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Variation in the genomic basis of parallel phenotypic and ecological divergence in benthic and pelagic morphs of Icelandic Arctic charr (*Salvelinus alpinus*)

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Abstract

Sympatric adaptive phenotypic divergence should be underlain by genomic differentiation between sub-populations. When divergence drives similar patterns of phenotypic and ecological variation within species we expect evolution to draw on common allelic variation. We investigated divergence histories and genomic signatures of adaptive divergence between benthic and pelagic morphs of Icelandic Arctic charr. Divergence histories for each of four populations were reconstructed using coalescent modelling and 14,187 single nucleotide polymorphisms. Sympatric divergence with continuous gene flow was supported in two populations while allopatric divergence with secondary contact was supported in one population; we could not differentiate between demographic models in the fourth population. We detected parallel patterns of phenotypic divergence along benthic-pelagic evolutionary trajectories among populations. Patterns of genomic differentiation between benthic and pelagic morphs were characterized by outlier loci in many narrow peaks of differentiation throughout the genome, which may reflect the eroding effects of gene flow on nearby neutral loci. We then used genome-wide association analyses to relate both phenotypic (body shape and size) and ecological (carbon and nitrogen stable isotopes) variation to patterns of genomic differentiation. Many peaks of genomic differentiation were associated with phenotypic and ecological variation in the three highly divergent populations, suggesting a genomic basis for adaptive divergence. We detected little evidence for a parallel genomic basis of differentiation as most regions and outlier loci were not shared among populations. Our results show that adaptive divergence can have varied genomic consequences in populations with relatively recent common origins, similar divergence histories, and parallel phenotypic divergence.

Introduction

The evolutionary mechanisms and processes that underlie adaptive phenotypic divergence in the presence of gene flow are complex and their effects on genomic signatures are not well understood (Feder et al., 2012; Ravinet et al., 2017; Samuk et al., 2017; Wolf and Ellegren, 2017). Considerable evidence suggests that divergent natural selection between different environments can lead to phenotypic divergence, as well as the subsequent build-up of reproductive isolating mechanisms causing reductions in gene flow between coexisting sub-populations (morphs or ecotypes) (Smadja and Butlin, 2011; Seehausen et al., 2014). Adaptive divergence, potentially leading to speciation, may occur in sympatry with continuous gene flow, but may also involve periods of allopatry followed by secondary contact events (Smadja and Butlin, 2011; Ravinet et al., 2017). Understanding how local adaptation leads to genomic differentiation of sub-populations in the face of gene flow will aid in determining the evolutionary characteristics of ecological speciation (Rundle and Nosil, 2005).

Genomic signatures of divergent selection are typically characterized by heterogeneity in the magnitude of differentiation across the genomes of diverging morphs (Via, 2001; Feder et al., 2012, 2013). Conceptual models propose that highly differentiated regions or 'islands' of differentiation are the result of divergent selection on loci located in those regions (Via, 2001; Wu, 2001; Via, 2012; Flaxman et al., 2013; Wolf and Ellegren, 2017). The size (width) of the genomic islands are predicted to increase with greater ecological divergence and form 'continents' of differentiation due to the indirect effects of selection causing reductions in the effective migration rate for linked loci (i.e. divergence hitchhiking) (Feder and Nosil, 2010; Nosil

and Feder, 2012; Via, 2012). The prediction that islands of differentiation should increase in size as divergence proceeds has received mixed support. Highly differentiated regions can be either widespread throughout the genome or found in only a few genomic regions due to the combined effects of time since divergence, gene flow, drift, divergent selection, and genomic structure (Yeaman and Whitlock, 2011; Foote, 2018). Thus, further studies into the consequences of divergent selection on patterns of genomic differentiation are needed.

Features of the genome and the genomic architecture of traits associated with adaptation have large impacts on genomic patterns of differentiation that affect the ability to detect signatures of divergent selection (Semenov et al., 2019). Background or purifying selection may cause similar peaks of genomic differentiation and discerning among these processes is challenging (Cruickshank and Hahn, 2014; Ravinet et al., 2017; Martin et al., 2019). One approach to disentangling the genomic consequences of divergent selection from that of linked selection is to incorporate information on the hypothesized agents and targets of divergent selection in analyses (Lotterhos and Whitlock, 2015; Forester et al., 2018). Including such information could also lead to inferences on the genomic location of highly differentiated loci associated with ecological and phenotypic divergence. Highly differentiated loci within genomic islands are thought to be resistant to gene flow and are referred to as barrier loci (Flaxman et al., 2013; Ravinet et al., 2017). Barrier loci are disproportionately associated with adaptive divergence and may have an effect on reproductive isolation as they contribute to reduced fitness of hybrids (Abbott et al., 2013). The identification of barrier loci is key to understanding the genomic basis of reproductive isolation between diverging sub-populations (Ravinet et al., 2017).

Closely related populations that show varied levels of ecological and phenotypic divergence into discrete resource-based morphs have provided significant insights into the genomic consequences of adaptive divergence (Conte et al., 2012; Elmer et al., 2014; Malinsky et al., 2015). Studies of diverging populations with shared ancestry may be especially useful as they will likely show fewer confounding effects of variation in genomic structure and evolutionary history. In some cases, diverging populations show similar (parallel) patterns of genomic differentiation (Conte et al., 2012; Conte et al., 2015) where they share the same highly differentiated loci (Roesti et al., 2014; De Lisle and Bolnick, 2020). Parallel genomic differentiation (i.e. divergence at the same locus across diverging morph pairs) may be more common in populations with a recent common origin due to the inheritance of the same standing genomic variation or shared *de novo* mutations (Conte et al., 2012; Via, 2012; Conte et al., 2015; Haenel et al., 2019; Rennison et al., 2020). However, many cases of non-parallel genomic differentiation have been observed in such populations (Elmer et al., 2014; Bolnick et al., 2018; Salisbury et al., 2020) and this can be explained by a variety of genomic, developmental, and evolutionary factors. For instance, subtle differences in the selective landscape and optimal phenotype within each population may result in a low degree of sharing of beneficial alleles, especially if the phenotype under selection is complex (high dimensionality) (Thompson et al., 2019). Furthermore, non-parallel genomic patterns may be associated with parallel phenotypic divergence if they are mediated by shared developmental pathways across populations (Losos, 2011; Uller et al., 2018), where shared environmental conditions promote the development of similar morphs (Parsons et al., 2020; Uller et al., 2020). In fact, recent studies have shown that parallel patterns of gene expression are associated with

phenotypically similar morphs, despite fewer instances of parallel genomic signatures of selection (Jacobs et al., 2020). Thus, the relationship between genomic and phenotypic parallelism is complex and not fully understood.

The ability to detect common signatures of selection across the genome is also impacted by variation in divergence history and the timing of gene flow between diverging sub-populations (Bierne et al., 2013; Ravinet et al., 2017). Divergence history is often characterized with reference to two extreme evolutionary scenarios: primary divergence and a period of allopatry followed by secondary contact. Primary divergence occurs when morphs diverge in sympatry with continuous gene flow while divergence with secondary contact occurs when morphs diverge in allopatry for a period of time followed by secondary contact and initiation of gene flow (Smadja and Butlin, 2011; Feder et al., 2013; Foote, 2018). Modelling studies have suggested that populations undergoing primary divergence should show a few genomic regions with low to modest differentiation and those peaks are expected to grow due to being resistant to gene flow and recombination (Yeaman and Whitlock, 2011). In contrast, populations diverging in allopatry should rapidly accumulate multiple differences across the genome due to isolation and these differences may become selected on or eroded by gene flow upon secondary contact (Pinho and Hey, 2010; Feder et al., 2013). Despite these contrasting expectations, little consistency in genomic patterns has been observed among taxa thought to have diverged under similar ecological contexts, which can now be partially explained by new insights into the divergence history of taxa. Reassessments of divergence history with demo-genetic modelling have revealed complex divergence histories for many species and presumed cases of primary divergence are better explained by complex scenarios involving periods of

spatial isolation, waves of colonization, and secondary contact (Roux et al., 2013; Rougeux et al., 2017; Jacobs et al., 2018; Rougemont and Bernatchez, 2018; Lehnert et al., 2019; Jacobs et al., 2020). Thus, patterns of genomic variation may be similar among species showing contrasting patterns of divergence histories (i.e., primary vs. secondary contact) due to the effects of selection and gene flow on a contemporary time-scale (Ravinet et al., 2017; Foote, 2018; Semenov et al., 2019). Moreover, studies that compare populations with relatively simple divergence and evolutionary histories are needed to better understand the genomic consequences of adaptive divergence and the degree to which genomic parallelism is expected in nature.

Due to their relatively simple evolutionary history and patterns of ecological and phenotypic divergence, Icelandic populations of Arctic charr (*Salvelinus alpinus*) are a powerful model system to disentangle the evolutionary factors promoting adaptive divergence and speciation. In contrast to many Arctic charr populations in other parts of the species range (Moore et al., 2015; Doenz et al., 2019; Salisbury et al., 2019; Jacobs et al., 2020), genetic analyses suggest rapid post-glacial recolonization of Icelandic populations from a single lineage (Brunner et al., 2001) and rapid reductions in gene flow between lakes and on small spatial scales (Wilson et al., 2004; Kapralova et al., 2011). Similar (parallel) sympatric resource-based morphs occur in different Icelandic lakes with different degrees of phenotypic, ecological, and genetic divergence (Gíslason et al., 1999; Woods et al., 2013; Brachmann et al., 2021). Benthic morphs have a subterminal mouth with a robust jaw for feeding on benthic invertebrates and stockier body shapes for maneuverability in structurally complex habitats. Pelagic morphs have a longer, more gracile jaw that improves suction feeding on zooplankton or fish, while their

slimmer body shape aids in sustained swimming in open-water habitats. Relationships between trophic and phenotypic variation suggests that resource use within benthic and pelagic habitats is the agent of selection driving divergence in body shape and size (targets of selection) (Franklin et al., 2018; Doenz et al., 2019; Brachmann et al., 2021). The findings that benthic and pelagic morphs are genetically differentiated (Gíslason et al., 1999; Kapralova et al., 2011; Guðbrandsson et al., 2019) and that greater divergence in resource use can be associated with less genetic admixture (Brachmann et al., 2021) suggest that diet divergence may be associated with reduced gene flow between morphs. Studies of the genomic basis of phenotypic divergence in Arctic charr indicate that the degree of genomic parallelism (identity of outlier loci) appear to be relatively low across populations despite high phenotypic parallelism (Jacobs et al., 2020; Salisbury et al., 2020). In contrast, transcriptome-wide gene expression is highly parallel across populations and exists despite variation in evolutionary histories ranging from primary divergence in sympatry to a period of isolation in allopatry (Jacobs et al., 2020; Jacobs and Elmer, 2021). One reason that genomic parallelism may be relatively weak is that few studies have taken the degree of trophic and morphological divergence across populations which vary at different stages of adaptive divergence into account. Thus, our goal is to evaluate how the degree of ecological and phenotypic divergence is related to genomic divergence in a set of populations with a relatively simple evolutionary history.

We investigated the genomic consequences of adaptive divergence among sympatric benthic and pelagic morphs of Icelandic Arctic charr from populations that show varied levels of phenotypic, ecological, and genetic divergence. Our first aim was to reconstruct the divergence history of each population using coalescent modelling with single nucleotide polymorphisms

(SNPs) because differences in divergence histories will shape the genomic background in ways that could influence subsequent patterns of genomic differentiation within populations. We compared models of sympatric divergence with continuous gene flow against models of allopatric divergence with secondary contact. Our second aim was to quantify benthic and pelagic body shape variation and assess the degree of phenotypic parallelism among all morph pairs. Parallel patterns of body shape divergence along common axes suggests the potential for common patterns of selection and similar optimal phenotypes across populations. Our third aim was to evaluate whether highly differentiated genomic regions could be signatures of divergent selection acting on ecologically related phenotypic variation. After characterizing genome-wide patterns of genomic differentiation among benthic and pelagic morphs, we identified outlier loci associated with variation in morphology and resource use traits. The identification of such outlier loci would suggest that the genomic regions in which they are located could reflect signatures of divergent selection from resource or habitat use on functionally relevant traits. In addition, if the degree of heterogeneity in genomic divergence is a consequence of the intensity of divergent selection, the degree of ecological and phenotypic divergence will be positively associated with the degree of genomic heterogeneity between sympatric morphs. Our fourth aim was to determine if the same genomic regions are associated with variation in both resource use and phenotype across populations as this would likely enhance phenotypic responses to selection. Genomic loci that are associated with both resource use and phenotypic variation may more readily differentiate benthic and pelagic morphs and represent putative barrier loci that disproportionately contribute to adaptation. Our fifth and final aim was to identify the degree of genomic parallelism of divergence into

benthic and pelagic morphs across Icelandic populations. A high degree of genomic parallelism would suggest a common pattern of genomic divergence from standing genetic variation. In contrast, a low degree of genomic parallelism across populations would suggest that there are many ways for selection to partition genomic variation and still lead to phenotypically and ecologically similar morphs across populations.

Methods

Sampling

We captured benthic and pelagic morphs of Arctic charr from four geographically separated lakes in Iceland (designated as populations hereafter) during 2013-2015 (Table 1). The current analysis is based on the same set of fish used previously to assess the relationships among phenotypic, ecological, and genetic variation (Brachmann et al., 2021). The sample sizes per morph per population used here ranged from 25 to 64 individuals. The lakes Galtaból, Svínavatn, and Vatnshlíðarvatn contain a single pair of sympatric benthic and pelagic morphs while Þingvallavatn contains three sympatric morphs, two benthic morphs and a single pelagic morph. Although earlier studies indicated that Svínavatn contains three morphs (two pelagic and one benthic morphs) (Gíslason et al., 1999), recent work (Brachmann et al., 2021) indicates that the two pelagic groups of fish are neither genetically nor ecologically differentiated so we consider them as a single morph for the current analysis. Fish were caught during morph specific spawning periods using gill nets ranging in mesh sizes from 5-50 mm. Fish were classified to morph in the field by eye based on body shape, body size, craniofacial traits, and coloration (Skúlason et al., 1989; Snorrason et al., 1994; Gíslason et al., 1999; Jonsson and

Skúlason, 2000). The fish were then taken to the laboratory and photographed on their left

side, fork length (from the tip of the snout to the fork of the tail) was measured, and a sample

of white muscle tissue was taken for stable isotope and genetic analyses. Muscle tissue was

stored in ethanol for the genetic analyses and dried (see next section) for the stable isotope

analyses. The fish used within the study were captured and euthanized humanely according to

protocols approved by the University of Guelph Animal Care Committee as per regulations of

the Canadian Council of Animal Care.

Morphological and resource use traits

To detect genome wide signatures of selection associated with body shape and size

variation, we characterized morphological variation using landmark-based geometric

morphometrics. The set of homologous landmarks used within these analyses have been

previously defined and used in Brachmann et al., (2021). Briefly, the analysis was based on 25

landmarks placed on individual images using TpsDig (Rohlf, 1990). Landmarks 1, 22-25 were

used to remove variation due to bending on the lateral axis, using TpsUtil (Rohlf, 1990) after

which landmarks 23-25 were removed from further analyses. A generalized Procrustes

superimposition (GPA) was performed to remove effects of scale, rotation, and translation for

all specimens using Coordgen8, part of the IMP8 software (Sheets, 2014). The effects of

allometry and sex were minimized from the body shape data (see supplementary methods) to

focus on phenotypic variation associated with ecologically relevant traits under divergent

selection and with habitat divergence. After minimization of sex and allometric variation, the

Procrustes landmark coordinates were input into PCAgen8 (Sheets, 2014) to obtain relative

warp scores, which are based on a principal component analysis of all partial warp and uniform component scores (Zelditch et al., 2012). A relative warp score is a quantitative estimate of an individual's body shape trait value along a major axis of body shape variation among all the fish in the dataset. We considered the first three relative warp scores and fork length as traits for genome association analysis.

We used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopic signatures as proxies of individual resource use and as trait values for the genome association analysis. Fish that consume greater proportions of benthic prey items were expected to show higher $\delta^{13}C$ signatures than those that consume more pelagic organisms (Post, 2002). $\delta^{15}N$ was used to infer the trophic level of prey items where those from a lower trophic level typically usually show a lower $\delta^{15}N$ signature than those at a higher trophic level (Post, 2002). White muscle was dried at 60°C for 48 hours and then homogenized, weighed to 1.25mg of tissue and placed into tin capsules. The samples were analyzed at the University of Calgary Isotope Science Laboratory with a continuous flow elemental analysis-isotope ratio mass spectrometer (Themo Finnigan Delta with Elementar vario ISOTOPE cube). An internationally known internal standard of Mussel tissue (NIST-2976) was used to standardize carbon and nitrogen measurements to a known reference. $\delta^{13}C$ values were lipid normalized using the equation: $\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C:N$, to account for effects of lipids on $\delta^{13}C$ for aquatic organisms (see Post et al., 2007). Stable isotopes of reference prey baselines were not available, and as such we examined broad patterns of resource use along a benthic-pelagic gradient rather than assigning morphs to specific resource niches.

Genetic variation

DNA was isolated from white muscle tissue using a phenol-chloroform extraction (Taggart et al., 1992; Bardakci and Skibinski, 1994). DNA purity was assessed using a NanoDrop-TM ND-8000 spectrophotometer and samples with high 260/230 readings were purified using a genomic DNA cleanup protocol. DNA concentrations were determined using a Qubit 2.0 Fluorometer and 10 ng/ml samples genotyped at the Clinical Genomics Centre, Mount Sinai Hospital (Toronto, Canada) with an Arctic charr 87K Affymetrix SNP array (Nugent et al., 2019). The Axiom Analysis Suite (Version 3.1.5.1) was used to process the genotypic data and the data were filtered following the best practice workflow specified by Affymetrix. The specifications included specifying a diploid genome, each individual had genotypes for at least 82% of the total SNPs on the array, the call rate for a particular SNP must be greater than 97% across all samples, and the average call rate for individual samples across all SNPs had to be greater than 98%. The analysis returned a set of 14,187 recommended SNPs that were polymorphic within at least one population. All polymorphic SNPs were located with the *Salvelinus* sp. Genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_002910315.2/).

Data analyses:

All data analyses were performed in R (version 4.0.0) unless otherwise stated.

(a)   Reconstruction of divergence history

To address our first aim, we reconstructed the divergence history of the morphs within each population using a coalescent modeling based on the site frequency spectrum performed

in *fastsimcoal2* (Excoffier et al., 2013). Prior to modelling, we identified loci that were

potentially under selection (outliers) and removed them from the dataset for the individual

analyses of each population. This was necessary because divergence history should be based on

neutral loci (Schrider et al., 2016), as inferred from test statistics between genetic sub-

populations that fall within the background genomic differentiation expected under neutrality.

We identified outlier loci for each population individually by performing genome scans. The

outlier loci were identified by comparing allele frequencies of different genetic sub-populations

(morphs) to the allele frequency of the total population for each SNP. Based on previous

population genetic analyses (Brachmann et al., 2021), two genetic sub-populations were

considered for each of Galtaból, Svínavatn and Vatnshlíðarvatn while three genetic sub-

populations were considered in Þingvallavatn. We used both *BayeScan* (Foll and Gaggiotti,

2008) and *pcadapt* (Luu et al., 2017) as they identify outlier loci in different ways leading to a

more conservative filtering process. *BayeScan* uses a logistic regression approach to decompose

F_{st} coefficients into population and locus components to estimate selection (Foll and Gaggiotti,

2008), while *pcadapt* uses Mahalanobis distances to calculate a per locus test statistic (Luu et

al., 2017). *BayeScan* calculates the posterior probability for models including selection (positive

or background selection) and calculates q-values for each model. *Pcadapt* calculates p-values

from Mahalanobis distances and then we converted them to q-values using the *q-value* package

(Storey et al., 2019) to account for false positive outliers. Any locus that had a q-value less than

0.01 from either the *BayeScan* or *pcadapt* analyses was removed from the population specific

data set using the –extract flag in PLINK ver. 1.07 (Purcell et al., 2007). As the identified non-

neutral loci will differ between populations due to population specific selective dynamics,

different populations will have different marker sets for these analyses (Table S1). We then calculated the site frequency spectrum (SFS) for each population using the neutral SNPs with *Arlequin* (Excoffier and Lischer, 2010).

After the removal of outlier loci, we reconstructed the divergence history of each population with a set of three models for each of the three populations with two genetic sub-populations (benthic and pelagic morphs) and a set of four models for the one population with three genetic sub-populations (Figure 1). For populations with two sub-populations, we modeled: (i) Divergence of benthic and pelagic morphs in sympatry with continuous gene flow (two morphs (2), **I**solation by **M**igration model (2IM)), (ii) Divergence of benthic and pelagic morphs in sympatry with a **C**hange (reduction) in **I**solation by **M**igration between morphs over time (2CIM), (iii) Divergence of benthic and pelagic morphs in allopatry followed by **S**econdary **C**ontact and the onset of gene flow (2SC). The models used for Þingvallavatn with its three morphs, were similar to the two morph models but also included models where benthic morph variants (small and large benthic morphs) evolved from the benthic **M**orph **T**ype (MT). The models were: (i) Divergence of three morphs in sympatry through **I**solation by **M**igration (3IM), (ii) divergence of benthic and pelagic morph types in sympatry through **I**solation by **M**igration with continuous gene flow followed by the evolution of the two benthic morph variants (3IM_MT), (iii) divergence of benthic and pelagic morphs in allopatry followed by **S**econdary **C**ontact and gene flow (3SC), (iv) divergence of benthic and pelagic morph types in allopatry followed by the evolution of the two benthic morph variants and subsequent gene flow occurring between benthic and pelagic morphs at a secondary contact event (3SC_MT). For each model, we performed 25 independent runs of 100,000 iterations using the flags: -m, -0, -n

100000, -L 50, -s 0, -M, -q. The best run of the 25 total runs was then determined by identifying the run with the greatest maximum likelihood value. Next, another 100 iterations of the best model (with the same parameters as above) were used to determine a range of maximum likelihood values. We then compared the maximum likelihood and Akaike information criterion (AIC) for each model in each population to identify which model represents the most likely evolutionary history. Delta-AIC was calculated for each model set. Models with a delta-AIC value less than two were given equal support as the model with the lowest AIC in the set, and we interpret a delta-AIC value greater and equal to two as a lack of support for a given model in its model set (Burnham et al., 2011; Aho et al., 2014).

(b)   Phenotypic parallelism

For our second aim, we characterized phenotypic variation in body shape among all populations and determined the degree of phenotypic parallelism among all benthic and pelagic morph pairs. To visualize overall patterns of phenotypic variation, we performed a principal component analysis (PCA) on all partial warp and uniform component scores with all populations combined. We then tested for parallelism in phenotypic divergence trajectories among benthic and pelagic morph pairs (Adams and Collyer, 2009; Oke et al., 2016; Stuart et al., 2017) by calculating the covariance relationship between the first and second principal component axes via the prcomp R package (Venables and Ripley, 2002). The covarying relationship between PC1 and PC2 describes the overall benthic and pelagic divergence trajectory. Next, we calculated the angle between pairs of divergence trajectories and the relative differences in trajectory lengths with the TestOfAngle and dist_mean_boot functions

within the GeometricMorphometricsMix R package

(https://github.com/fruciano/GeometricMorphometricsMix). The TestOfAngle function

compares the angles of each vector in multivariate shape space, where random vectors are

drawn from a uniform distribution in an n-dimensional hypersphere (Li 2011). This method is

similar to tests of angles used in MorphoJ (Klingenberg 2011) and Morpho (Schlager and

Jefferies, 2013). A small angle indicates similar (parallel) divergence trajectories and a large

angle indicates non-parallel phenotypic trajectories. The dist_mean_boot function provides a

bootstrap estimate of the phenotypic distance between two groups, effectively estimating how

phenotypically similar each benthic and pelagic morph pair are to one another. A small distance

measurement indicates a high degree of phenotypic similarity between benthic and pelagic

morph pairs while a large distance measurement indicates a high degree of phenotypic

divergence between benthic and pelagic morph pairs.

(c)   Patterns of genomic differentiation

We addressed our third aim of evaluating whether highly differentiated genomic regions

could be signatures of divergent selection in three steps. First, we measured per-locus FST

between benthic and pelagic morphs using a sliding window analysis. We calculated per locus

FST between each benthic and pelagic morph within a population using the –fst flag within

PLINK 1.9 (Purcell et al., 2007). We then averaged FST continuously with a window size of 200

kilobases (Kb), which overlapped by 1Kb, using the *winScan* function within the *WindowScanR*

package (github.com/tavareshugo/WindowScanR). We tested a range of window sizes from 50

– 250Kb in 50Kb increments and used a 200Kb window based on the high reliability of results.

Any windows that contained fewer than three SNPs were removed from the dataset. SNP

density was similar among most chromosomes except for AC12, AC21, and AC34 (see Figure S1)

as they had many areas with less than three SNPs compared to other chromosomes. We then

classified the various windows (regions) as either outliers and non-outliers for each benthic and

pelagic morph pair based on the average FST values (see Rennison et al., 2019). We classified

outlier regions as having a mean FST value within the top 5% (95th quantile) of the FST

distribution.

Second, we identified significant associations (RDA outliers) between SNP genotypes

and either (1) $\delta^{13}C$ or $\delta^{15}N$ stable isotope signatures or (2) the first three relative warps and fork

length values with redundancy analysis (RDA) (Forester et al., 2018). An RDA was performed for

each of the five pairs of benthic and pelagic morphs (one in each of Galtaból, Vatnshlíðarvatn,

Svínavatn, and two in Þingvallavatn). A locus was considered an RDA outlier if it was equal to or

greater than four standard deviations away from the mean along any RDA axis. Outlier loci from

RDA analyses are commonly identified as being greater than three standard deviations from the

mean (Forester et al., 2018). However, we defined outlier loci as being greater than four

standard deviations to provide a conservative estimate of the number of outliers and control

for false positives in outlier detection. We imputed missing genotypic data using the most

common allele for each locus missing from the dataset (1.4% of loci). We used the RDA

approach to identify outlier loci rather than other approaches as it generally has a higher

threshold for false positives as well as high power to detect polygenic outlier loci (Capblancq et

al., 2018). The RDA models were:

Genotype matrix ~ $\delta^{13}C + \delta^{15}N$

Genotype matrix ~ Relative warp 1 + Relative warp 2 + Relative warp 3 + Fork length

Third, we determined whether RDA outliers co-localized with regions of high FST values. Such co-localization would suggest that loci significantly associated with variation in resource use and morphology differ in allele frequencies between benthic and pelagic morphs. We first retained SNPs that were 200Kb up- and down-stream of all trait associated outlier loci for each benthic and pelagic morph pair. Next, we identified the average FST values for the RDA outlier loci and SNPs surrounding the RDA outlier loci. This was done using a sliding window analysis centered around the loci associated with resource use and/or morphological traits. The sliding window analysis was performed using the *winScan* function within the *WindowScanr* package (github.com/tavareshugo/WindowScanR). As defined previously, the window size was 200Kb and there was 1Kb overlap between windows. We tested whether the average FST values of the RDA outlier loci were significantly higher than loci surrounding the RDA outlier loci and non-outlier loci (Via, 2012) with ANOVA followed by Tukeys HSD post-hoc tests. The observation that RDA outlier loci have significantly higher FST values than presumed neutral loci located upstream and downstream would suggest that RDA outliers are possible signatures of selection. Lastly, for each 200Kb region that contained an RDA outlier locus we calculated the peak width (last SNP position in the region – first SNP position in the region) and then averaged the peak widths for each benthic and pelagic morph pair. We then calculated the average FST per peak for each benthic and pelagic morph pair.

To address our fourth aim, we tested whether the same genomic regions are associated with variation in both resource use and phenotype across populations. To do this, we first determined the frequency of RDA outlier loci and RDA outlier regions unique to each trait and then the number expected for both traits by chance alone (product of the frequency of each trait). The observed and expected numbers were evaluated against a 1:1 ratio with a Chi Square Goodness of Fit test. We then assessed whether the number of SNPs/regions uniquely associated with variation in a specific trait and shared between traits (3 columns) differed across the four benthic-pelagic morph pairs (4 rows) with a Contingency chi square test. The chisq.test function was used to perform a Pearson's chi-squared test where the expected frequencies are from a joint distribution made up of the product of the row and column marginal. Given that Þingvallavatn contains two non-independent morph pairs, we repeated the analysis after the removal of one pair and then repeating the test with the other pair and observed no difference compared to the full data set. We then used an ANOVA to test if the number of RDA outliers associated with both phenotypic and ecological variation was greater than for each trait alone (aov function).

To address our fifth aim of assessing the degree of genomic parallelism across morph pairs, we determined the number of FST outliers, RDA outliers, and RDA outlier regions that were shared among benthic and pelagic morph pairs. A high number of overlapping and highly differentiated loci or genomic regions across populations would indicate that genomic parallelism is likely due to benthic and pelagic divergence from standing genomic variation. A low number of overlapping loci and genomic regions across populations would indicate that genomic differentiation is non-parallel and due to the differential partitioning of standing

genomic variation within each population. We also calculated a metric for pairwise parallelism to quantify the fraction of outlier loci and genomic regions that are shared across populations using the equation:

(Shared loci/Total loci in population 1 + Shared loci/ Total loci in population 2)*0.5

Results

Reconstruction of divergence history

Reconstruction of divergence history supported both models of isolation with migration (a period of divergence in sympatry) and secondary contact (a period of divergence in allopatry) depending upon the geographic population analyzed (Figure 1 & Figure 2). A model of isolation by migration with a reduction in gene flow between sub-populations over time (2CIM model) had the greatest maximum likelihood value and lowest AIC and ΔAIC scores for Galtaból and Svínavatn (Table S2). In contrast, the best supported model within Þingvallavatn was a model with a period of divergence in allopatry followed by secondary contact (3SC) (Table S2). The 3SC model suggests the simultaneous and rapid radiation of all three morphs within the population, rather than a benthic-pelagic divergence event followed by the divergence of the two benthic morph variants. For Vatnshlíðarvatn, we could not differentiate between a model of isolation by migration (2IM model) and a model of secondary contact (2SC model) (Table S2) as the maximum likelihood and AIC values were similar

Parallel patterns of benthic-pelagic phenotypic divergence

Phenotypic divergence in body shape among benthic and pelagic morphs appears to be largely parallel among populations (Figure 3; Figure S5). Thus, benthic and pelagic morphs from different populations appear to be diverging along common phenotypic trajectories. The benthic-pelagic divergence trajectories were parallel among all pairs of populations except between Galtaból and Vatnshlíðarvatn (Table S3; above diagonal). The magnitude of body shape divergence did not differ significantly among all benthic-pelagic pairs (Table S3; below diagonal), suggesting that the body shape of each morph pair is similar among populations.

Patterns of genomic differentiation

The degree of genomic divergence between sympatric benthic and pelagic morphs varied across populations (Figures S2-S4). The morphs in Galtaból and Vatnshlíðarvatn showed the greatest (Mean F_{ST} = 0.19) and least (Mean F_{ST}= 0.028) divergence across the 200Kb F_{ST} outlier regions, respectively. Morphs from Svínavatn (Mean F_{ST} = 0.073) and Þingvallavatn (Mean F_{ST} Benthic1-Pelagic = 0.069; Mean F_{ST} Benthic1-Pelagic = 0.073) showed intermediate levels of divergence (Figure S2-S4). The total number of 200Kb F_{ST} outlier regions, from the top 5% of the distribution of F_{ST} windows (regions) was similar across all benthic and pelagic morph pairs from all four lakes (Figure S2; Table 2). However, the genomic location of the F_{ST} outlier loci and regions varied across the benthic and pelagic morph pairs (Figure S4). For instance, the two most closely related morph pairs in Þingvallavatn had a single 200Kb F_{ST} outlier region in common which is located on linkage group AC18.

Significant associations between variation in resource use and morphological traits and genomic variation were observed in the three most phenotypically differentiated populations

with the RDA analysis (Figure 3, Table 2). The most genetically differentiated population (Galtaból) showed the greatest number of RDA outlier loci and regions. No significant associations were detected in Vatnshlíðarvatn. All sympatric benthic and pelagic morphs separated along a combination of RDA axes 1 and 2 whereas the morphs within Vatnshlíðarvatn did not (Figure 3). RDA outlier loci had significantly higher FST values than non-RDA loci in the three populations where significant associations were detected (Figure 5; Table S4). RDA outliers associated with both phenotypic and ecological variation had the greatest FST values within Galtaból (Table S4). However, within all other benthic-pelagic morph pairs the RDA outliers associated with morphology and ecology had roughly equivalent FST values. RDA outlier regions (200Kb windows) also had high average FST values (Figure S3). FST values of RDA outlier loci were significantly higher than FST values of loci surrounding RDA outlier loci and neutral loci (Figure 6). FST values of loci surrounding RDA outliers were not significantly different than neutral loci.

In the vast majority of cases, RDA outlier loci associated with resource use were also associated with morphological traits. The frequency of overlap between the association analyses was greater than chance alone (p-value <0.001 for all morph pairs) (Table 2). The frequency of RDA outlier loci (X^2 = 54.24, df = 6, p-value = <0.001) and RDA outlier regions (X^2 = 23.88, df = 6, p-value <0.001) associated with both traits varied across morph pairs. The frequency of RDA loci and RDA outlier regions shared between traits was greater than expected in Svínavatn and Þingvallavatn and lower than expected in Galtaból. The mean size of highly differentiated regions associated with morphology and resource use were similar across all

benthic-pelagic morph pairs (Table 3). All highly differentiated regions were relatively narrow, showing low mean peak sizes and standard deviation.

Very few RDA outlier loci and regions were shared across the four pairs of benthic and pelagic morphs from the three lakes where significant associations were detected (Figure 7; Table 4; Table S5). Only 13 RDA outlier loci were shared among the four morph pairs and all of which were on separate chromosomes except for two loci on AC10. Of the shared RDA outliers, only the four located on AC13, AC24, AC25, and AC33 were associated with variation in both trait types. Only a single RDA outlier region associated with variation in resource use was shared between the two morph pairs in Þingvallavatn with a handful of others associated with both trait types in other morph pairs. The pairwise metric of genomic parallelism was relatively low among most morph pairs, except for the two morph pairs in Þingvallavatn which had the greatest fraction of shared RDA outliers and 200Kb outlier regions among all morph pairs (Table 4).

Discussion

We assessed the genomic consequences of divergence history and the effects of divergent selection in sympatric morphs of benthic and pelagic Arctic charr from Icelandic lakes. Reconstruction of divergence histories indicate that benthic and pelagic morphs across two lakes appear to be diverging in sympatry with continuous gene flow. However, the most probable scenario in Þingvallavatn is a period of divergence in allopatry (possibly within the lake) with subsequent secondary contact and gene flow between morphs. Despite varied divergence histories, we identified outlier loci associated with resource use and phenotypic

variation in the three most phenotypically divergent populations. Trait associated loci that differentiated benthic and pelagic morphs had high FST values while loci surrounding them were relatively undifferentiated, suggesting that the genomic peaks of differentiation are relatively narrow and represent signatures of selection. A large proportion of outlier loci were associated with both resource use and phenotypic variation and these loci had the greatest FST values compared to loci associated with resource use or phenotypic variation alone, suggesting that they may be barrier loci that readily differentiate benthic and pelagic morphs. Lastly, the degree of genomic parallelism associated with benthic and pelagic divergence was low, suggesting that the genomic underpinnings of parallel adaptive divergence differs across lakes, even though populations have a relatively recent common origin. Overall, despite a high degree of parallelism in phenotypic and ecological divergence between benthic and pelagic morphs across populations, we see very weak parallel genomic divergence across populations. The varied demographic history among population may, to some extent, be responsible for the limited parallel genetic divergence.

Divergence history

The divergence history of phenotypically and ecologically differentiated morphs appears to differ across populations. Models of sympatric divergence with continuous gene flow were supported within Galtaból and Svínavatn while a model of allopatric divergence followed by secondary contact between morphs was supported in Þingvallavatn. No consensus could be made on the divergence history within Vatnshlíðarvatn due to modelling constraints as multiple models of divergence history were supported. Our findings in Þingvallavatn are consistent with

a previous study where an allopatric phase in the evolution of morphs could not be rejected (Kapralova et al., 2011). Although the timing of a potential period of allopatry is unknown, one possible scenario is that phenotypically divergent populations colonized the lakes from the Atlantic glacial refuge at different times (Brunner et al., 2001), similar to the double invasion (or transporter) hypothesis in stickleback (Taylor and McPhail, 2000; Schluter and Conte, 2009; Liu et al., 2016). However, geological data suggests that repeated colonization event are unlikely as fish would have only been able to colonize Iceland immediately following deglaciation when the isostatic depression of the region caused relatively high sea levels (Norddahl and Pétursson, 2005). Subsequent rapid isostatic rebound would have created barriers in outflowing streams causing populations (lakes) to become and remain physically isolated precluding a second colonization event (Wilson et al., 2004). An alternative scenario is that benthic and pelagic morphs became spatially separated within the lake in more recent times. Þingvallavatn has experienced geological events, such as volcanic eruptions (Saemundsson, 1992), with the potential to impact the genetic connectivity of sub-populations. Despite this uncertainty, our results are consistent with studies of other Arctic charr populations, even those originating from different glacial refugia (Doenz et al., 2019; Jacobs et al., 2020) and other northern fishes (Nichols et al., 2015; Le Moan et al., 2016; Rougeux et al., 2017; Lehnert et al., 2019) which indicates that parallel patterns of phenotypic divergence occurs despite variation in divergence history. Our findings provide additional evidence that the response to selection in contemporary environments plays a key role in driving the phenotypic patterns of divergence in this species (Jacobs et al., 2020).

Patterns of genomic differentiation

The detection of highly differentiated loci associated with variation in resource use and morphology suggests that the patterns of genomic differentiation are signatures of divergent selection. Loci significantly associated with variation in stable isotopic signatures and morphology (body shape and size) co-localized with F_{ST} peaks of differentiation between benthic and pelagic morph pairs across three populations. Not unexpectedly, the least morphologically, ecologically, and genetically differentiated morph pair (in Vatnshlíðarvatn) showed no detectable RDA outlier loci. However, F_{ST} outlier regions not associated with trait variation were detected within this lake. This suggests that the F_{ST} outlier analysis alone may not be sufficient to detect signatures of divergent selection given that such outliers can arise from other mechanisms such as background or purifying selection (Ellegren et al., 2012; Burri, 2017b; Ravinet et al., 2017; Matthey-Doret and Whitlock, 2019). Unfortunately, our genotyping approach does not allow for the accurate detection of background or purifying selection, which may also affect genomic differentiation observed within these fishes.

We found mixed support for the prediction that ecological and phenotypic divergence will be positively associated with the degree of genomic heterogeneity between sympatric morphs. Þingvallavatn and Svínavatn showed the expected pattern with significant numbers of RDA outlier loci and regions located in high F_{ST} regions. Moreover, the population with a relatively low amount of phenotypic and ecological divergence (Vatnshlíðarvatn) (Brachmann et al., 2021) showed no detectable RDA outliers. However, the low degree of phenotypic and ecological divergence between morphs in Vatnshlíðarvatn likely limits the statistical power of

outlier detection methods, resulting in no detectable outliers. Unexpectedly, the fourth

population (Galtaból) showed the greatest number of RDA outliers and genomic divergence and

yet has much lower ecological divergence than observed in Þingvallavatn and Svínavatn. There

are multiple explanations for the patterns seen across the four lakes. One explanation is that

the prey communities within benthic and pelagic habitats differ slightly across populations,

which would result in different isotopic signatures across populations and different patterns of

phenotypic and ecological divergence. Assessment of benthic and pelagic prey baselines within

each population is necessary although, isotopic signatures have been shown to be diverging in

parallel across the Icelandic populations within this study (Brachmann et al., 2021). Another

explanation for this apparent disparity between phenotypic and ecological divergence relates to

the observation that the four lakes are at different stages of the divergence process, specifically

the degree of reproductive isolation between sympatric benthic and pelagic morphs. Unlike,

the three populations that show the expected pattern between the number of RDA outliers and

morphological divergence, our recent study found no evidence of hybridization between

sympatric morphs in Galtaból (Brachmann et al., 2021). It is possible that the development of

reproductive isolation as seen in this lake alters the relationships among phenotypic, ecological,

and genomic differentiation such that the genomic patterns we observe could be the

consequences of past divergent selection, rather than selection on a contemporary timescale.

Thus, reproductive isolation and differential mating strategies due to ecology, rather than

divergent selection, could be maintaining phenotypic divergence between morphs even though

the effects of resource use appear to be relatively low. Future investigation into the strength of

pre- and post-zygotic isolating mechanisms in relation to the strength of divergent selection within populations of Icelandic Arctic charr are needed to test this hypothesis.

Highly differentiated regions of the genome between benthic and pelagic morphs were relatively narrow and did not vary in size across morph pairs. High genomic differentiation of outlier loci linked to loci under selection did not extend to loci in close physical linkage regardless of population or degree of adaptive divergence. Given that all regions of genomic differentiation were relatively narrow, divergence hitchhiking of neutral loci surrounding loci under selection has not effectively taken place (Feder and Nosil, 2010; Via, 2012; Feder et al., 2013; Flaxman et al., 2013). Divergence hitchhiking may not have had extensive effects on increasing the background level of genomic variation in these populations due to ongoing gene flow, time since divergence, and high recombination rates. Ongoing or recent gene flow events may erode and counter the effects of divergence hitchhiking and limit genomic differentiation to regions of the genome that are under or closely associated with selection (Gagnaire et al., 2013; Lamichhaney et al., 2015; Han et al., 2017). Gene flow occurs between benthic and pelagic morphs within most populations except Galtaból, where morphs are reproductively isolated (Gislason et al., 1999; Brachmann et al., 2021). However, we were not able to measure the time since divergence and reproductive isolation within these populations. If adaptive divergence is recent in all populations and differences in divergence times are relatively small, we would see relatively narrow peaks of genomic differentiation within all populations. Recent patterns of divergence may limit the capacity for divergence hitchhiking to extend to background levels of genomic variation (Flaxman et al., 2013). Another caveat is that recombination rates among loci are unknown and could, in combination with recent or ongoing

gene flow, result in narrow peaks of differentiation during periods of strong divergent selection (Wellenreuther and Bernatchez, 2018; Oomen et al., 2020). The observation that benthic and pelagic morphs within Icelandic lakes show consistent and relatively narrow peaks of genomic differentiation contrasts to other studies showing much larger peaks of differentiation in other species (Via et al., 2012; Renaut et al., 2012; Rennison et al., 2019).

The observation that many genomic regions were co-associated with variation in both morphology and resource use suggests the presence of putative barrier loci promoting genomic differentiation between benthic and pelagic morphs. The number of RDA outliers associated with both trait types varied across morph pairs but was consistently high. The RDA outliers co-associated with both morphology and resource use had greatest FST values than loci associated with only one trait and neutral loci, suggesting that they differentiated benthic and pelagic morphs to a greater extent than other outlier loci. Effective barrier loci promote adaptive divergence in sympatry as they are effectively resistant to the effects of gene flow (Abbott et al., 2013; Ravinet et al., 2017). These loci may also facilitate the rapid evolution of adaptive divergence as allele frequency changes at a single locus will cause correlated responses to selection in both ecological and phenotypic traits (Ellegren and Sheldon, 2008; Casto and Feldman, 2011; Ragland et al., 2017; Mark et al., 2019; Archambeault et al., 2020; Duntsch et al., 2020). It is important to note, however, that the high number of genomic outliers associated with variation in both resource use and morphology might be a statistical artifact of the association approach used as resource use and phenotypic variation are highly correlated in these populations. The techniques for identifying signatures of divergent selection are still

being refined for wild populations (Lotterhos and Whitlock, 2015) and it might soon be possible to differentiate between the effects of highly correlated predictor variables.

The genomic basis of phenotypic and ecological divergence of closely related benthic and pelagic morphs of Icelandic Arctic charr appears to differ across morph pairs even though populations share a relatively recent common origin. Despite phenotypic divergence in body shape variation being parallel across most populations, the genomic basis of phenotypic (and ecological) adaptation was non-parallel. Benthic and pelagic morphs pairs shared few outlier loci (two FST outliers and thirteen trait-associated outliers) and genomic regions (two 200Kb genomic regions) indicating a low degree of genomic parallelism. Even the two closely related morph pairs in Þingvallavatn showed limited genomic parallelism, although slightly greater than the other morph pairs. Limited genomic parallelism appears to be a feature of this species (see Jacobs et al., 2020 and Salisbury et al., 2020), even when populations have a relatively simple evolutionary history such as those studied here. Limited genomic parallelism may be due to a high degree of genomic redundancy where many loci may provide the same function (Louis, 2007; Elmer et al., 2014; Barghi et al., 2020). Genomic redundancy could facilitate adaptation to common ecological problems and cause parallel phenotypic outcomes to selection (Barghi et al., 2019). The degree of genomic redundancy might also be affected by phenotypic plasticity where common environmental differences between benthic and pelagic habitats across populations could cause the common expression of traits throughout development (Noble et al., 2019; Parsons et al., 2020; Radersma et al., 2020; Uller et al., 2020). The observation that patterns of gene expression show greater parallelism across populations compared to outlier loci and highly differentiated regions (Jacobs et al., 2020; Jacobs et al., 2021) supports the

hypothesis that environmentally mediated expression of traits due to plasticity plays a key role in the process of adaptive divergence (Skúlason et al., 2019).

Conclusion

We have implemented an integrative approach by linking phenotypic, ecological, and genomic variation to determine the extent to which resource use and phenotypic variation influences genomic differentiation during adaptive divergence along a benthic-pelagic ecological axis. Benthic and pelagic morphs of Icelandic Arctic charr appear to be diverging in sympatry with continuous gene flow for morphs in Galtaból and Svínavatn while morphs in Þingvallavatn diverged during a period of allopatry. However, despite varied divergence histories across populations, narrow regions of high differentiation between benthic and pelagic morphs appear to be associated with morphological and ecological variation except in Vatnshlíðarvatn, where no trait-associated loci were found. Thus, genomic differentiation appears to be related to the degree of phenotypic divergence as well as the degree of gene flow between morphs within populations, where highly divergent populations show a greater degree of genomic differentiation. Despite low degrees of genomic parallelism across populations, differential resource use and phenotypic variation appear to shape the degree of genomic differentiation between benthic and pelagic morph pairs. Due to the low degree of genomic parallelism across populations, we suggest a role for conserved developmental pathways which produce similar phenotypic outcomes across populations which are most likely due to a high degree of phenotypic plasticity. Overall, this study provides novel insight into the

mechanisms and processes driving large scale genomic differentiation and potential speciation of Icelandic Arctic charr.

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Tables and figures

Table 1. Sample sizes of benthic and pelagic morphs of Arctic charr collected from four Icelandic populations and analyzed for variation in stable isotope signatures, morphology, and single nucleotide polymorphisms (SNPs). The specific descriptor is the name used for each morph in previous studies. The two pelagic morphs originally described in Svínavatn were grouped together in analyses of divergence history and genomic differentiation in the current study (see methods for justification). A common set of 14,187 SNPs were used for all analyses. Morphs and populations varied in the identity of polymorphic vs monomorphic SNPs.

Population	Coordinates	Morph	Specific descriptor	Sample size: Morphology and isotopes	Sample size: SNPs	SNPs per morph	SNPs across morphs
Galtaból	Latitude: 65˚16′0′′N Longitude: 19˚43′60′′W	Benthic	Small benthic	34	32	3126	4473
		Pelagic	Piscivorous	25	25	1772	
Svínavatn	Latitude: 65˚12′0′′N Longitude: 20˚1′0′′W	Benthic	Large benthic	26	26	2926	6247
		Pelagic 1	Planktivorous	33	32	4065	
		Pelagic 2	Piscivorous	33	32	3820	
Þingvallavatn	Latitude: 64˚18′33′′N Longitude: 21˚15′00′′W	Benthic 1	Large benthic	33	32	2337	4465
		Benthic 2	Small benthic	33	32	2831	
		Pelagic	Planktivorous	33	31	2839	
Vatnshlíðarvatn	Latitude: 64˚31′0′′N Longitude: 19˚37′0′′W	Benthic	Silver	33	32	3705	5114
		Pelagic	Brown	33	32	3752	

Table 2. The number of FST outlier loci, FST outlier regions, and SNPs associated with variation in resource use and morphological traits for pairs of sympatric benthic and pelagic morphs from four populations of Icelandic Arctic charr. FST outlier were determined as the top 5% of the FST distribution for each benthic and pelagic morph pair. The RDA outlier loci were identified genome wide association analysis with RDAThe term regions references to 200Kb regions. G – Galtaból, S – Svínavatn, T – Þingvallavatn, V – Vatnshlíðarvatn.

Benthic-pelagic morph pair	Number FST outlier loci	Number FST outlier regions	Trait type	Total trait associated SNPs	SNPs unique to trait type	SNPs shared across trait types	Percent (%) shared outliers	Regions unique to trait type	Regions shared across trait types	Percent (%) shared regions
G: benthic-pelagic	467	26	Resource use	325	77	248	58.1	15	45	53.6
			Morphology	350	102			24		
S: benthic-pelagic	482	26	Resource use	294	43	251	76.3	4	51	89.5
			Morphology	286	35			2		
T: benthic 1-pelagic	365	26	Resource use	264	29	235	79.1	6	42	71.2
			Morphology	268	33			11		
T: benthic 2-pelagic	371	26	Resource use	260	38	222	74.7	8	39	73.6
			Morphology	259	37			6		
V: benthic-pelagic	435	26	Resource use	0	0	0	0	0	0	0
			Morphology	0	0			0		

Table 3. Estimated size of peaks of differentiation (Mb) for 200Kb RDA outlier regions associated with variation in morphological traits and resource use traits in four benthic-pelagic morph pairs from three Icelandic lakes. The size of highly differentiated peaks were calculated from all SNPs located 200Kb up- and down-stream of trait associated outlier loci.

Population	Trait type	Peak size (Mb)	Peak size standard deviation	Average FST per peak
G: Benthic-pelagic	Both traits	0.156	0.112	0.002
	Resource use	0.120	0.120	0.002
	Morphology	0.156	0.127	0.002
S: Benthic-pelagic	Both traits	0.151	0.115	0.007
	Resource use	0.121	0.117	0.004
	Morphology	0.168	0.124	0.0004
T: Benthic 1-pelagic	Both traits	0.169	0.103	0.0002
	Resource use	0.157	0.111	<0.0005
	Morphology	0.157	0.117	<0.0005
T: Benthic 2-pelagic	Both traits	0.164	0.108	<0.0005
	Resource use	0.147	0.112	0.0003
	Morphology	0.148	0.116	0.0004

Table 4. Parallelism of RDA outliers and regions for pairs of benthic-pelagic morphs from three populations of Icelandic Arctic charr. Genomic parallelism was quantified with a metric of genomic parallelism based on the shared amount of total outlier loci across benthic-pelagic morph pairs (see text for details). Above the diagonal shows the parallelism of RDA outlier loci across each benthic and pelagic morph pair. Below the diagonal shows the parallelism of 200Kb RDA outlier regions across each benthic and pelagic morph pair. The greater the number indicates a higher degree of shared loci among morph pairs (greater genomic parallelism) while a lower number indicates a lower degree of shared loci (less genomic parallelism).

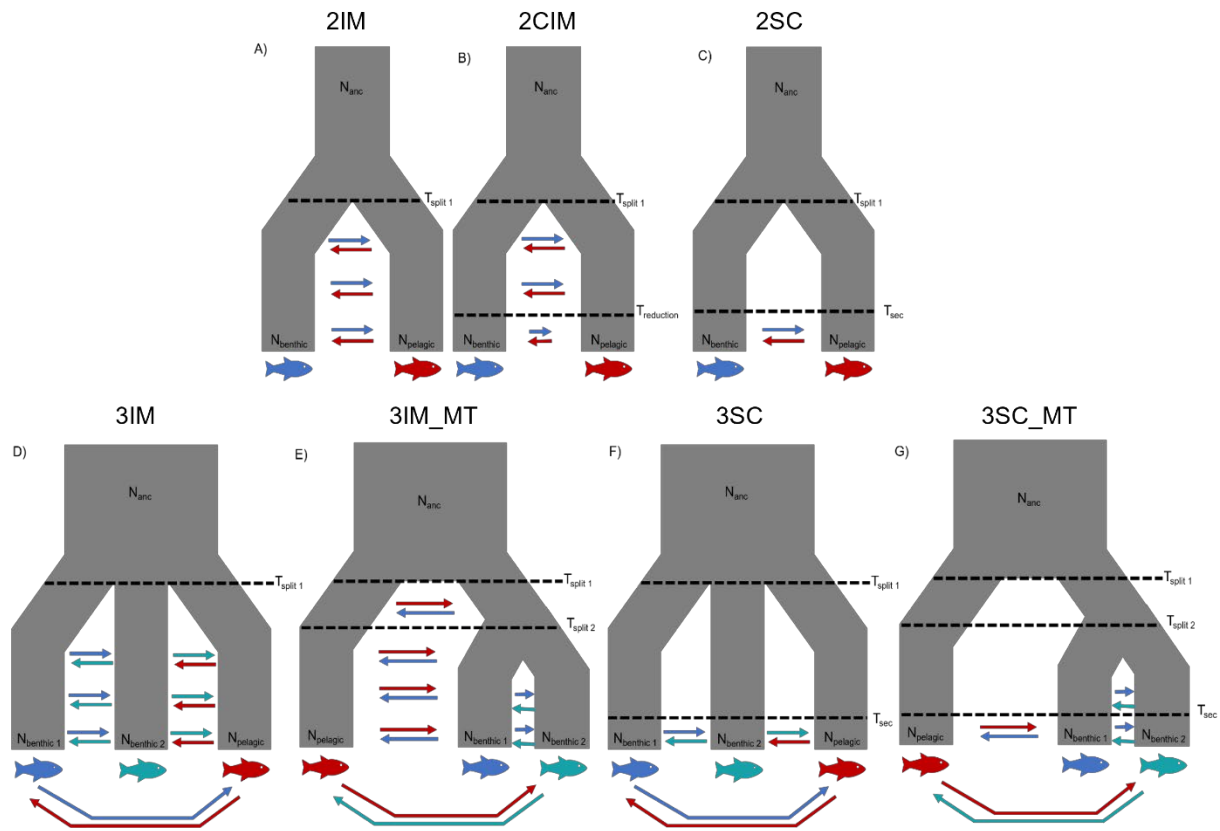| Benthic-pelagic morph pair	G: Benthic-pelagic	S: Benthic-pelagic	T: Benthic 1-pelagic	T: Benthic 2-pelagic
G: Benthic-pelagic	—	0.15	0.16	0.18
S: Benthic-pelagic	0.03	—	0.20	0.16
T: Benthic 1-pelagic	0.01	0.03	—	0.48
T: Benthic 2-pelagic	0.00	0.00	0.07	—

Figures

Figure 1. Demographic models of population divergence for two (A-C) and three (D-G) genetic sub-populations. Two sub-population models were used to model divergence history in Galtaból, Vatnshlíðarvatn, and Svínavatn while three sub-population models were used in Þingvallavatn. Benthic and pelagic morphs are represented as blue and red, respectively. The length of the arrows between demes indicates the amount of gene flow at any moment in time which increases from top to bottom in each figure. Morphs are defined based on distinct resource use patterns, morphology, and population genetic differences. A) divergence of benthic and pelagic morphs in sympatry with continuous gene flow (two morphs, **I**solation by **M**igration, 2IM). B) Divergence of benthic and pelagic morphs in sympatry with a **C**hange (reduction) in **I**solation by **M**igration between morphs over time (2CIM). C) Divergence of benthic and pelagic morphs in allopatry followed by **S**econdary **C**ontact and the onset of gene flow (2SC). D) Divergence of three morphs in sympatry through **I**solation by **M**igration with continuous gene flow (3IM). E) Divergence of benthic and pelagic morph types (MT) in sympatry through **I**solation by **M**igration with continuous gene flow followed by the divergence of two benthic morph variants (3IM_MT). F) Divergence of three benthic and pelagic morphs in allopatry followed by **S**econdary **C**ontact (3SC). G) Divergence of benthic and pelagic morph types in allopatry followed by the evolution of two benthic morph variants and **S**econdary **C**ontact among the three morphs (3SC_MT).
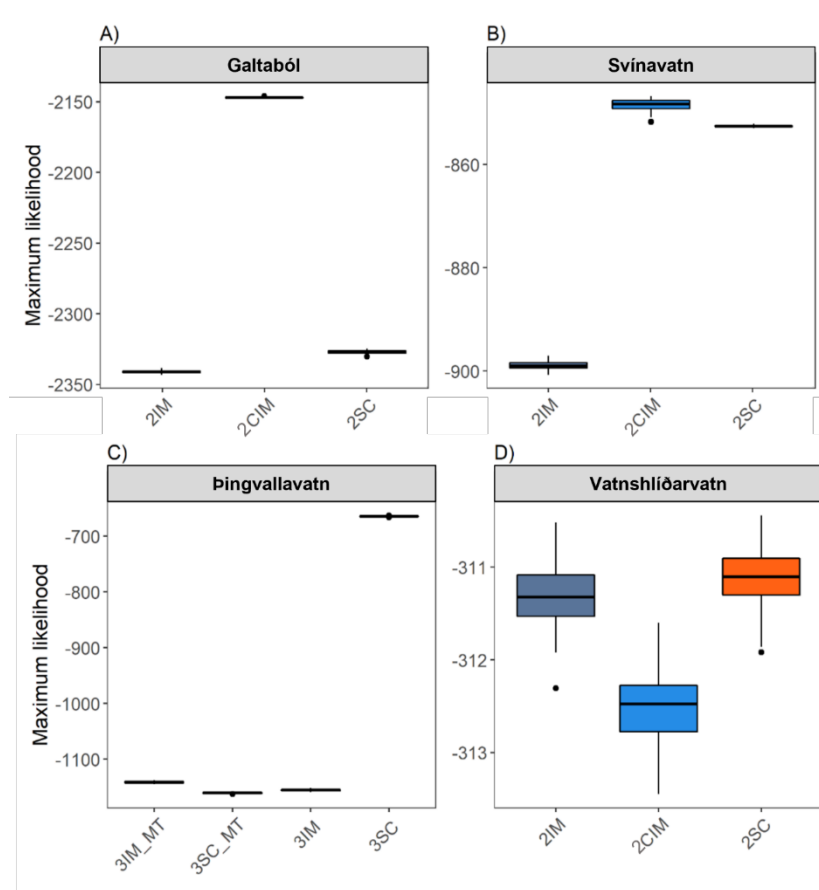
Figure 2. Models of divergence history within four populations of Icelandic Arctic charr. The best supported model within each population had the greatest maximum likelihood value. The boxplot whiskers represent the 95% confidence intervals for each model which were calculated using 1000 bootstraps. A) Galtaból. B) Svínavatn. C) Þingvallavatn. D) Vatnshlíðarvatn. The demographic models used in Galtaból, Svínavatn, and Vatnshlíðarvatn were the 2IM model (divergence of benthic and pelagic morphs in sympatry by isolation by migration with continuous gene flow), 2CIM model (divergence of benthic and pelagic morphs in sympatry by isolation by migration with a change (reduction) in gene flow over time), and the 2SC model (divergence of benthic and pelagic morphs in allopatry and subsequent secondary contact). Within Þingvallavatn four separate models were used to assess demographic history, the 3IM_MT model (divergence of benthic and pelagic morph types (MT) in sympatry through isolation by migration with continuous gene flow followed by the divergence of two benthic morph variants), the 3SC_MT model (divergence of benthic and pelagic morph types in allopatry followed by the evolution of two benthic morph variants and secondary contact among the three morphs), the 3IM model (divergence of three morphs in sympatry through Isolation by Migration with continuous gene flow), and the 3SC model (divergence of three benthic and pelagic morphs in allopatry followed by secondary contact).
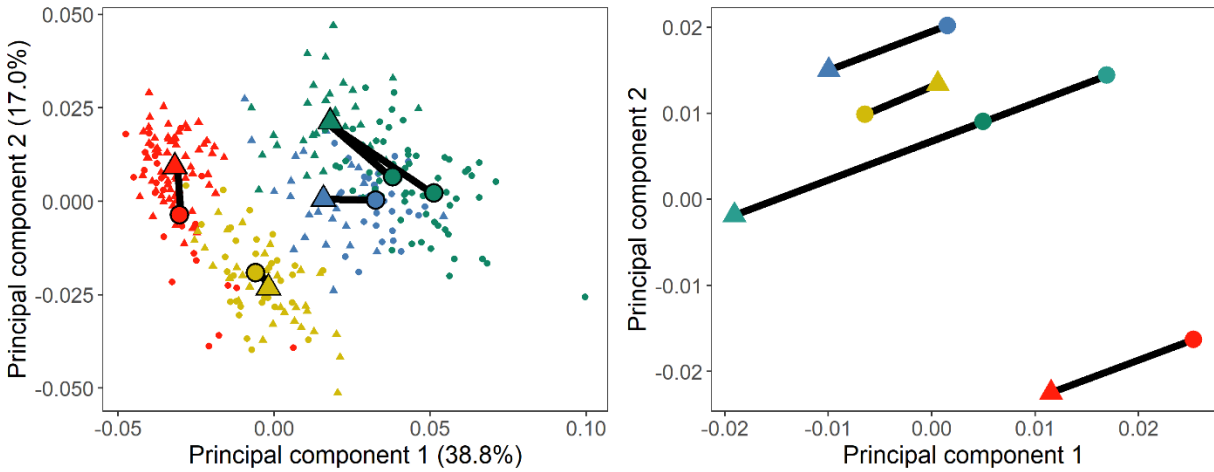
Figure 3. Phenotypic divergence trajectories across five benthic and pelagic morph pairs of Arctic charr from four geographically separated lakes in Iceland. Galtaból is shown in blue; Svínavatn in red; Þingvallavatn in green, and Vatnshlíðarvatn in yellow. Circles and Triangles represent benthic and pelagic morphs, respectively. A) PCA of all partial warp and uniform component scores describing body shape variation. B) Phenotypic divergence trajectories for each benthic and pelagic morph pair. Benthic and pelagic divergence trajectories are represented as the mean PC1 and PC2 values for each benthic and pelagic morph among all morph pairs. All partial warp and uniform component scores were projected onto PC1 and PC2, the major axes of phenotypic variation, with all populations combined. Divergence trajectories were then calculated by summarizing the mean covariance between PC1 and PC2 for further tests of parallelism.
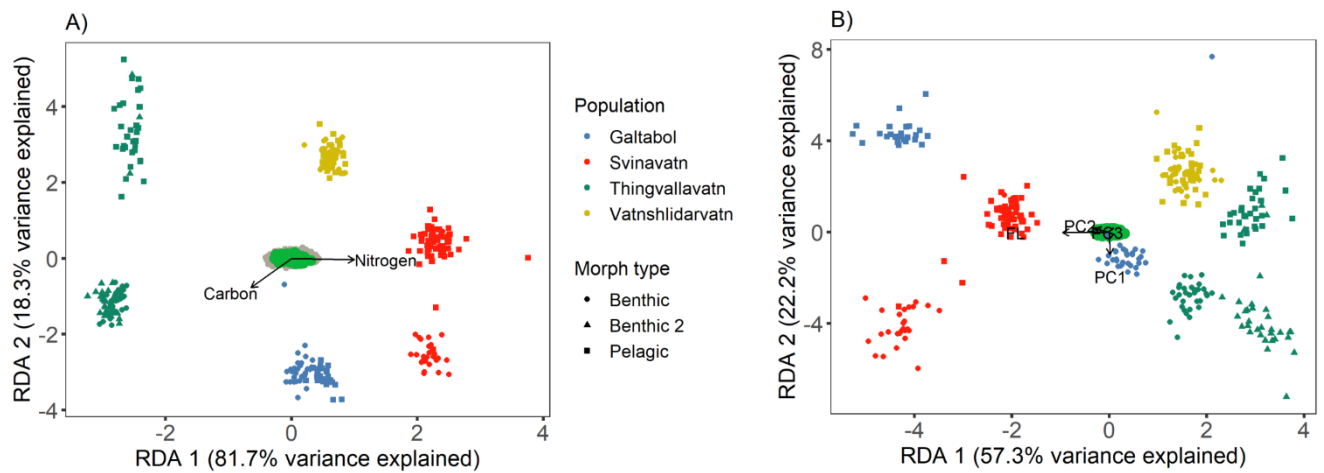
Figure 4. RDA outlier analysis of 14,187 polymorphic SNPs across four populations of Icelandic Arctic charr containing benthic and pelagic morphs. Populations are shown in different colours while benthic and pelagic morphs are represented as different shapes. A) SNP associations with variation in resource use traits. B) SNP associations with variation in morphological traits.
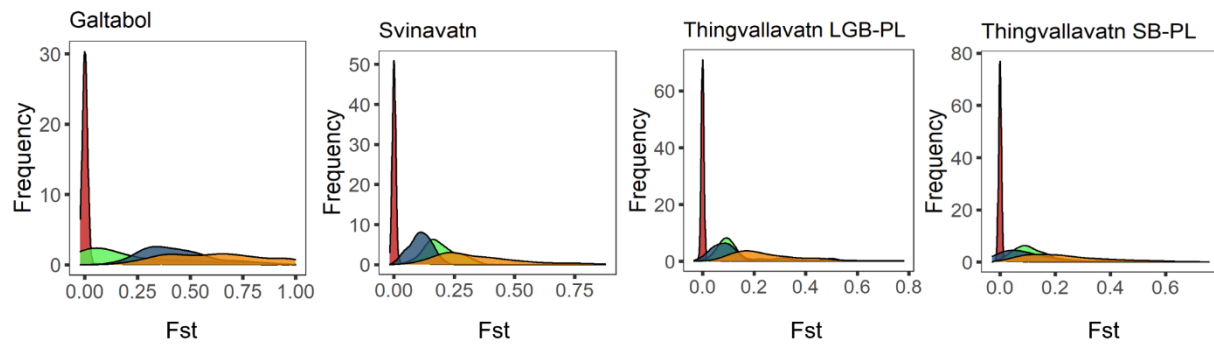
Figure 5. Distribution of neutral and RDA outlier SNPs for four benthic and pelagic morph pairs from three lake populations of Icelandic Arctic charr. Neutral SNPs are shown in red while SNPs associated with morphological and ecological variation are highlighted in blue and green, respectively. SNPs that are associated with both morphological and ecological variation are highlighted in orange.
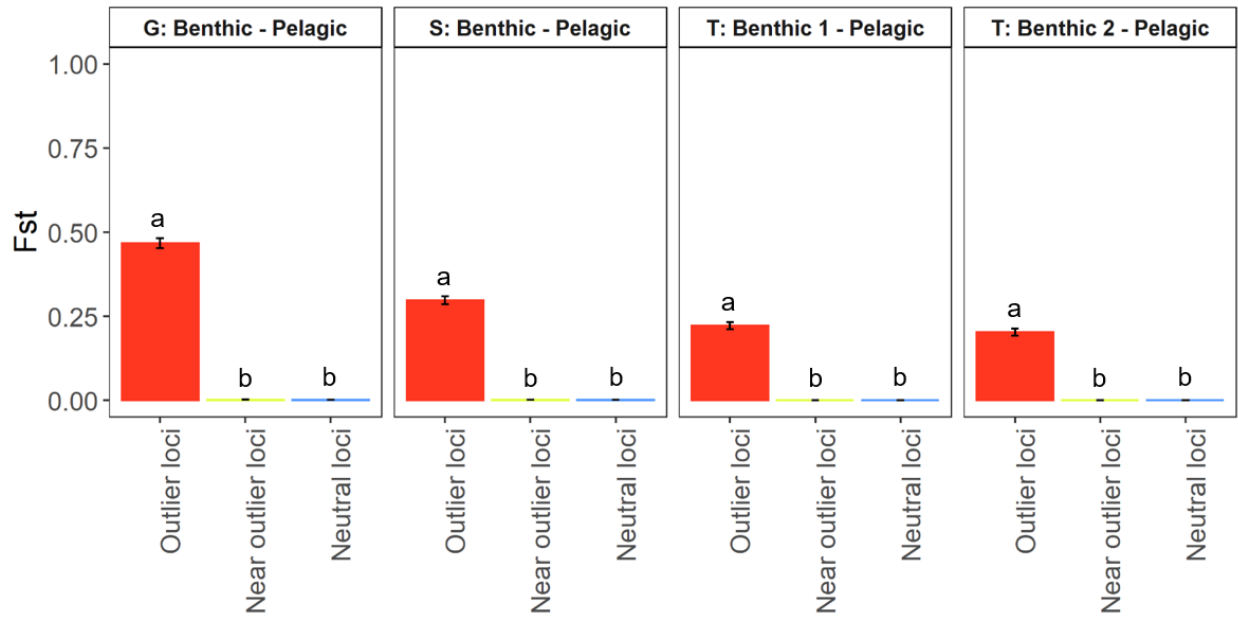
Figure 6. Colocalization of FST and RDA outlier loci for four pairs of sympatric morphs of Icelandic Arctic charr. Red, yellow, and blue bars indicate outlier loci, loci surrounding outliers, and non-outlier loci, respectively. Different letters indicate statistical significance between the different groups of loci in each population comparison
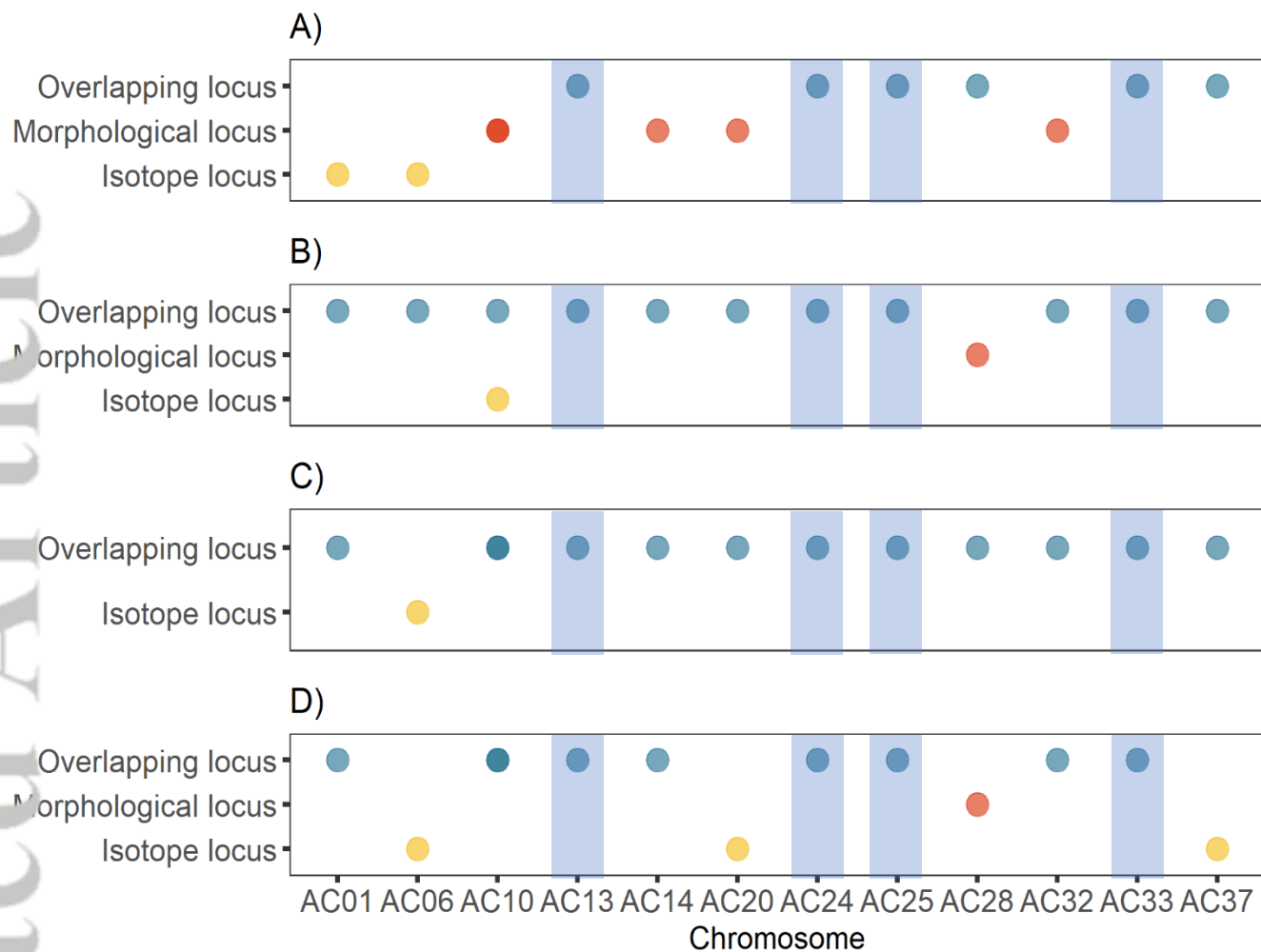
Figure 7. Parallelism of RDA outlier loci across four benthic-pelagic morph pairs from three distinct populations of Icelandic Arctic charr. A total of thirteen loci were identified as being parallel across various chromosomes. Chromosome AC10 had two out of the thirteen outlier loci located on it. A) Galtaból, B) Svínavatn, C) Þingvallavatn benthic 1 – pelagic morph pair, D) Þingvallavatn benthic 2 - pelagic morph pair. No outlier loci were identified within Vatnshlíðarvatn.