Genomic underpinnings of head and body shape in Arctic charr ecomorph pairs

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Abstract

Across its Holarctic range, Arctic charr (Salvelinus alpinus) populations have diverged into distinct trophic specialists across independent replicate lakes. The major aspect of divergence between ecomorphs is in head shape and body shape, which are ecomorphological traits reflecting niche use. However, whether the genomic underpinnings of these parallel divergences are consistent across replicates was unknown but key for resolving the substrate of parallel evolution. We investigated the genomic basis of head shape and body shape morphology across four benthivore-planktivore ecomorph pairs of Arctic charr in Scotland. Through genome-wide association analyses, we found genomic regions associated with head shape (89 SNPs) or body shape (180 SNPs) separately and 50 of these SNPs were strongly associated with both body and head shape morphology. For each trait separately, only a small number of SNPs were shared across all ecomorph pairs (3 SNPs for head shape and 10 SNPs for body shape). Signs of selection on the associated genomic regions varied across pairs, consistent with evolutionary demography differing considerably across lakes. Using a comprehensive database of salmonid QTLs newly augmented and mapped to a charr genome, we found several of the head and body shape associated SNPs were within or near morphology QTLs from other salmonid species, reflecting a shared genetic basis for these phenotypes across species. Overall, our results demonstrate how parallel ecotype divergences can have both population-specific and deeply shared genomic underpinnings across replicates, influenced by differences in their environments and demographic histories.

Genomic underpinnings of head and body shape in Arctic charr ecomorph pairs

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Abstract:

Across its Holarctic range, Arctic charr (*Salvelinusalpinus*) populations have diverged into distinct trophic specialists across independent replicate lakes. The major aspect of divergence between ecomorphs is in head shape and body shape, which are ecomorphological traits reflecting niche use. However, whether the genomic underpinnings of these parallel divergences are consistent across replicates was unknown but key for resolving the substrate of parallel evolution. We investigated the genomic basis of head shape and body shape morphology across four benthivore-planktivore ecomorph pairs of Arctic charr in Scotland. Through genome-wide association analyses, we found genomic regions associated with head shape (89 SNPs) or body shape (180 SNPs) separately and 50 of these SNPs were strongly associated with both body and head shape morphology. For each trait separately, only a small number of SNPs were shared across all ecomorph pairs (3 SNPs for head shape and 10 SNPs for body shape). Signs of selection on the associated genomic regions varied across pairs, consistent with evolutionary demography differing considerably across lakes. Using a comprehensive database of salmonid QTLs newly augmented and mapped to a charr genome, we found several of the head and body shape associated SNPs were within or near morphology QTLs from other salmonid species, reflecting a shared genetic basis for these phenotypes across species. Overall, our results demonstrate how parallel ecotype divergences can have both population-specific and deeply shared genomic underpinnings across replicates, influenced by differences in their environments and demographic histories.

Keywords: parallel evolution, ecomorphology, QTL, genomics, fish, population genomics

Introduction:

Parallel evolution is an evolutionary process and outcome by which similar phenotypes arise and establish in multiple independent populations in separate environments . These replicate evolutionary outcomes suggest that similar environments impose similar selective pressures on organismal phenotypes, with only a small number of phenotypic solutions favoured in that context. While the existence of parallel phenotypes is well established in natural populations , the extent to which those are associated with similarly shared genomic underpinnings is rarely examined . Indeed, the appearance of the same phenotypes through parallel evolution processes does not mean that the same genomic processes underpin those similar evolutionary outcomes across replicates . Similar phenotypic outcomes could result from alternative genetic pathways. This might arise because of differing demographic histories , variable genetic backgrounds or the involvement of alternative splicing, differential gene expression or post-translational modifications resulting in phenotypic parallelism .

One classic example of parallel evolution is the replicated divergence across northern freshwater lakes of distinct trophic specialists, also known as ecomorphs or ecotypes. These occur abundantly in salmonid fishes in recently glaciated lakes, such as lake whitefish (*Coregonus clupeaformis*) , lake trout or lake charr (*Salvelinus namaycush*) , and Arctic charr (*Salvelinus alpinus*) . In Arctic charr, ecomorphs associated with divergence along the depth axis and ecological niche, typically forming pelagic and benthic foraging specialists . Two key traits involved in this divergence are head shape and body shape, both having functional significance; head shape being important for foraging and prey specialisation and body shape important for swimming behaviour and niche use Additionally, ecomorphs often differ in other complex traits such as body size, colouration and spawning time .

Previous research on benthivorous-planktivorous ecomorph pairs of Arctic charr has shown that the repeatability into ecomorph is highly parallel and that individual traits related to head shape also show some parallelism . Genomic analysis to-date suggested limited genetic parallelism across lakes, with many differences in demography and colonisation history across the breadth of charr distribution . However, this previous research on parallelism between Arctic charr ecomorphs focused on the patterns of the genomic response to selection such as outlier loci, which are known to have high rates of false-positives and false-negatives and are indirect approaches to understanding morphology, strongly influenced by evolutionary genomic and demographic histories . Therefore, we lack knowledge of the genomic regions associated with key phenotypic differences in charr and the extent to which these are shared across ecomorph pairs .

Because of their vital role in foraging and swimming, the genetics of head and body morphology has been explored as QTL studies in many fish species, including salmonids . For example, in lake whitefish, as many as 138 different quantitative trait loci (QTLs) related to body shape have been identified, implying that the trait is highly polygenic in this species . In other species, body shape has been found to be less polygenic, with a small number of QTLs identified related to benthic-limnetic differences between ecomorphs of lake trout and a single region that differentiates ecomorphs in Atlantic cod (*Gadus morhua)* However, the extent to which parallel phenotypes have the same genomic underpinnings across replicates seems to vary greatly between species and locality. Studies in three-spine stickleback (*Gasterosteus aculeatus*), rainbow trout (*Oncorhynchus mykiss*), and sockeye salmon (*Oncorhynchus nerka*) all suggest that while some genetic variation that underlies morphological variation can be shared across replicates, this is rarely the case across the species' entire range and the degree of genetic parallelism that is shared is often low . Often only a few key genes or loci are shared across replicates, and in some cases, they underlie morph differentiation in multiple species .

In this study, we investigated the genomic underpinnings of head and body shape morphology in replicate ecomorph pairs of Arctic charr across four independent lakes in Scotland. First, we determined the extent of phenotypic parallelism in head and body shape morphology across ecomorph pairs. We examined these two traits separately as they are known to involve different axes of ecological specialisation and can have different genetic bases . Second, we investigated the genome-wide underpinnings of these phenotypes by identifying SNPs strongly associated with head shape variation and body shape variation. We then evaluated the genomic organisation of these head shape- and body shape-associated SNPs, specifically to determine if they were distributed widely across the genome, co-localised in genes or genomic regions, or within known salmonid QTLs. To do this, we updated and augmented an existing QTL database and mapped this to the orthologous location in the *Salvelinus sp.* genome. Third, we examined evolutionary genomic background by analysis of selection on those loci we found to be associated with head shape and with body shape. Shared signals of selection and elevated differentiation and divergence in independent ecomorph pairs would suggest similar processes across evolutionary replicates. Finally, we synthesised the findings to identify shared genomic underpinnings and similarities in phenotypic divergence between head and body shape, as two interlinked components of ecomorph.

Materials and Methods:

Study populations:

Fish populations were examined from four lakes in Scotland: Loch Awe, Loch Dughaill, Loch na Sealga, and Loch Tay (Figure 1A). Each lake contains two ecomorph populations, one benthivorous and the other planktivorous, with each lake representing an ecomorph pair . Explictily, we investigated the simiarlities in divergences of head and body shape between the two different ecomorphs across pairs. Short-read genomic data (ddRADseq NCBI short read bioproject: PRJNA607173) and fish photographs were drawn from previous research and reanalysed here.

Morphological analyses:

To investigate patterns in head morphology and body shape separately, landmarks for head (16 landmarks) and body shape (14 landmarks) were placed using *ImageJ v1.50i* (Figure 1E-F). To allow us to cover the whole-body shape of each individual, several markers related to head shape were retained in the body shape analysis. As such, the head and body shape analyses are not completely independent but are focused on each specific phenotype and use a distinct set of landmarks. In total, 341 individual fish were landmarked for head shape (Awe_Bn=37, Awe_Pl=31, Dughaill_Bn=43, Dughaill_Pl=54, naSealga_Bn=42, naSealga_Pl=20, Tay_Bn=41, Tay_Pl=73) and 335 were landmarked for body shape (Awe_Bn=34, Awe_Pl=30, Dughaill_-Bn=43, Dughaill_Pl=54, naSealga_Bn=42, naSealga_Pl=19, Tay_Bn=40, Tay_Pl=73).

General Procrustes Analysis (GPA) was performed to standardise each individual's shape for size and orientation using *geomorph v3.0.7*(R package) . Following this procedure, shape data were standardised for any residual size effects using the log of centroid size to correct for allometry. Size corrected data then were used in all subsequent analyses. Principal Component Analyses (PCA) were conducted on head shape and body shape separately using the *plotTangentSpace* and*arrayspecs* functions and plots were made in *ggplot2v3.3.5* (R package) .

Analysis of variance (ANOVA) models were used to determine the relative contributions of parallel and non-parallel aspects of the morphological divergence across ecomorph pairs . Rather than using a single PC (Principal Component) score for the ANOVAs (ANOVA model: PC ˜ ecomorph + lake + ecomorph x lake), we used a combined weighted PC variable with scores from multiple PCs to account for a higher proportion of biologically important variance in a single variable. For this variable, we combined all consecutive PCs that explained more than 5% of the total variance in shape. For both head shape and body shape, this was PC1 to PC5 (Figure S1). To weight the scores within the combined variable, each PC score was multiplied by the amount of variance explained by that PC (i.e., each individual PC1 score x proportion of variance explained by PC1) before all five weighted scores were summed for each individual. The *EtaSq* function in the*BaylorEdPsych v0.5* (R package) was used to estimate the effect size of each model term. In our model, the ecomorph term represents the parallel or shared term, the ecomorph*lake interaction is the non-parallel (non-shared) term, and the lake term represents unique evolutionary history. ANOVA results for PC1 through PC5 separately can be found in Table S3.

Phenotypic Trajectory Analyses (PTA) were performed on the procrustes scores using the *trajectory.analysis* function in *geomorph*to look at the extent and direction of phenotypic change between ecomorphs. Magnitude of divergence is described by the length of trajectories (L) while the angle between trajectories (ϑ) describes their direction in phenotypic space. This approach allows us to determine how parallel the trajectories of each ecomorph pair are to one another by using the difference in phenotypic trajectory length (ΔL_P) and the direction of phenotypic trajectories (ϑ_P). The significance of differences in trajectory lengths and differences in trajectory direction were assessed using 1,000 permutations . Phenotypic divergence between ecomorphs in different lakes was considered to be parallel if the direction and/or magnitude of phenotype change did not differ significantly between the pairs ($P < 0.05$).

Population genomics:

Filtered 75bp reads for each individual, generated via ddRADseq from Jacobs *et al* . (2020) and accessed from (ddRADseq NCBI short read bioproject: PRJNA607173), were mapped using *bwa mem* and*SAMtools* using settings described in that paper to the*Salvelinus* sp. genome from NCBI (ASM291031v2). The number of reads per individual ranged from 1 to 3.5 million. RAD loci were built in the *gstacks* module of *Stacks v2.53* for 200 individuals (Awe_Bn= 26, Awe_Pl=29, Dughaill_Bn=28, Dughaill_Pl=27, naSealga_Bn=18, naSealga_Pl=20, Tay_Bn=21, Tay_Pl=31). SNPs were retained in the *populations* module of *Stacks* if they met the following criteria: present in 66% of all individuals in each population and across all populations, a minimum minor allele frequency of 0.05, maximum observed heterozygosity of 0.5. Each ecomorph within a lake was considered to be a discrete population. The script filter_hwe_by_pop.pl to filter out sites outside Hardy-Weinberg equilibrium within populations (available at https://github.com/jpuritz/dDocent). *vcftools v0.1.13* was used to filter to a minimum coverage of 5x and a maximum of 50x. A principal component analysis was performed to identify the major axes of genetic variation using *SNPRelate v1.22.0* (R package) .

Genotype-phenotype association analyses:

To determine the association between genotypes and phenotypic variation in head or body shape, we ran Linear Mixed Models (LMM) in *Gemma v0.98.1* . Univariate and Multivariate LMMs with Wald's test were run using PCs 1-5 for head shape and body shape, the SNP dataset generated for the population genomics analyses, and lake of origin as a co-variate . Missing genotypes were imputed using *LinkImpute v1.1.4* and a relatedness matrix was generated using *Gemma* before running the models. We determined significant associations using bonferroni-corrected P-values (0.05/7329 unlinked SNPs) from the Wald's tests. The number of unlinked SNPs was determined by LD-pruning the full SNP dataset using the snpgdsLDpruning function in *SNPRelate* . Bayesian Sparse Linear Mixed Models (BSLMM) were run using PC1-5 variables to determine how much of the phenotypic variation is explained by the SNPs in our dataset (PVE) and secondly how much of that variation is explained by large-effect loci (PGE), and finally how polygenic each phenotype is ([?]). Manhattan plots were made using *CMplot v4.0* (R package) (https://github.com/YinLiLin/CMplot).

We subsequently determined if SNPs showing significant associations with head shape or body shape morphology were found within annotated genes in the *Salvelinus* sp. reference genome using *BEDtools v2.27.1* . The functions of genes containing, or ± 1kbp of, associated SNPs were investigated using GO term overrepresentation analysis (ORA) and gene set enrichment analysis (GSEA). These analyses were run using*topGO v2.40.0* (R package) with all genes containing any RAD loci as the full comparison dataset. Results were summarised using*REVIGO* before visualisation in *Cytoscape v3.91* .

Comparisons to known QTLs:

Using existing information on the genetics of important phenotypes from other salmonid species, we mapped a database of 1,338 QTL markers to the*Salvelinus* sp. genome. This was based on a previously published database of QTLs involved in traits related to morphology and life history, derived from a range of salmonid species and previously mapped to the *Salmo salar* genome . Additionally, a literature search was conducted up to April 2021 to augment the existing database with more recently published QTLs. This literature search was conducted in Web of Science and Google Scholar using the search terms "QTL", "quantitative trait loci", "salmonid", and the common and scientific names for rainbow trout, Atlantic salmon, Arctic charr, lake whitefish, Chinook salmon, coho salmon, brook trout, and lake trout. QTL marker sequences were gathered for 17 different phenotypes: body length, body shape, body weight, Fulton's condition factor, directional change, disease resistance, embryonic development, gill rakers, growth rate, hatching time, head shape, parasite resistance, salinity tolerance, sexual maturation, smolting, spawning time, upper temperature tolerance (Table S1).

Following Jacobs *et al.* (2017), the strategy of mapping the QTL-linked markers to the *Salvelinus* sp. genome depended on the QTL marker type: RAD loci were mapped using *Bowtie2 v2.4.4* and the *very sensitive* pre-set; microsatellite primer sequences, which are shorter, were mapped using *Bowtie v1.3.1* allowing for 3 mismatches. QTLs for which the flanking markers mapped to different chromosomes were removed. Redundant QTLs, i.e., where two QTLs for the same trait from the same species mapped to the same location, were removed only keeping the QTL with the higher PVE or LOD score (following . For QTLs where more than one marker was reported, we attempted to map all markers. Position values for the QTLs markers were then compared to positions of the phenotype associated SNPs using *BEDtools* , with a cut-off of ±100kbp. This value was used so that we could consider SNPs within the range to be proximal to a QTL peak while also accounting for the large size of many of the QTLs in the database. In total, we successfully mapped 669 QTL-linked sets of markers to the*Salvelinus* sp. genome after removing redundant QTLs (Table S2).

Genomic response to selection:

We investigated if the phenotype-associated SNPs identified in our analyses showed signals of a response to selection and if those signals were replicated across ecomorph pairs. To test this, for each ecomorph pair we compared F_{ST} and D_{XY} values for phenotype-associated SNPs to a random background subset of SNPs. This random subset was 100 SNPs randomly selected from the whole dataset and the mean F_{ST} and D_{XY} values for those SNPs were calculated. This was repeated 10,000 times and the means for F_{ST} and D_{XY} were taken across all permutations. These permuted values were then compared to the empirical mean F_{ST} and D_{XY} values for the phenotype-associated SNPs using the *t.test* function in R.

Analyses of recombination rate variation:

To test the effect of the recombination landscape on phenotype-genotype association, we first estimated recombination rates using the published Arctic charr linkage map (N=3,636) using *MareyMap v1.3.6* . RAD loci from the linkage map were aligned to the *Salvelinus* sp. reference genome with Bowtie2 using the *-very-sensitive* setting. Loci were kept if they uniquely mapped to one location, mapped to the same chromosome as all other loci on their linkage group, and followed the orientation of the linkage map (i.e., not reversed). The filtered dataset was used to estimate the recombination rate across each chromosome using a spline algorithm. Spar values were varied for each chromosome from 0.5 to 0.9, depending on chromosome size, to best fit the data . Subsequently, *WindowScanR v0.1* (available at: https://github.com/tavareshugo/WindowScanR) was used to summarize recombination rate values in 1 MB windows along the genome. All SNPs were assigned to these windows using *BEDtools* . A random subset of 100 SNPs was then selected and the mean recombination rate for those SNPs was calculated based on their windows. This was repeated 10,000 times to generate a background mean recombination rate, which was then compared to the mean recombination rate of the phenotype-associated SNPs (based on their windows) using a t-test.

Results:

Ecomorph divergence in head shape:

For head shape the benthivore and planktivore ecomorphs were separated across PC1 (31% variance explained), except from Loch Dughaill where the ecomorphs separate along PC2 (17.3%) (Figure 1C). Individuals with a positive PC1 score had shallower heads with larger eyes than those with negative PC1 scores (Figure 1E). For PC2, a more positive score suggested a longer head shape. The benthivore ecomorphs generally have a more negative PC2 score suggesting their heads are shorter than the planktivore ecomorphs (Figure S2).

We compared the magnitude and direction of phenotypic change for head shape between the ecomorphs across pairs, to determine how similar the divergences were, through a phenotypic trajectory analysis (PTA). We

found that for all pairwise comparisons, the angle of difference in phenotypic trajectories (ϑ) was significantly different ($P < 0.05$) (Table 1). Similarly, almost all differences in trajectory lengths between ecomorphs pairs (ΔL) were significantly different with the exception of the Awe vs na Sealga comparison. These results suggest that head shape morphology is variable across lakes. This is in agreement with our ANOVA model (PC ~ ecomorph + lake + ecomorph x lake) which found that the ecomorph x lake interaction term explained most variation ($\eta^2_{\text{Eco*Lake}} = 0.565$) suggesting that the effect of lake environment and/or evolutionary history strongly impacts the direction and magnitude of head shape divergence between ecomorphs, and that head shape is not strictly parallel across lakes (Table S3).

Genomic regions associated with head shape:

To determine the genomic variation underpinning head shape, we performed a genome-wide association analysis (GWAS) on a set of 13,071 SNPs (Figure S3). Using the PC scores from the head shape analysis (PCs 1-5), a Bayesian Sparse Linear Mixed Model (BSLMM) showed that the proportion of phenotypic variance in head shape explained by genetic variation (PVE_{Head}) in the SNP dataset was 0.62 with the proportion of phenotypic variance explained by large ("sparse") effect loci (PGE_{Head}) was 0.82. This is supported by the [?] (rho) value for head shape that suggests that the head shape phenotype is controlled largely by a few large-effect loci ($[?]_{\text{Head}} = 0.792$).

Applying Linear Mixed Models to identify SNPs highly associated with head shape variation found a total of 82 SNPs (66 SNPs mapped on 27 of 39 chromosomes, 16 mapped to unanchored scaffolds) that showed a significant association with variation in head shape (Bonferroni corrected P-value < 0.05; Fig. 2A) with these SNPs broadly distributed across the genome.

Genomic differentiation at SNPs associated with head shape:

We investigated whether these head shape-associated SNPs were highly diverged between the ecomorphs in all lakes, consistent with a shared genomic bases for these phenotypes, or whether they were specific to certain populations suggesting the deployment of different genetic pathways leading to the similar phenotypes across pairs. We found a total of three SNPs that were diverged in all four lakes (Fig. 3A).

We aimed to identify if those SNPs associated with head shape showed signs of response to divergent or positive selection in all four lakes. Mean genetic differentiation (F_{ST}) and absolute divergence (D_{XY}) between ecomorphs in lochs Dughaill and Tay were elevated amongst associated SNPs when compared to the background (Figure 4, 5) (Table S4). F_{ST} and D_{XY} were significantly lower than background between ecomorphs in Loch Awe for the associated SNPs. There was no significant difference in F_{ST} or D_{XY} between associated SNPs and the background in na Sealga. These results suggest that the SNPs associated with head shape are not similarly responding to, or are under selection, across all lakes. These patterns were not influenced by linkage, because recombination rates for genomic regions around head shape-associated SNPs did not differ significantly from genomic background (Figure S4).

Genes and QTLs associated with head shape variation:

Focusing on the location of the head shape-associated SNPs relative to known genes in the charr genome, we found that 38 of the 82 SNPs were located within annotated genes (Table S5). GO term analyses found that the genes containing head-shape associated SNPs showed over-enrichment for GO terms related to odontogenesis (GO:0042476), cranial skeleton system development (GO:1904888) among other processes and functions (Table S6) when compared to all genes containing SNPs in our dataset.

To examine if any of these genomic associations were shared across other species, we compared the positions of the head shape-associated SNPs to QTL markers from across salmonid species. We found that three of the head shape-associated SNPs were found within ±100,000 bp of the peak positions of two mapped QTLs

(Table 2). These two QTLs were previously found to be associated with body shape morphology in lake trout and lake whitefish .

Ecomorph divergence in body shape:

For body shape, all four ecomorph pairs showed separation along PC1 (30.3%) (Figure 1D) however, the pair from Loch Dughaill diverged in a different direction, along PC2 (24.9%). Individuals with a positive PC1 score (e.g., the benthivore morphs at Awe, na Sealga, and Tay) have shallower, more elongated body shapes (Figure 1F). The patterns across PC2 suggest that a more positive score is associated with a deeper body (Figure S5).

In the PTA, we again found that all differences in the magnitude of phenotypic change between the pairs (ΔL) was significant with the exception of the Awe-na Sealga comparison (Table 1). When comparing angle of difference in trajectories (ϑ), we found all angles between significant with the exception of the na Sealga-Tay comparison suggesting body shape may show some parallelism across lakes. The ecomorph term in the ANOVA model ($\eta^2_{\text{Eco}} = 0.301$) for body shape explained more variation than the ecomorph x lake term ($\eta^2_{\text{Eco*Lake}} = 0.135$), suggesting the ecological niche is a stronger determinant of body shape, indicating some level of phenotypic parallelism across lakes. However, the unique evolutionary history (the lake term) explained most variation ($\eta^2_{\text{Lake}} = 0.414$) suggesting that the absolute position of ecomorph pairs in the multivariate space differs between lakes and is strongly influenced by differences in the evolutionary histories or environment of each of the pairs (Table S3).

Genomic regions associated with body shape:

A BSLMM found that the proportion of phenotypic variance in body shape explained by the SNP dataset (PVE_{Body}) was 0.82 and the proportion of phenotypic variance explained by large ("sparse") effect loci (PGE_{Body}) was 0.39 and the [?] (rho) value ($[?]_{\text{Body}}$) was 0.425. By analysis with LMMs, we found 180 SNPs significantly associated with body shape variation (144 SNPs mapped to 34 chromosomes, 36 SNPs mapped to unplaced scaffolds) (Figure 2B). We found that 10 of these SNPs were present in all four ecomorph pairs (Figure 3B) and broadly distributed across the genome (on 7 chromosomes).

Genomic differentiation at SNPs associated with body shape:

For F_{ST}, we found that the body shape-associated SNPs had a higher mean value than the background at Loch Dughaill and Tay (Figure 4, 5, Table S4) but no notable difference at Loch Awe or na Sealga. D_{XY} was significantly higher at Tay for the associated SNPs while it was significantly lower at Loch Awe. There was no significant difference in D_{XY} between associated SNPs and the background at Loch Dughaill and naSealga. The mean recombination rate in regions containing body shape SNPs did not differ from the background (Figure S4).

Genes and QTLs associated with body shape:

Relative to known genes in the *Salvelinus sp.* genome, 89 of the body shape-associated SNPs were located within annotated genes (Table S5). The body shape-associated genes showed overrepresentation for genes involved in skeletal system (GO:0001501), face (GO:0060324), eye (GO:0060041, GO:0001745), and mouth development (GO:0060021) among other processes and functions (Table S6). We found five SNPs in close proximity to four known QTLs (Table 2). These QTLs were previously found to be associated with body shape morphology in lake whitefish, body weight in Arctic charr and body shape morphology in lake trout .

Comparisons between head shape and body shape:

We found in our PTA that comparisons in head shape showed greater mean differences in the magnitude and direction of phenotypic change ($\Delta L_{Head} = 0.053 \pm 0.035$ s.d., mean $\vartheta_{Head} = 69.87^{\circ} \pm 17.54$ s.d. mean) compared to body shape (mean $\Delta L_{Body} = 0.016 \pm 0.011$ s.d., mean $\vartheta_{Body} = 61.62^{\circ} \pm 22.63$ s.d.) (Table S7). Both our PTA and ANOVA suggest that body shape shows slightly stronger patterns of phenotypic parallelism than head shape, however, both phenotypes show substantial deviations from strict parallelism across lakes.

Our association analyses indicated a significant shared genetic basis behind body shape and head shape. 50 of the SNPs found in our study appeared associated both with head and body shape morphology (212 SNPs identified: 32 SNPs associated with head shape, 130 associated with body shape, and 50 associated with head and body shape), which exceeds random expectation (hypergeometric test; $P = 6.633e^{-16}$). Of these head- and body-shape shared SNPs, 38 mapped to 20 chromosomes and 12 mapped to unplaced scaffolds (Figure 2). These SNPs show overrepresentation for terms related to brain (GO:0030900, GO:0021575) and heart development (GO:0003007), and regulation of cell shape (GO:0008360) among other processes (Table S6). With a number of SNPs shared between both head and body shape, two of the QTLs we identified as near associated SNPs were near SNPs shared for both phenotypes. These QTLs related to body shape in lake trout and lake whitefish respectively (Table 2) .

This shared genetic basis is reflected in the PTA, which showed that there was a positive linear relationship when comparing trajectory lengths for head shape and body shape across lakes. Specifically, pairs in which the ecomorphs have diverged to a similar extent in head shape have also diverged to a similar extent in body shape (adjusted $R^2 = 0.99$, $P < 0.001$) (Figure 6). A similar positive relationship was seen when comparing differences in the direction of phenotypic change, although this was non-significant (adjusted $R^2 = 0.29$, $P = 0.154$) (Figure S6).

Discussion:

Through our analyses, we identified genomic regions that underlie head shape and/or body shape morphology in ecomorph pairs of Arctic charr. Of these phenotype-associated SNPs, many (approximately one quarter: 50 of 212) were shared between head and body shape. The extent and direction of divergence in head and body shape morphology were positively correlated, suggesting a shared developmental basis for the two phenotypes. The SNPs we found associated to each phenotype were often found in genes related to morphology or anatomical development. Indeed, independently previously identified QTLs in the genomic region of body or head associated SNPs were often those related to morphology.

We found limited parallelism in shape morphology and in genomic underpinnings with many population-specific patterns in the divergence of head and body shape morphology between ecomorphs across pairs. Many of the phenotype-associated SNPs were not present in all four pairs likely due to polygenic genomic architectures and the incomplete representation of the genome in our approach. The phenotype-associated SNPs that were found to be shared were not highly diverged in all pairs and did not appear to be under the same selective pressures. Body shape in particular appears to be rather polygenic allowing for the genomic underpinnings of the phenotypes to vary across lakes.

Limited parallelism in head and body shape divergences across replicates

While previous work on parallelism in these pairs suggested substantial phenotypic parallelism in some linear traits related to head shape our more sensitive geomorphometric approach to describe shape suggests considerable phenotypic variation across replicated ecomorphs in multivariate space for both head shape and body shape.

The lack of phenotypic parallelism between Loch Dughaill and the other ecomorphs pairs may arise from its ecological distinctiveness compared to the others. While benthivore morphs typically occupy the shallow littoral zone of lakes as they do at the other three lakes in our study, the benthivore morph at Loch Dughaill is a 'profundal' benthivore and as such utilises a much deeper part of the lake environment, and the lake has a smaller area . The divergence we see between the ecomorphs at Loch Dughaill, as indicated by the PCA, is in line with other 'profundal' charr morphs found across the Holarctic, with the benthivore ecomorph having a deeper head and body than the planktivore morph . This is the inverse pattern of the more common benthivore-planktivore divergence where the benthivore morph has the shallower, longer head and body shape that is seen in the other three ecomorph pairs .

Evidence for the parallel evolution of head and body shape morphology across ecomorphs has been shown in previous studies of Arctic charr . However, even disregarding the notably distinct Dughaill ecomorph pair, the other three ecomorph pairs show limited evidence of parallelism in shape morphology despite the parallel evolution of ecomorphs themselves. Evolutionary divergence and thus phenotypic trajectories are influenced by the interaction between environmental variation and adaptive genetic variation. As a result, repeated ecomorph divergences often have very different phenotypic trajectories for key components of phenotypes, as seen in three-spine stickleback . Thus, the lack of strict parallelism seen in our study is likely the result of known differences in the evolutionary histories of these pairs and differing selective pressures in their local environments, for example from differences in their ecosystems or diets . Parallelism can also be generated by other mechanisms, such as differences in gene expression, post-translational modifications, and/or alternate splicing . Indeed, differences in splicing and gene expression patterns showed parallel patterns across ecomorph pairs in a study on three of the ecomorph pairs we investigated and provide explanations of alternative adaptive paths.

Of the two traits we tested, head shape had longer phenotypic trajectory lengths and greater angles, suggesting that the ecomorphs are more differentiated in head shape from one another and that head shape has evolved in more distinct directions across lakes. Head shape is well recognised as an important phenotype for foraging and prey specialisation, while body shape is important for swimming behaviour and habitat complexity . The considerable divergence in head shape between ecomorphs within lakes suggests different prey specialisations (Garduño-Paz et al. 2012; Hooker et al 2016) while their body shapes and therefore perhaps swimming behaviours are more subtly different. The high trajectory angles for head shape suggest notable difference in foraging across lakes, whether that be due to the lake environments or the actual species available as prey . For both traits, Loch Tay and Dughaill showed notably higher trajectory lengths than Awe and na Sealga and more evolutionary divergence, also seen in the ecomorphs' genomic divergence (F_{ST} ~1% between ecomorphs in Awe and na Sealga vs 9% in Dughaill and Tay). This reflects likely what were previously inferred to be recent sympatric divergences of ecomorph pairs in Awe and na Sealga while Tay and Dughaill each have complex histories of divergence and secondary contact between colonising lineages .

Genomic underpinnings of head and body shape across lakes:

From our total of 212 SNPs that showed high associations with head and/or body shape (Figure 2), we found more SNPs associated with body shape than for head shape. Head shape was controlled by more large-effect loci relative to body shape and may suggest that head shape is controlled by fewer genes/pathways. In both cases these will be an underestimate of actual associations because we have reduced representation of the genome captured. We found for that for both head and body shape only a small number of associated SNPs were diverged between ecomorphs in all four pairs (Figure 3). This is line with what has been suggested both in other Arctic charr studies and other salmonid species, in which genetic differentiation between ecomorphs is largely nonparallel across pairs bar at a few key genes . Further to previous work, we found that the SNPs shared across pairs were not highly differentiated between ecomorphs in all pairs suggesting that while present, they are not critical to underlying the phenotypic differences in each pair (Figure 4). These results also suggest that the genomic underpinnings of each phenotype varies across the lakes, likely contributing to the phenotypic differences we see between pairs. The polygenic genomic underpinnings of both phenotypes,

as indicated by the numbers of associated SNPs identified, indicate that there are multiple pathways that can achieve the same phenotypes hence the lack of high divergence for the same SNPs across all lakes .

Loch Dughaill and in particular Loch Tay often showed notable high genomic divergence between ecomorphs for many of the associated SNPs for each trait. Additionally, the associated SNPs for both traits showed high D_{XY} values compared to the background subsets at both of these lakes. While increased levels of D_{XY} or F_{ST} compared to genomic background can be indicative of positive selection , they might also be expected for loci resisting introgression following secondary contact , as is likely the case in Loch Tay and Loch Dughaill . The associated SNPs we found are widespread across the genome (Figure 2) indicating these are not single linked regions of divergence as found in studies on Atlantic cod and rainbow trout but instead are diffused and highly polygenic, similar to patterns for body shape in lake whitefish .

Functional genomic regions for head and body shape:

Roughly half of the associated SNPs identified for each of head and body shape were found within or proximal to an annotated gene in the charr genome. A number of the GO terms that appeared as significantly overrepresented or enriched in our study have been identified in other studies investigating adaptive divergences or parallel evolution in various fish species. Odontogenesis (GO:0042476), sensory perception of sound (GO:0007605), blood vessel remodelling (GO:0001974), response to muscle activity (GO:0014850), ventricular trabecula myocardium morphogenesis (GO:0003222), common-partner SMAD protein phosphorylation (GO:0007182), cellular response to ethanol (GO:0071361), and neuromuscular synaptic transmission (GO:0007274) have shown significance in other Arctic charr studies investigating ecomorph divergence . The GO terms for associative learning (GO:0008306), regulation of cell shape (GO:0008360), and UDP-glucuronate biosynthetic process (GO:0006065) also appear in overrepresented groups in a study on the divergence of a sympatric lake whitefish species pair (*Corgeonus clupeaformis*) in the USA . Finally, in pupfishes (*Cyprinodon. sp.*), the divergent expression of a number of genes involved in cranial skeletal system development was seen between different trophic specialists with the GO term for this process (GO:1904888) significant in our study . Differences in ossification rate have been related to adaptive morphological differentiation in other freshwater fish and the over enrichment or overexpression of genes related to formation of various bones in our study indicates a similarly important role in adaptive divergences between ecomorphs of Arctic charr . Indeed, previous work has noted the importance of differences in bone structure and sizes between different ecomorphs of Arctic charr .

The QTL database that we have developed allows us to explore whether rapid replicated diversification of ecomorphs in different salmonid species is underlined by the use of the same functional regions as has been previously suggested for salinity tolerance . Our results suggest that this is true to some extent with QTLs related to body shape in lake trout and whitefish found in proximity to SNPs that we identified as being associated with phenotypic differences in Arctic charr ecomorphs. Whilst we only identified a small number of QTLs located near the associated SNPs, this is line with other work which suggests that shared basis for ecomorph divergence across species may be limited . This QTL marker database will be a valuable resource for future salmonid research.

Conclusion:

Our results indicate differences in head and body shape responses to ecological selection regimes across four replicate lakes. These differing responses are likely enabled through the use of largely different genetic bases across independent replicate ecomorph pairs. Specifically, we found that only a small number of SNPs were shared across all four pairs, suggesting limited genetic parallelism with these shared SNPs under varying selective pressures across lakes. We found that head and body shape morphology have a level of shared genetic underpinnings in Arctic charr and that the genetics of these phenotypes is shared to an extent across different salmonid species. Our analyses highlight the complexity of the evolutionary genetics that underlie

parallel phenotypes across replicates. Further it we demonstrate the power of using population replicates to resolve fundamental genetic and evolutionary patterns from the noise of local variation.

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References:

Data Accessibility and Benefit-Sharing:

The SNP VCF file, landmark data, and the QTL database are available on the University of Glasgow's data repository, Enlighten (DOI: http://dx.doi.org/10.5525/gla.researchdata.1502 [closed until acceptance]). Benefits from this research accrue from the sharing of our data and results on public databases.

Author Contributions:

KRE and AJ conceived the study with input from CB and CA. Read mapping and landmarking was done by AJ. Data analyses were performed by SF with analytics support from AJ. SF wrote the original draft with support from KRE and all authors contributed to subsequent versions of the manuscript.

Disclosure statement:

The authors report there are no competing interests to declare.

Table 1:

Table 1. Phenotypic trajectory analysis comparisons across ecomorph pairs for head shape and body shape. The difference in trajectory length, the magnitude of change, between ecomorph pair is indicated by ΔL. The angle between trajectories is indicated as ϑ. The significance values are provided for each comparison.

Phenotype	Ecomorph pair comparison	$\Delta\Lambda$ (μαγνιτυδε οφ ςηανγε)	p value	ϑ (διρεςτιον οφ ςηαν
Head shape	Awe-Dughaill	0.0347	0.003	67.51°
	Awe-naSealga	0.0032	0.777	65.74°
	Awe-Tay	0.0901	0.001	57.29°
	Dughaill-naSealga	0.0379	0.002	78.37°
	Dughaill-Tay	0.0554	0.001	100.56°
	naSealga-Tay	0.0933	0.001	47.88°
Body shape	Awe-Dughaill	0.0097	0.004	92.48°
	Awe-naSealga	0.0013	0.726	55.27°
	Awe-Tay	0.028	0.001	39.64°
	Dughaill-naSealga	0.011	0.001	70.65°
	Dughaill-Tay	0.0183	0.001	79.24°

Phenotype	Ecomorph pair comparison	ΔΛ (μαγνιτυδε οφ ςηανγε)	p value	ϑ (διρεςτιον οφ ςηαν
	naSealga-Tay	0.0293	0.001	34.20°

Table 2:

Table 2: Table of associated SNPs found within ±100kbp of salmonid QTLs mapped to the *Salvelinus sp.* genome. Which phenotype the SNP is associated with, its position, and position of QTL marker in question are all indicated. The QTL type and species of origin are indicated. QTL name refers to the designated the QTL was given in the whole QTL database found in Table S1.

SNP phenotype	SNP phenotype	QTL species	QTL marker	QTL type	Chromosome	SNP positi
Head and Body	*C.clupeaformis*	*C.clupeaformis*	Cocl_BS_096	Body shape	NC_036838.1	46764741
Head and Body	*S.namaycush*	*S.namaycush*	Sna_BS_069	Body shape	NW_019942687.1	358354
Head and Body	*S.namaycush*	*S.namaycush*	Sna_BS_069	Body shape	NW_019942687.1	358355
Body	*S.alpinus*	*S.alpinus*	Sal_BW_053	Body weight	NC_036871.1	32030838
Body	*S.namaycush*	*S.namaycush*	Sna_BS_092	Body shape	NC_036854.1	21330886

Figure 1:

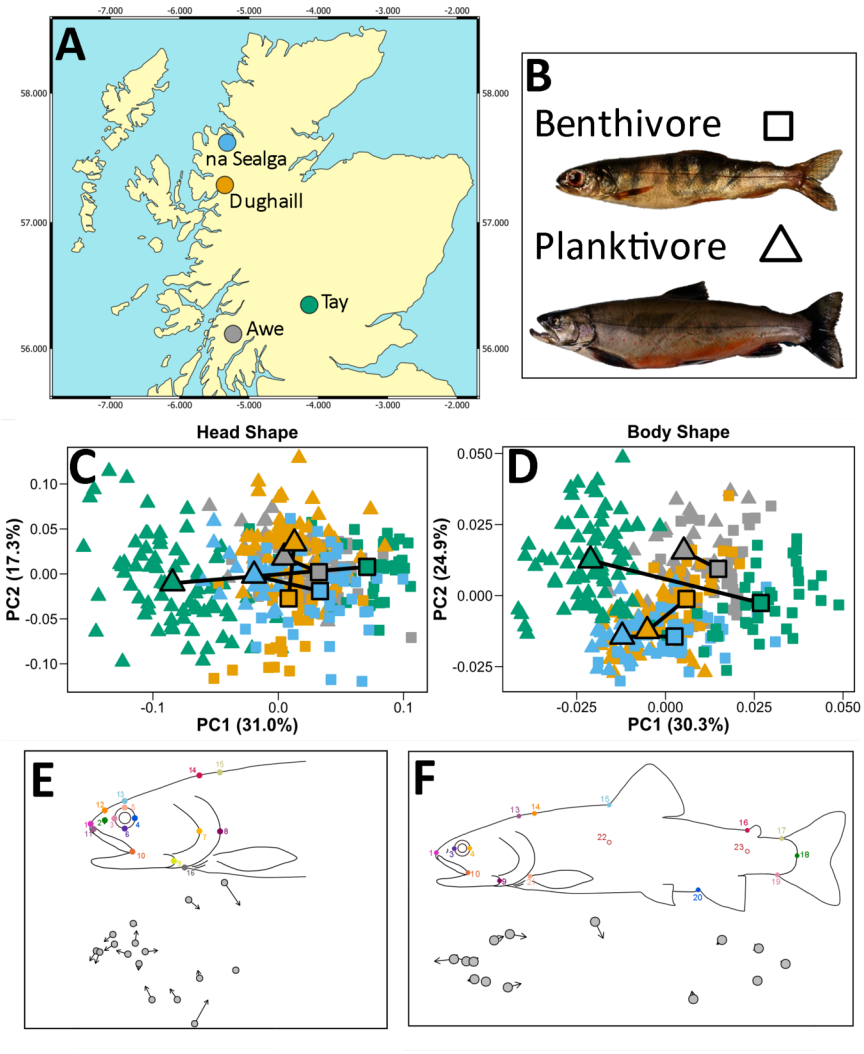

Figure 1. Sample locations and morphological analysis. (A) Map of Scotland showing the sampling locations. (B) Pictures showing an example ecomorph pair, here from Loch Tay. Principal component analysis for landmark analysis for Head shape (C) and Body shape (D). Individuals are coloured by lake with larger points representing the mean values for each ecomorph with a line connecting means in each pair. Vector plots display how head shape (E) and body shape (F) morphology changes across PC1 with diagrams showing where each landmark was placed on the fish for each phenotype. Grey points show landmark positions at the minimum PC1 score and vectors show how landmark positions changes at the maximum PC1 score.

Figure 2:

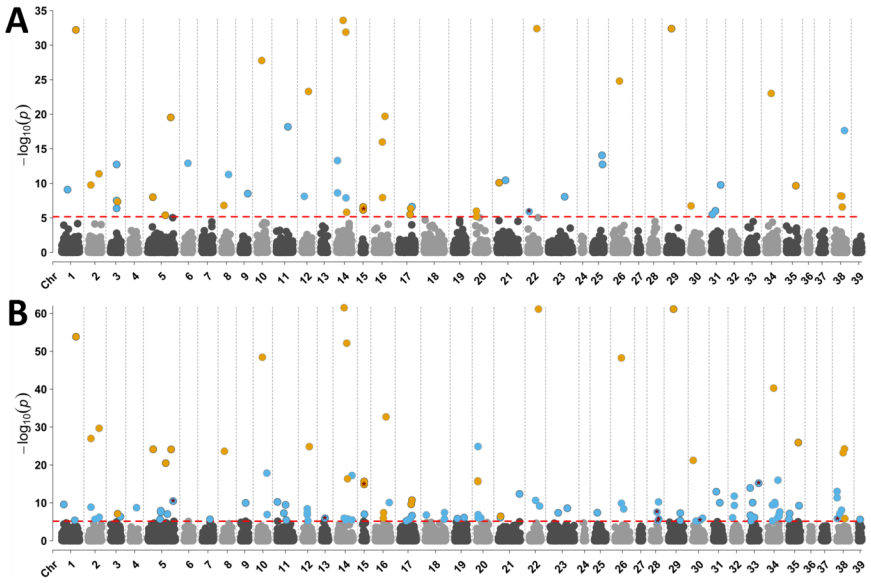

Figure 2: Manhattan plots for genomic location of SNPs highly associated with morphology. A) Shown are 67 SNPs associated with head shape across the four lakes of two ecomorphs of charr. B) Shown are 144 SNPs associated with body shape. SNPs associated with head or body shape are highlighted in blue; SNPs associated with both head shape and body shape are highlighted in orange (38 SNPs). Red asterisk indicate SNPs shared across all four ecomorph pairs for head shape (N=2) and body shape (N=9). SNPs on unanchored scaffolds are not pictured.

Figure 3

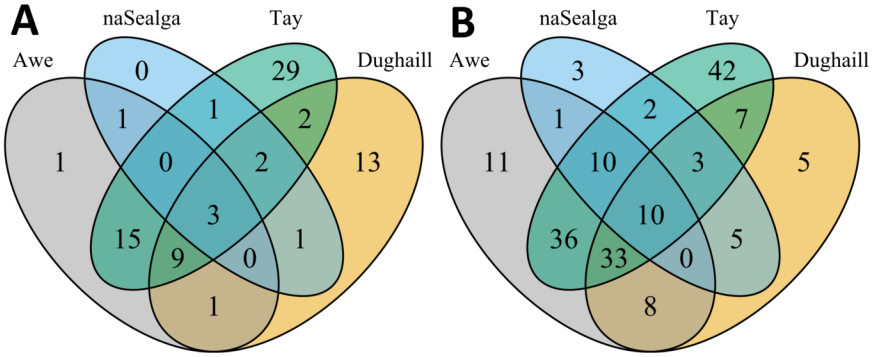

Figure 3: Venn diagram of SNPs associated with head shape (A) and body shape (B) and how they are shared across each combination of different lake pairs.

Figure 4:

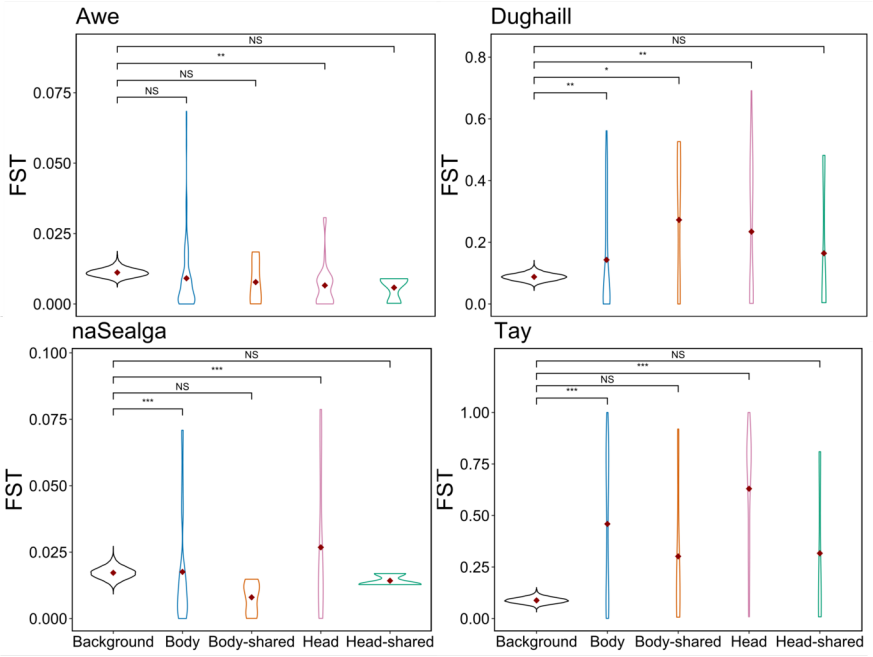

Figure 4: Genetic differentiation (F_{ST}) between ecomorphs at each lake for different associated SNPs datasets. Body refers to all SNPs associated for body shape and Head refers to all SNPs associated with head shape. Body-shared dataset is just the body shape SNPs found in all four ecomorph pairs and Head-shared is the equivalent for head shape SNPs. Background SNPs refers to a randomly selected background subset of SNPs used for comparisons. Red diamonds are the mean value for that dataset. Significance of difference in means is indicated by NS ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Figure 5:

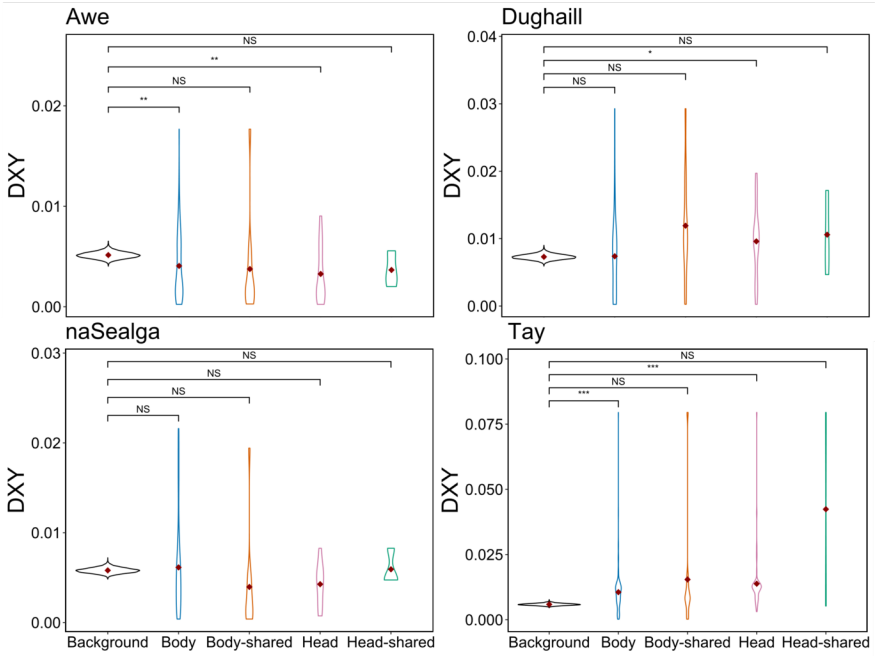

Figure 5: Absolute divergence (D_{XY}) between ecomorphs at each lake for different associated SNPs datasets. Body refers to all SNPs associated for body shape and Head refers to all SNPs associated with head shape. Body-shared dataset is just the body shape SNPs found in all four ecomorph pairs and Head-shared is the equivalent for head shape SNPs. Background SNPs refers to a randomly selected background subset of SNPs used for comparisons. Red diamonds are the mean value for that dataset. Significance of difference in means is indicated by NS ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Figure 6:

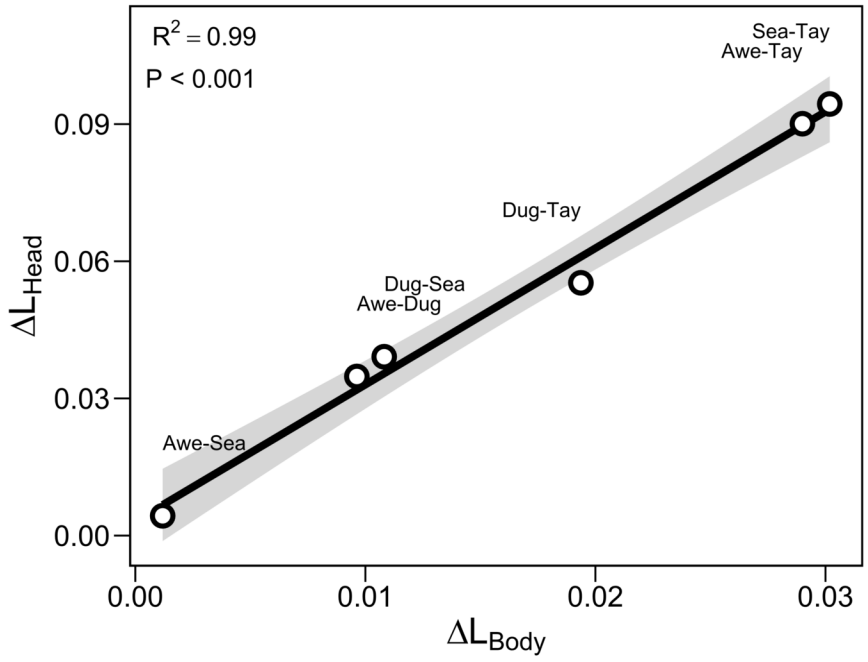

Figure 6. Comparing differences in the magnitude of phenotypic change between ecomorphs for head (ΔLhead) and body shape (ΔL_{Body}) across ecomorph pairs. Each ecomorph pair comparison is indicated on the plot. Dug refers to Dughaill and Sea to na Sealga.