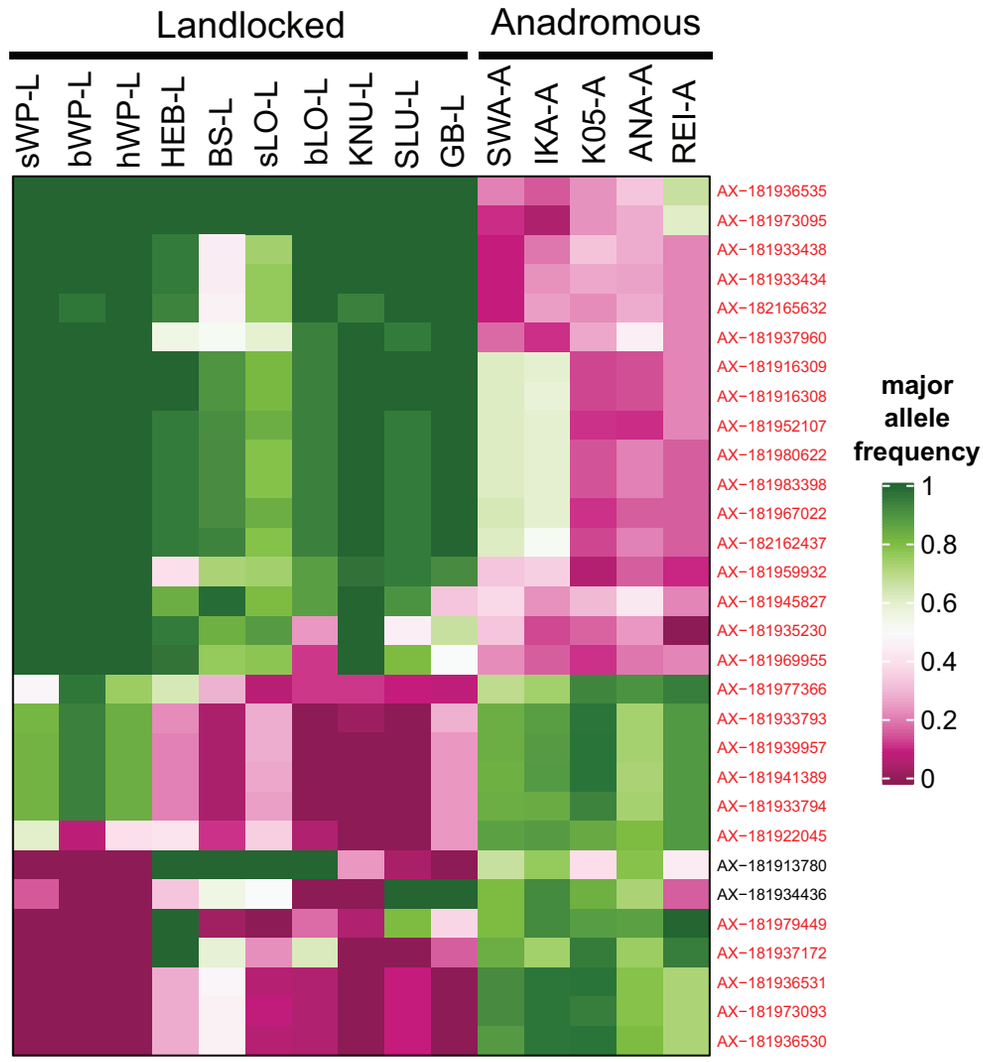

Figure 5: Heat map of allele frequencies for those loci detected as outliers for five or more of seven paired landlocked and anadromous populations: (1) WP-L versus SWA-A (either small morph WP-L vs. SWA-A or big morph WP-L vs. SWA-A), (2) HEB-L versus IKA-A, (3) BS-L versus K05-A, (4) LO-L versus K05-A (either small morph LO-L vs. K05-A or big morph LO-L vs. K05-A), (5) KNU-L versus ANA-A, (6) SLU-L versus REI-A, and (7) GB-L versus REI-A. The names of SNPs that show parallel allelic trends across the locations in which a SNP was detected as an outlier are highlighted in red.

However, the functionally relevant genes found to consistently differentiate landlocked versus anadromous populations suggest the potential for a common genetic basis for the loss of anadromy.

Limited Overall Genetic Parallelism

An overall limited amount of genetic parallelism was found in this study between paired landlocked and anadromous populations. No single locus consistently differentiated all paired comparisons, conflicting with recent observations that migratory life history in rainbow trout (*Oncorhynchus mykiss*; Arostegui et al. 2019; Pearse et al. 2019) and in Japanese grenadier anchovy (*Coilia nasus*; Zong et al. 2020) are dictated by consistent inverted genomic regions. Threespine stickleback also demonstrate strong parallel genetic differentiation at loci like *Eda* and *Pitx* (Nelson and Cresko 2018; Xie et al. 2019). However, even for these loci of large effect, parallelism is not always perfect (DeFaveri et al. 2011; Weinstein et al. 2019), and other loci across the genome may show little evidence of parallelism (Hale et al. 2013; Liu et al. 2018). Indeed, despite high genome-wide genetic differentiation between replicate landlocked populations of *O. mykiss* and a downstream anadromous population,

Table 3: Genes containing one or more outlier loci in five or more of seven landlocked versus anadromous population comparisons

						Method of outlier detection by landlocked vs. anadromous comparison								
Protein name	Linkage group	Protein code	SNP code	Position (Mbp)	Distance to CDS (kbp)	Small morph WP-L vs. SWA-A	Big morph WP-L vs. SWA-A	HEB-L vs. IKA-A	BS-L vs. K05-A	Small morph LO-L vs. K05-A	Big morph LO-L vs. K05-A	KNU-L vs. ANA-A	SLU-L vs. REI-A	GB-L vs. REI-A
1-acyl-sn-glycerol-3-phosphate acyltransferase gamma	AC02	XP_023860472.1	AX-181934436	17.1	0		P	P			P, F	P	P, F	P, F
Neurexin-3a-like	AC04q.2	XP_023842075.1	AX-181947935	28.8	0								P, F	
			AX-181915470	28.9	0	P		P		F	P	P		
			AX-181937420	29.2	0	P	P							
Low-quality protein: protocadherin-11 X-linked-like	AC08	XP_023847824.1	AX-181937960	.8	0	P	P				P	P	P	P, F
Extended synaptotagmin-1	AC11	XP_023852472.2	AX-181939957	30.7	0			P	P, F	P, F	P, F	P	P, F	
E3 ubiquitin-protein ligase DTX3L isoform X1	AC11	XP_023852474.1	AX-181933794	30.7	0			P	P, F	P, F	P, F	P	P, F	
			AX-181933793	30.7	0			P	P, F	P, F	P, F	P	P, F	
			AX-181941389	30.7	−.9			P	P, F	P, F	P, F	P	P, F	
EEF1A lysine methyltransferase 3 isoform X2	AC11	XP_023852598.1	AX-181922045	32.8	.6		P		F		P, F	P	P, F	
Uncharacterized protein LOC111970338	AC11	XP_023852785.1	AX-181945827	38.6	−4.5	P			F		P	P	P	
Partner of Y14 and mago B	AC17	XP_023861958.1	AX-181916308	22.6	.1				P, F	P, F	P, F	P	P, F	P, F
			AX-181916309	22.6	0				P, F	P, F	P, F	P	P, F	P, F
PAN2-PAN3 deadenylation complex catalytic subunit PAN2 isoform X1	AC17	XP_023861482.1	AX-181952107	22.7	−2.6				P, F	P, F	P, F	P, F	P	P, F
Nuclear envelope integral membrane protein 1-like isoform X1	AC17	XP_023862374.1	AX-181967022	22.7	0				P, F	P, F	P, F	P	P, F	P, F

Low-quality protein: serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit C-like	AC17	XP_023862177.1	AX-181980622	22.9	2.7				P, F	F	P, F	P	P, F	P, F
			AX-181983398	22.9	2.7				P, F	F	P, F	P	P, F	P, F
Inactive dipeptidyl peptidase 10	AC17	XP_023860785.1	AX-182162437	22.9	0				P, F	P, F	P, F	P	P, F	P, F
			AX-181987181	23.0	0				P, F	P, F	P, F	P		P, F
E3 ubiquitin-protein ligase BRE1B isoform X2	AC18	XP_023862569.1	AX-181935230	13.0	0			P, F	P, F	P, F		P		P
			AX-181935231	13.0	0			P, F	P, F	P, F		P		
Parafibromin	AC19	XP_023865178.1	AX-181969955	34.1	0	P	P	P, F	P	F		P		
Calpain-9	AC21	XP_023869744.1	AX-181973095	1.4	0	P	P	P, F	P, F	P, F	P	P		
Uncharacterized protein LOC111982472	AC21	XP_023869810.1	AX-181936535	1.5	0	P	P	P, F	P, F	P, F	P	P		
Myomesin-2	AC21	XP_024003594.1	AX-181973093	1.5	0	P	P	P		P, F	P, F	P		P
			AX-181936531	1.6	−1.8	P	P	P		P, F	P, F	P		P
Low-quality protein: lengsin	AC21	XP_023869550.1	AX-181936530	1.6	.8	P	P	P		P, F	P, F	P		P
Peroxisome assembly protein 12	AC23	XP_023824703.1	AX-181933438	19.3	0	P	P				P	P	P, F	P, F
Exonuclease V-like isoform X2	AC23	XP_023823204.1	AX-181933434	19.7	0	P	P				P	P	P, F	P, F
Collagen alpha-1 (XXVI) chain-like	AC23	XP_023823891.1	AX-182165632	20.0	−3.2	P	P				P	P	P, F	P, F
Ubl carboxyl-terminalhydrolase 18-like	AC24	XP_023825191.1	AX-181937172	9.6	−.3	P	P			P, F	P	P	P, F	P
			AX-181944480	9.6	−1.9	P	P			P, F				
DET1- and DDB1-associated protein 1	AC32	XP_023833368.1	AX-181924007	10.3	−1.2		P				P	P		
			AX-181924006	10.3	−1.2			P						P

Note: Population comparisons are as follows: (1) WP-L versus SWA-A (either small morph WP-L vs. SWA-A or big morph WP-L vs. SWA-A), (2) HEB-L versus IKA-A, (3) BS-L versus K05-A, (4) LO-L versus K05-A (either small morph LO-L vs. K05-A or big morph LO-L vs. K05-A), (5) KNU-L versus ANA-A, (6) SLU-L versus REI-A, and (7) GB-L versus REI-A. The method by which each SNP was identified as an outlier is denoted for each landlocked versus anadromous population comparison (P = pcadapt; F = F_{ST}).

few loci demonstrated parallelism across replicate landlocked populations apart from inversions on Omy05 and Omy20 (Campbell et al. 2021).

Similarly, only a single outlier SNP differed between small and big morphs in both landlocked locations, and this overlap was not greater than expected by chance on the basis of C-score analyses. Interestingly, pappalysin-2, which was detected as an outlier between small and big morphs in all locations studied by Salisbury et al. (2020) and has been associated with growth in mice and humans (Conover et al. 2011; Dauber et al. 2016), was not detected as an outlier in either WP-L or LO-L. Limited amounts of genetic parallelism have also been found to underlie intralacustrine radiations in other populations of char (Jacobs et al. 2020) and cichlids (Elmer et al. 2014).

Several mechanisms could be responsible for this overall lack of genetic parallelism underlying morph differentiation. Nonparallel genetic pathways may have been employed to achieve similar adaptive differentiation (Campbell and Bernatchez 2004), potentially facilitated by the abundance of genomic material provided by the whole genome duplication at the base of Salmonidae (Campbell et al. 2021). Alternatively, inconsistent environmental conditions across populations could have resulted in local adaptation and reduced genetic parallelism (Campbell and Bernatchez 2004). Given the colonization of Labrador by three glacial lineages (Salisbury et al. 2019), landlocked populations could have also been founded by different glacial lineages, thereby reducing shared genetic variation and the probability of genetic parallelism. However, though some landlocked populations differ by mtDNA haplotypes, different haplotypes could have been fixed in each population due to drift if all landlocked populations were founded by a common admixed population. Given the isolation of landlocked populations, drift could have contributed to the nonparallel fixation of not only mtDNA haplotypes but also nDNA. Though alternative sources of nonparallelism require further study, we speculate that drift has primarily contributed to a lack of genetic parallelism in this system.

Genetic Parallelism across Landlocked versus Anadromous Comparisons

Despite the isolation of landlocked populations, a few key loci seem to consistently differ between landlocked and anadromous populations. The parallel allelic trends demonstrated by 28 of the 30 outliers SNPs detected in at least five pairs of landlocked lakes and anadromous populations strongly suggest that these loci are responding to consistent directional selection. Our significant C-score analysis also suggests parallelism at the level of the SNP.

Of the outlier gene/proteins detected in five or more comparisons, several have putative functions relevant to the loss of anadromy. Myomesin-2 is associated with cardiac and fast-twitch muscle function (Schoenauer et al. 2008), traits expected to differentiate anadromous and nonanadromous populations (Delgado and Ruzzante 2020). In addition, myomesin-1 is significantly upregulated during Atlantic salmon smoltification (Seear et al. 2010). The outlier inactive dipeptidyl peptidase 10 is associated with neuronal potassium regulation (Jerng et al. 2004) and genetically differentiates resident and diadromous populations of *Galaxias maculatus* (Delgado et al. 2019).

Interestingly, seven of the 30 outlier SNPs detected in at least five landlocked versus anadromous population comparisons were also detected as outliers between sympatric small and big morphs in either Ramah, Brooklyn, or Esker North lakes (Salisbury et al. 2020; fig. S18; table S14). Five of these SNPs were in an ~240-kb region of AC17 and were detected as outliers between sympatric small and big morphs in Ramah Lake. For all five SNPs, the most prevalent allele in anadromous populations was also so for the Ramah big morph (fig. S18). This is notable, as the big putative anadromous morph in Ramah was more genetically similar to the anadromous populations studied here than those big morphs from Brooklyn and Esker North (fig. 3). This region in AC17 (containing the gene inactive dipeptidyl peptidase 10 discussed above) might therefore be key to the loss of anadromy both in sympatry (between resident and anadromous morphs) and in allopatry (between landlocked and anadromous populations).

Multiple genes also demonstrated evidence for parallelism at the paralog level in five or more landlocked versus anadromous population comparisons. Most putative paralogs were located on distinct mapped linkage groups, though some were unmapped and may not represent distinct paralogous copies. The presence of some paralogs on homeologous linkage groups suggests the potential importance of the recent whole genome duplication in salmonids (Macqueen and Johnston 2014) on contemporary adaptive differentiation. Furthermore, several of the genes demonstrating parallelism at the paralog level were associated with functionally relevant functions. One was chloride channel protein 2, which is associated with osmoregulation and is differentially expressed in salinity-tolerant and salinity-sensitive populations of Sacramento splittail (*Pogonichthys macrolepidotus*; Jeffries et al. 2019; Mundy et al. 2020). Another—pro-neuregulin-3, membrane-bound isoform—is associated with neural development in mice (Zhang et al. 1997; Anton et al. 2004) and genetically differentiates migratory and nonmigratory brown trout (*Salmo trutta*; Lemopoulos et al. 2018). Interestingly, paralogous copies of this gene were also found to genetically differentiate

sympatric small and big morphs in Ramah, Brooklyn, and Esker North (Salisbury et al. 2020). Our results therefore suggest that parallelism at the level of the paralog could play a key role in morph differentiation in char and other species. Though we had limited ability to detect paralogs using the SNP data employed in this study, our results argue for further investigation of this overlooked level of parallelism.

Genetic Structure of Sympatric Morphs

The ecologies of the small and big morphs in WP-L and LO-L remain ambiguous. Genetically distinguishable morphs within WP-L had previously been described as cryptic on the basis of 11 microsatellites, as no size difference had been detected (Salisbury et al. 2018). This was, however, likely due to a failure to remove putative hybrid individuals prior to size comparisons (see fig. S19). Size-differentiated char morphs have been observed within lakes in Alaska (May-McNally et al. 2015), Europe (Westgaard et al. 2004), and Canada (Kess et al. 2021). Indeed, the size difference between morphs in LO-L and WP-L is similar to that between genetically distinguishable small, littoral morph and the large benthic morphs within Lake Aigneau in northern Quebec (Power et al. 2009). Small and large morphs are also present within Charr Lake in Hebron Fjord, Labrador (Bouillon and Dempson 1989). Though we suspect these size-differentiated morphs arose in sympatry within these lakes, we cannot rule out the possibility of recent allopatry with subsequent secondary contact. For example, the putative hybrids identified in WP-L could reflect an earlier stage of incipient speciation prior to complete reproductive isolation or could alternatively be due to a collapse of morph differentiation. Sympatric morphs were not, however, founded by different glacial lineages, as both morphs from WP-L had Atlantic lineage mtDNA, whereas both morphs from LO-L had Arctic lineage mtDNA (Salisbury et al. 2019; table S5). Further investigation of these morphs' ecologies as well as the environmental factors driving/maintaining this genetic differentiation is therefore needed.

In addition, of those sea-accessible populations with sympatric big (putative anadromous) and small (putative resident) char investigated in Salisbury et al. (2020), only the big morphs from Ramah—but not those of Brooklyn or Esker North—were genetically similar to the anadromous populations. Though Anderson (1985) suggests that both Esker North and Brooklyn are sea accessible, given their remoteness there is some uncertainty about this status, particularly Esker North, which was considered landlocked by Van der Velden et al. (2013). It is possible then that big morphs from Esker North and Brooklyn could be nonanadromous, but as stated in Salisbury et al. (2020), it would be useful to verify the migratory phenotype of these morphs using telemetry and stable isotopes. Regardless, the genetic distinctiveness of Brooklyn and Esker North char likely contributed to the limited genetic parallelism observed between sympatric small and big morphs across Ramah, Brooklyn, and Esker North (Salisbury et al. 2020).

Conclusions

Despite the isolation of landlocked populations, our results demonstrate that their genetic diversity was sufficient to allow for both incipient speciation as well as their consistent, potentially adaptive genetic differentiation from anadromous populations. While the former result has previously been observed (e.g., Guðbrandsson et al. 2019; Jacobs et al. 2020; Østbye et al. 2020), the latter has rarely been assessed using a replicated pairwise design of natural populations as studied here. Our experimental design allowed us to uncover a number of candidate genes and paralogs demonstrating genetic parallelism across drainages, many of which were associated with ecologically relevant functions. Furthermore, some of the genes that consistently genetically differentiated landlocked and anadromous populations had also previously been found to differentiate sympatric putative resident and putative anadromous Arctic char in other Labrador populations. Some of these genes had also been associated with migratory and nonmigratory life histories in other fish species. Our results propose the intriguing possibility that the underpinnings of migration in both Arctic char and other fishes may share a genetic commonality.

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Statement of Authorship

S.J.S. and D.E.R. conceptualized the study. S.J.S., D.E.R., R.P., D.K., and I.R.B. developed and conducted sampling design. S.J.S. and G.R.M. conducted lab work. J.S.L., C.M.N., B.F.K., and M.M.F. contributed to the data collection (sequencing and genotyping). D.E.R., G.R.M., I.R.B., K.K.S.L., and T.K. contributed to data analysis, visualization, and interpretation. S.J.S. analyzed data and led the writing under the supervision of D.E.R. All authors contributed to editing the paper.

Data and Code Availability

Genepop files of SNP data as well as metadata are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.cz8w9gj42; Salisbury et al. 2022). Genepop files of SNP data for Ramah, Brooklyn, and Eskar North locations have been published in the Dryad Digital Repository (https://doi.org/10.5061/dryad.cz8w9gj1f).

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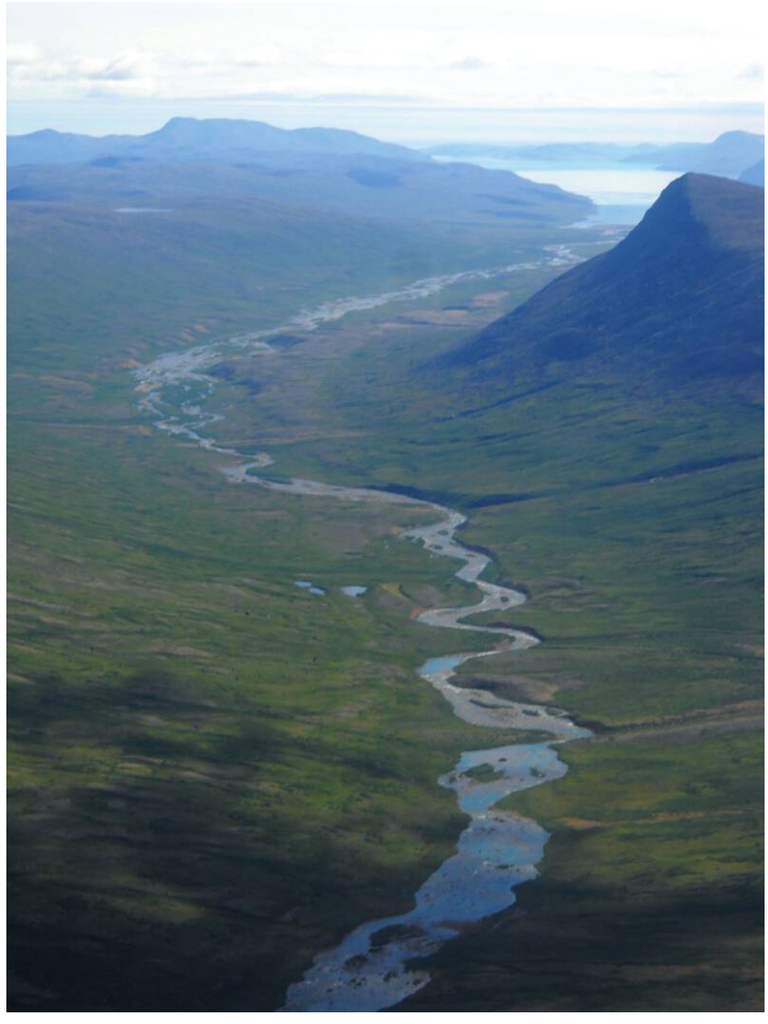

River in Labrador, Canada. Photo: Sarah J. Salisbury.