



# Effects of population, family, and diet on craniofacial morphology of Icelandic Arctic charr (*Salvelinus alpinus*)

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We evaluated hypotheses of intralacustrine diversification and plastic responses to two diet environments in Icelandic Arctic charr (*Salvelinus alpinus*). Full-sib families of progeny of wild polymorphic charr from two lakes where morphs vary in their degree of phenotypic and ecological divergence were split, with half of the offspring reared on a benthic and half on a limnetic type of diet to estimate family norms of reaction. We focused on variation in craniofacial traits because they are probably functionally related to diet and complement a previous study of body shape in these charr. A hierarchical analysis of phenotypic variation between lakes, pairs of morphs within each lake, and two families within each morph found that phenotypic variation partitioned between families relative to morphs was reduced in the more ecologically diversified population, which is consistent with adaptive diversification. The effect size of plastic responses between lake populations was similar, suggesting little difference in the degree of canalization in contrast to a previous analysis of body form plasticity. Thus, the role that plastic morphological responses play in the adaptive diversification of morphs and different lake populations of Arctic charr may depend on the trait. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, 108, 702–714.

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## INTRODUCTION

Considerable intraspecific diversity exists as discrete or continuous polymorphisms in populations facing low species-diversity but heterogeneous environments (Skúlason & Smith, 1995; Robinson & Schluter, 2000). The coexistence of divergent phenotypes ecologically specialized for alternate niches can reflect the developmental divergence of individuals through adaptive phenotypic plasticity (West-Eberhard, 1989; Adams & Huntingford, 2004; Schlichting, 2004; Crispo, 2007) or the adaptive genetic divergence of populations

(Pigliucci, Murren & Schlichting, 2006; Crispo, 2007). Phenotypic plasticity may also contribute to adaptive divergence (Ghalambor *et al.*, 2007; Pigliucci, 2010; Thibert-Plante & Hendry, 2011) because it can evolve in polymorphic populations (Adams & Huntingford, 2004; Pigliucci, 2005; Parsons & Robinson, 2006; Svanback & Eklov, 2006; Chevin & Lande, 2011; Michimae & Emura, 2012). In the present study, we evaluated hypotheses about the intralacustrine diversification of polymorphic Icelandic Arctic charr (*Salvelinus alpinus*) using a hierarchical analysis of phenotypic variation between two lakes, pairs of morphs within each lake, and families within each morph, split and reared under different diet treatments.

Adaptive divergence arises as selection sorts genetic variation that underlies functionally useful phenotypic variation into alternate niches. The

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variation providing the raw material for selection initially exists among individuals and families, although additional genetic variation may be exposed through plastic responses to changed environments (Skulason & Smith, 1995; Crispo, 2007). As adaptive divergence progresses, morphs that are increasingly more ecologically and phenotypically specialized should have less variation expressed among families relative to morphs that are less specialized (Robinson & Schluter, 2000). Similarly, genetic variation among families should generally decline in populations as they evolve greater specialization.

Phenotypic plasticity may facilitate adaptive diversification by allowing a population to colonize a new environment and come under selection for a new phenotypic optimum (Ghalambor *et al.*, 2007; Pigliucci, 2010; Fierst, 2011). Adaptation to the new environment may occur via genetic accommodation, first involving the rapid evolution of increased plasticity (reaction norm slope), followed by the evolutionary loss of plasticity compensated by the evolution of reaction norm elevation, and referred to as genetic assimilation (Lande, 2009). Arctic charr morphology is developmentally responsive to rearing conditions (Andersson, 2003; Garduno-Paz & Adams, 2010; Parsons, Skulason & Ferguson, 2010) and so may contribute to the evolution of polymorphism. If Arctic charr polymorphism evolves via genetic accommodation, then more ecologically specialized forms will be more or less plastic than less specialized forms, depending on the stage of the process.

Arctic charr display pronounced levels of morphological variation in head and body shape within and among populations, making them useful for studies of adaptive divergence (Jonsson & Jonsson, 2001). Ancestral anadromous Arctic charr, probably from Europe, colonized Iceland (Brunner *et al.*, 2001) and contemporary lake populations vary in mean phenotype and in the degree of intralacustrine polymorphism. For example, four phenotypically distinct charr subpopulations differ in morphology, diet, and habitat use, and also exhibit modest genetic differentiation in the environmentally heterogeneous lake Thingvallavatn in southern Iceland (Skulason, Noakes & Snorrason, 1989; Malmquist *et al.*, 1992; Sandlund *et al.*, 1992; Kapralova *et al.*, 2011). Two benthic morphs feed mainly on large benthic invertebrates and differ in adult body size, whereas two limnetic morphs include a larger piscivorous form and a smaller zooplanktivorous morph that feeds mainly on crustacean zooplankton and chironomid pupae (Malmquist *et al.*, 1992; Snorrason *et al.*, 1994). By contrast, relatively low genetic, morphological, and reduced ecological diversity occurs between a pair of charr forms from Vatnshlidarvatn (Gislason *et al.*, 1999; Jonsson & Skulason, 2000; Wilson *et al.*, 2004)

in northern Iceland, which has a shallow and mostly uniform muddy bottom. Nonetheless, these two charr morphs diverge in life-history and ecology at 2 years of age (Jonsson & Skulason, 2000), with a brown morph feeding mainly on the water flea *Eurycerus* sp. and a silver morph feeding on a wider array of invertebrate prey (Jonsson & Skulason, 2000), except in late summer when *Eurycerus* sp. is superabundant. Thus, polymorphism differs between these two populations, both in the number of sympatric morphs and their degree of ecological, phenotypic, and genetic divergence.

In a previous study of these Arctic charr populations, Parsons *et al.* (2010) not only found developmental plasticity in external body shape in response to diet, but also that the magnitude of responses differed among morphs, demonstrating the evolutionary divergence of plasticity. The more ecologically specialized large benthic morph from Thingvallavatn and the brown morph from Vatnshlidarvatn (both with narrower diet breadths) exhibited more canalized (less plastic) development of external body form in response to diet than their respective less ecologically specialized morphs. Thus, some Thingvallavatn charr forms have evolved greater ecological and phenotypic specialization along with more canalized development than other charr (Parsons *et al.*, 2010, 2011). Furthermore, variation in external body form among morphs within lakes exceeded variation induced by plastic developmental responses to diet (Parsons *et al.*, 2010, 2011), suggesting that certain morphs in each population may have genetically assimilated plastic developmental responses of whole body form (Lande, 2009). However, the proximal causes of variation in external body features are unclear. Focusing on variation in osteological traits may clarify whether plastic developmental responses in body form involve soft tissues or both soft and hard tissues.

In the present study, we analyzed variation in the internal craniofacial traits of cleared and stained skulls of fish from families of laboratory reared morphs of charr used in a previous analysis of external body form by Parsons *et al.* (Parsons *et al.*, 2010; 2011). Our focus on skull morphology arises because it is probably functionally related to diet and so extends those previous studies. If diversifying selection drives the evolution of polymorphism in Arctic charr, then the more diversified Thingvallavatn population should exhibit less variation among families within morphs compared to the less diversified Vatnshlidarvatn population. If polymorphism evolves via genetic assimilation, then the more ecologically specialized and divergent charr from Thingvallavatn should have reduced plastic responses to diet than charr from Vatnshlidarvatn. Similarly, at a finer scale

within lakes, the ecologically more specialized large benthic morph in Thingvallavatn and the more diet-specialized brown morph in Vatnshlidarvatn will express less phenotypic plasticity than the less derived planktivorous morph (Thingvallavatn) and the less ecologically specialized silver morph (Vatnshlidarvatn) respectively.

## MATERIAL AND METHODS

A total of 570 fish were analyzed and are the F<sub>1</sub> progeny of wild-caught Arctic charr collected in 2003 from the lakes Thingvallavatn and Vatnshlidarvatn in Iceland. We focus on the silver (VS) and brown (VB) morphs from Vatnshlidarvatn, and planktivorous (TPL) and large benthic (TLB) morphs from Thingvallavatn (the small benthic and piscivorous morph from Thingvallavatn were unavailable because of low fertility and rarity respectively). Descriptions of collection and rearing are reported previously (Parsons *et al.*, 2010). In short, replicate full-sib families were created within each morph and sibs from each family were randomly allocated between two exclusive diet treatments that mimicked the spatial placement of either benthic or limnetic prey without varying nutritional content (Robinson & Wilson, 1995) because nutritional changes can induce shape changes (Wimberger, 1993). The 'benthic' diet treatment was characterized by larger frozen food particles delivered at the bottom necessitating downward feeding by fish against a surface, whereas the 'limnetic' diet treatment was finely ground and dropped onto the water surface where it suspended in the water column. Thus, developmental responses to diet treatment are probably more related to biomechanical aspects of food capture rather than prey processing. Developmental responses represent a mean 'family' rather than 'genotypic' reaction norm because they were assessed between full-sibs. Fish were reared on diet treatments for 160 days after which they were euthanized with phenoxyethanol (0.5 mL L<sup>-1</sup>), fixed in Bouin's solution and stored in 75% ethanol. Two families were analyzed per morph (Table 1), although human error made available only one TLB family reared on the benthic diet.

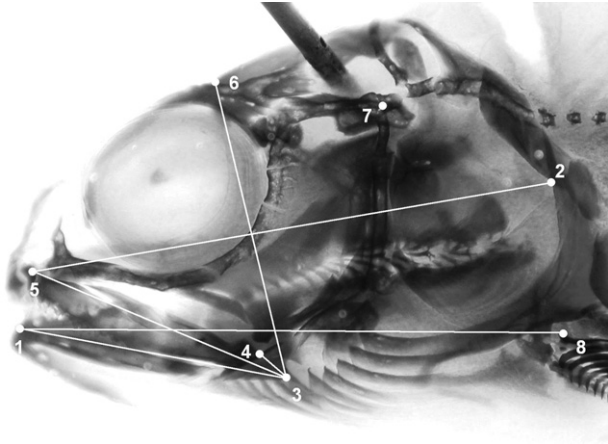
### CLEARING AND STAINING

Heads were separated from the body at the posterior of the occipital crest and sequentially rehydrated from 70% ethanol to 100% distilled water in two steps (placed for 24 h in 35% ethanol/65% distilled water, transferred for 24 h to distilled water, then washed in distilled water). Specimens were cleared and stained under a protocol modified from Klymkowsky & Hanken (1991). Specimens were bleached for 4 h in

**Table 1.** Experimental design, morph acronyms, and sample sizes of full sib families, each split into diet treatment (benthic or limnetic diets), within families of four morphs of Arctic charr from two Icelandic lakes: Thingvallavatn and Vatnshlidarvatn

Lake	Thingvallavatn				Vatnshlidarvatn			
	Large Benthic		Planktivorous		Silver		Brown	
Morph	TLB1	TLB2	TP1	TP2	VS1	VS2	VB1	VB2
Diet	Lim	Ben	Lim	Ben	Lim	Ben	Lim	Ben
N <sub>total</sub>	38	34	23	45	39	44	43	37
Length (cm)	6.2 ± 0.3	7.2 ± 0.9	6.4 ± 0.4	6.6 ± 0.3	5.0 ± 0.7	6.0 ± 1.2	5.8 ± 0.5	6.8 ± 1.0

Mean ± SD fork lengths at 160 days of age are given for each family and diet treatment (in cm). TLB, large benthic morph from Thingvallavatn; TP, planktivorous morph from Thingvallavatn; VS, silver morph from Vatnshlidarvatn; VB, brown morph from Vatnshlidarvatn; Lim, limnetic; Ben, benthic.

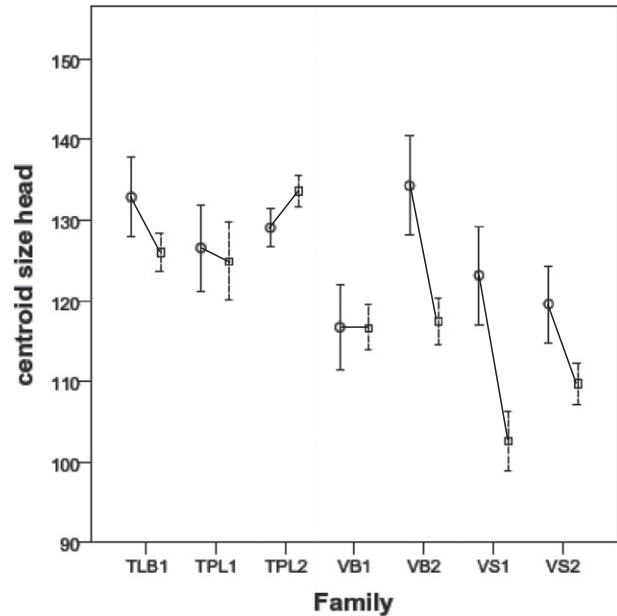


**Figure 1.** Landmarks recorded on the right side of head of Icelandic Arctic charr (*Salvelinus alpinus*) [1, anterior tip dentary; 2, pectoral girdle; postcleithrum/supracleithrum; 3, posterior end articular; 4, upper end joint articular/quadrates; 5, anterior tip premaxilla; 6, supraorbital#2/sphenotic; 7, pterotic, posterior point, connection to post-temporal; 8, pectoral fin proximal; landmark descriptions follow those of salmon skulls *sensu* Gregory (1959) and Rojo (1991)]. Linear measurements taken between landmark pairs are shown as white lines (UHL: LM2 – LM5 upper head length, LHL: LM1 – LM8 lower head length, HD: LM3 – LM6 head depth, LJL: LM1 – LM3 lower jaw length (ventral side of dentary and articular), UJL: LM3 – LM5 upper jaw length, AH: LM3 – LM4 articular height).

four parts 3% H<sub>2</sub>O<sub>2</sub> and six parts 1% KOH, rinsed twice with H<sub>2</sub>O, and then transferred overnight into 0.5% KOH. Specimens were then immersed in an Alizarin red solution (in 0.5% KOH) for 4 h; rinsed twice with H<sub>2</sub>O and transferred overnight to 0.5% KOH. The destaining and clearing process was monitored over the next 1–2 days and the KOH solution changed as necessary until sufficient clearing. Specimens were transferred to 100% glycerol by increasing the glycerol content by 25% per day. Digital photographs were taken of the right side of each head pinned to bees wax under glycerol and including a scale bar (96 × 96 dpi; Nikon coolpix 4500; no image distortion was detected using vertical and horizontal grids). A common head orientation was ensured by pinning through the anterior of the pectoral fin and the uncalcified portion of the neurocranium (Fig. 1).

#### MORPHOMETRIC DATA AND ANALYSIS

Eight homologous landmarks (Fig. 1) were digitized from the image of each head using TPSDIG2 (Rohlf, 2009). Variation in landmark coordinates among individuals as a result of size, position and orientation was removed by the generalized least squares



**Figure 2.** Mean  $\pm$  SEM and norms of reaction of centroid head size (vertical axis) to diet treatment for each family and morph of Arctic charr. The horizontal axis reflects morph categories for Thingvallavatn (TLB, large benthic; TPL, planktivorous) and Vatnshlidarvatn (VB, brown morph; VS, silver morph), followed by a family number (e.g. 'VB1' and 'VB2' refer to Vatnshlidarvatn brown morph families 1 and 2, respectively). The full-sib progeny of each family were divided, with half reared for 160 days exclusively on a benthic (pictured first with solid error bars) and half on a limnetic diet (pictured second with dotted error bars).

Procrustes superimposition method in CoordGen6 [Integrated Morphometrics Program (IMP) suite; <http://www3.canisius.edu/~sheets/moremorph.html>]. Landmark 1 was excluded as a result of variation in lower jaw position. The remaining variation was attributed to shape differences among individuals. The centroid size (CS) of each head was calculated as the square root of the sum of the squared distances of landmarks 2–8 from the centroid, and mean family centroids were plotted (Fig. 2). Repeatability of landmark placement was verified by re-digitizing 50 specimens and performing discriminant function analysis using replicate sets of landmarks.

To evaluate shape variation in specific head features, six linear distances were calculated between landmark pairs for each specimen from the subset of size and position-corrected landmarks above using tmmorphgen6 from the IMP series (for landmark description, see Fig. 1). Upper jaw length was measured from the anterior tip of the premaxilla (LM1) to the ventroposterior end of the articular (LM3) rather than the posterior end of the maxilla because this was

hidden behind the articular. Variation in length measures and centroid size were tested for normality and heterogeneity of variance using Shapiro–Wilk and Levene’s tests respectively.

We evaluated variation in trait allometry using an analysis of covariance (ANCOVA) model with centroid size as covariate for each of the six measurements, treating lake, morph, and family as fixed factors in three separate analyses for each of the six linear measurements. The absence of a statistically significant interaction between head centroid size and a fixed factor was interpreted as similarity in trait allometry among the fixed factor levels (correcting for the dependence among three analyses of each trait using a Bonferroni significance threshold of  $P = 0.017$ ). We then removed most of the remaining variation in size, by linearly regressing each of the six craniofacial measurements against centroid size and estimating residual variation for each individual (combining fish from all factors).

Residual linear measurements were used as dependent variables in a full multivariate analysis of variance (MANOVA) model that included lake as a fixed effect, family and morph as random effects (sequentially nested in lake), and diet as an among-individual fixed effect (Table 1). Factors having a significant effect on multivariate head shape were then evaluated by univariate ANOVA models of each residual linear trait (between-subject Tukey’s tests identified differences between factor levels). The relative effect size of each factor in the full multivariate and each univariate model were estimated as the proportion of explained variance for the model (PPV: the sum of squares for each factor divided by total sum of squares).

The large effect of lake on head shape may influence estimates of phenotype at lower levels (morphs, families, and diet treatments), and so we explored variation as a result of morphs and diet treatments in separate MANOVA models for Thingvallavatn and Vatnshlidarvatn, treating morph as a fixed effect, with family and diet treated as above. Relative PPV was estimated for each lake-specific model and compared between lakes after adjusting the total sum of squares by dividing by degrees of freedom for each model (Kaps & Lamberson, 2009). Unfortunately, family TLB2 reared on the benthic diet was absent from Thingvallavatn and so family and morph effects could not be distinguished for the large benthic morph (Table 1). Therefore, we subdivided the Thingvallavatn dataset into separate benthic and a limnetic diet datasets and performed separate MANOVAs on each diet group of Thingvallavatn, treating morph as a fixed factor and family nested within morph. As a result, family TLB2 was included in the analysis of limnetic but not of the benthic diet. All statistical

analyses were performed in PASW STATISTIC 18 (SPSS Inc.).

## RESULTS

Discriminant analysis could not distinguish between repeated sets of landmarks indicating reliable landmark placement. Linear craniofacial measurements and centroid size were normally distributed, although their variance was unequal among families combined across morphs and lakes [ $F$ -value and  $P$ -values of Levene’s test of equality of error variances for each trait: lower head length (LHL):  $F = 2.0$ ,  $P = 0.02$ , upper head length (UHL):  $F = 2.3$ ,  $P = 0.007$ , head depth (HD):  $F = 3.1$ ,  $P < 0.001$ , upper jaw length (UJL):  $F = 4.8$ ,  $P < 0.001$ , lower jaw length (LJL):  $F = 4.0$ ,  $P < 0.001$ , articular height (AH):  $F = 2.9$ ,  $P = 0.001$ , centroid size (CS):  $F = 8.8$ ,  $P < 0.001$ ]. The greatest ratio in variance of any linear measure between families was 1 : 11 for LHL. We proceed with our analysis below because the MANOVA  $F$ -statistic is robust to violations of up to one order of magnitude differences in variance among comparison groups (Lindman, 1974), recognizing some potential loss of statistical efficiency.

### DEPENDENCE OF CRANIOFACIAL VARIATION ON HEAD SIZE

The six cranial–facial traits varied significantly among families, morphs and populations, although the allometric relationship with head size differed among factor levels for only two traits. UJL and LJL Allometry varied significantly among lakes and morphs (ANCOVA head size  $\times$  factor interactions for LJL and UJL, all  $P < 0.005$ ; see Supporting information, Table S1). The remaining four craniofacial lengths all co-varied with head size in a similar way among all factor levels (remaining size  $\times$  factor interactions for UHL, LHL, HD and AH, all  $P > 0.06$ ). The adjusted mean values of these four craniofacial traits almost always varied significantly among lakes, morphs, and families (except for the effect of lake on the AH trait; see Supporting information, Table S1). This reflected differences in mean head size between Thingvallavatn and Vatnshlidarvatn (Table 1) because separate ANCOVA analyses of each lake population found little variation between morphs or families in each population for these four traits.

### DEPENDENCE OF CRANIOFACIAL VARIATION ON LAKE ORIGIN, FAMILY AND DIET (LAKE POPULATIONS COMBINED)

The six craniofacial traits (size corrected) responded to diet treatment (multivariate Diet Factor,  $P < 0.001$ ;

Fig. 2, Table 2), although univariate between subject tests found evidence of Diet effects only for UHL and HD. The diet main effect explained little of the variation in the six traits (all a percentage of phenotypic variation, PPV < 2.5%), although this ignored additional diet effects that differed between lakes. There was considerable variation among morphometric norms of reaction between charr from Thingvallavatn and Vatnshlidarvatn, particularly for LHL, UJL and AH (lake  $\times$  diet interactions, all  $P < 0.016$ ; Table 2). The greatest variation in reaction norms between lakes arose from the response of LHL which accounted for almost one-third (7.5%) of the total percentage (25.0%) of phenotypic variation explained by all factors for this trait (Table 2). We evaluate phenotypic plasticity between morphs within each lake in more detail in the lake-specific analyses below.

The main effects of lake and family that did not developmentally respond to diet treatment explained the most variation in craniofacial form. Lake tended to account for more variation in each trait than family, ranging from 6.8% for UHL to 28.4% for UJL (Fig. 3, Table 2). The means of each trait, except for LHL, were smaller in morphs from Thingvallavatn compared to the morphs from Vatnshlidarvatn and also varied significantly among families (Table 2). The proportion of total phenotypic variation accounted for by family ranged from 6.3% for UHL to 17.3% for AH and were generally less than that explained by lake effects (Table 2).

DEPENDENCE OF CRANIOFACIAL VARIATION  
ON MORPH, FAMILY AND DIET:  
LAKE POPULATIONS SEPARATED

Separate MANOVA models applied to Thingvallavatn or Vatnshlidarvatn charr accounted for similar amounts of total explained variation in craniofacial traits after adjusting for total degrees of freedom (Table 3). Total explained variance differed between lakes for only two traits (Table 3): HD in Thingvallavatn (5.7%) was half again as large as in Vatnshlidarvatn (3.9%), whereas LjL in Vatnshlidarvatn (11.5%) was almost half again as large as in Thingvallavatn (8.1%).

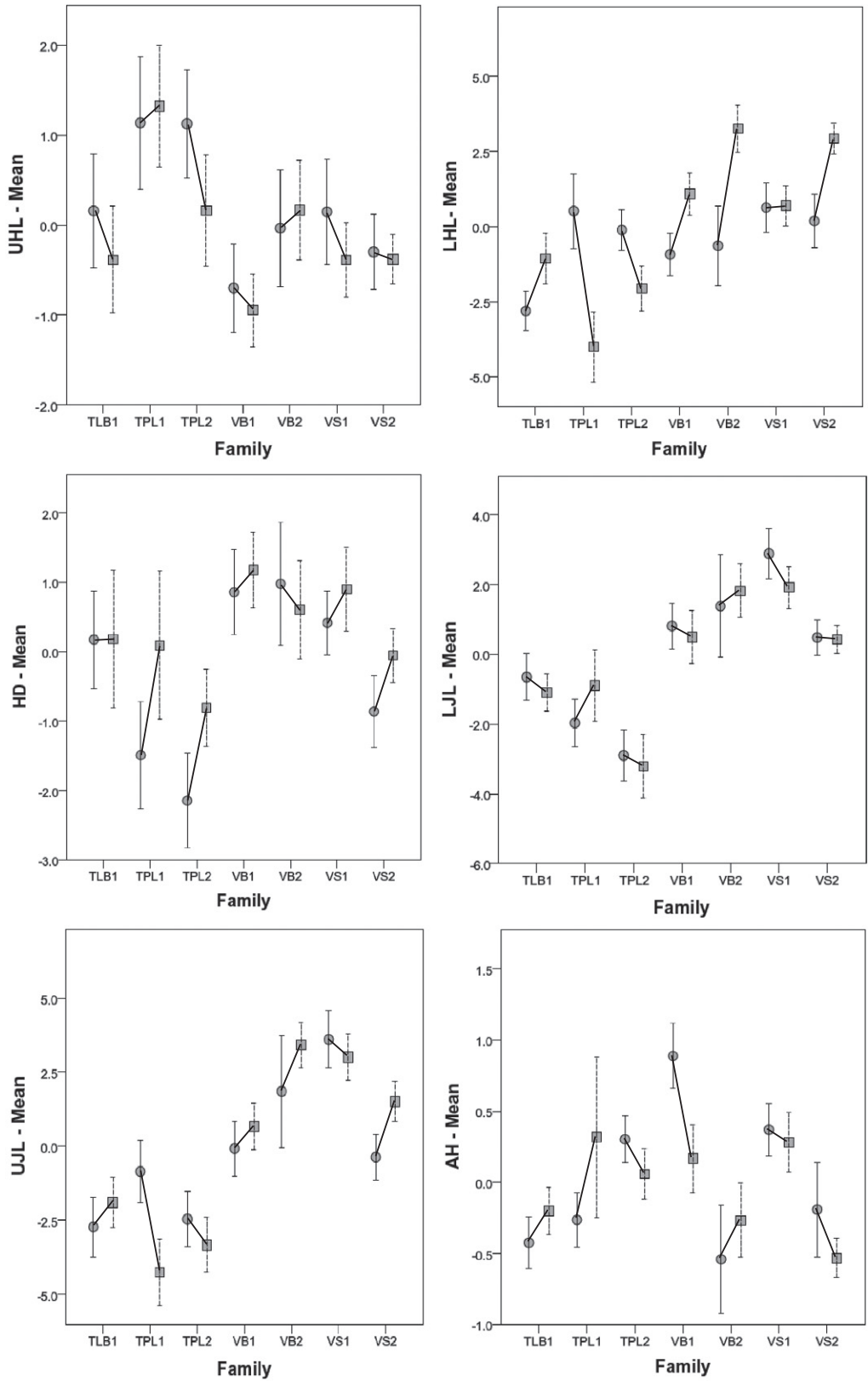
In each lake, diet affected multivariate variation in craniofacial traits (both Diet  $P < 0.03$ ; Table 3) and this was supported by univariate diet effects on LHL, HD, UJL, and AH in Vatnshlidarvatn, and UHL, LHL, and HD in Thingvallavatn. However, the main effect size of Diet was small for most traits (0–2.6%) relative to other factors (morph and family; see below), with the exception of LHL in charr from Vatnshlidarvatn, where the limnetic diet induced strong positive responses compared to the benthic diet (effect size of 12.9%). There was also some evidence that the PL families reared on the benthic diet developed deeper heads (HD) than those reared on the limnetic diet (Fig. 2).

As with the between-lake comparisons noted above, a variety of complex diet effects were revealed

**Table 2.** Results of the full multivariate analysis of variance of six size-corrected linear measurements (for trait descriptions, see Fig. 1) where family is nested within morph nested within lake, and diet is an among-individual effect

Factors		Multivariate	UHL	LHL	HD	LJL	UJL	AH
Lake	<i>F</i> (df)	74.3 (6, 529)	41.9 (1, 534)	105.6	45.6	210.6	243.4	1.1
	<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.289
	PPV		6.8	14.6	7.0	25.3	28.4	0.2
Family [Morph (Lake)]	<i>F</i> (df)	12.0 (30, 2118)	7.8 (5, 534)	3.8	12.2	14.2	12.6	22.7
	<i>P</i>	< 0.001	< 0.001	0.111	< 0.001	< 0.001	< 0.001	< 0.001
	PPV		6.3	2.6	9.2	8.5	7.3	17.3
Diet	<i>F</i> (df)	9.1 (6, 529)	5.3 (1, 534)	2.0	15.0	0.1	0.0	0.9
	<i>P</i>	< 0.001	0.022	0.156	< 0.001	0.793	0.932	0.343
	PPV		0.9	0.3	2.3	0.0	0.0	0.1
Diet $\times$ Lake	<i>F</i> (df)	14.9 (6, 529)	2.2 (1, 534)	54.0	2.8	0.3	12.9	5.8
	<i>P</i>	< 0.001	0.137	< 0.001	0.093	0.61	< 0.001	0.016
	PPV		0.4	7.5	0.4	0.0	1.5	0.9
PPV total (explained)			14.4	25.0	18.9	33.8	37.2	18.5

The complete dataset includes two morphs in each of two lakes: Thingvallavatn (large benthic and planktivorous) and Vatnshlidarvatn (silver and brown morph). Percentages of explained phenotypic variation (PPV) for each factor and totalled over all factors are estimated under each trait. Degrees of freedom provided for the univariate *F*-tests under UHL apply for all other traits. UHL, upper head length; LHL, lower head length; HD, head depth; LJL, lower jaw length; UJL, upper jaw length; AH, articular height.



**Figure 3.** Mean  $\pm$  SEM and norms of reaction of six linear measures to diet treatment for each family grouped inside morphs of Arctic charr. The vertical axis reflects residual variation in mean linear trait length for each of six traits: (UHL, upper head length; LHL, lower head length; HD, head depth; LJJ, lower jaw length; UJJ, upper jaw length; AH, articular height; descriptions in Fig. 1). Mean residuals of each measurement are shown, as estimated from linear regressions against head size. The horizontal axis and diet treatments are as described in Figure 2. TLB, Thingvallavatn large benthic; TPL, Thingvallavatn planktivorous; VB, Vatnshlidarvatn brown morph; VS, Vatnshlidarvatn silver morph.

**Table 3.** Multivariate analysis of variance on each lake population for six size-corrected linear measurements with factors: Family (nested within Morph) and Diet, plus their interaction

Factors		Multivariate	UHL	LHL	HD	LJJ	UJJ	AH
Vatnshlidarvatn								
Morph	<i>F</i> (df)	6.7 (6, 321)	0.3 (1, 331)	3.2	17.5	0.8	1.4	1.0
	<i>P</i>	< 0.001	0.561	0.077	< 0.001	0.385	0.238	0.312
	PPV		0.1	0.8	4.8	0.2	0.4	0.3
Family (Morph)	<i>F</i> (df)	14.1 (12, 642)	7.6 (2, 331)	5.9	6.5	17.4	27.4	40.8
	<i>P</i>	< 0.001	0.001	0.003	0.002	< 0.001	< 0.001	< 0.001
	PPV		4.4	3.0	3.6	9.6	14.0	19.6
Diet	<i>F</i> (df)	23.8 (6, 321)	0.5 (1, 331)	51.5	3.4	0.3	8.1	6.3
	<i>P</i>	< 0.001	0.493	< 0.001	0.068	0.564	0.005	0.013
	PPV		0.1	12.9	0.9	0.1	2.1	1.5
Diet $\times$ Morph	<i>F</i> (df)	2.1 (6, 321)	0.1 (1, 331)	7.6	2.4	0.3	0.2	0.1
	<i>P</i>	< 0.001	0.772	0.006	0.125	0.591	0.661	0.808
	PPV		0.0	1.9	0.7	0.1	0.1	0.0
PPV total		4.6	18.6	18.6	10.0	10.0	16.6	21.4
SS total/df		2.47	8.38	8.38	3.89	6.47	11.47	0.85
Thingvallavatn								
Morph	<i>F</i> (df)	14.7 (6, 201)	20.3 (1, 206)	3.8	22.4	18.7	1.1	18.0
	<i>P</i>	< 0.001	< 0.001	0.077	< 0.001	< 0.001	0.299	< 0.001
	PPV		8.7	1.4	9.0	7.6	0.5	7.9
Family (Morph)	<i>F</i> (df)	6.2 (6, 201)	2.4 (1, 206)	1.1	3.5	14.4	0.5	1.8
	<i>P</i>	< 0.001	0.126	0.297	0.064	< 0.001	0.486	0.184
	PPV		1.0	0.4	1.4	5.9	0.2	0.8
Diet	<i>F</i> (df)	2.3 (6, 201)	5.0 (1, 206)	3.6	6.5	0.1	1.5	1.6
	<i>P</i>	0.033	0.027	0.059	0.011	0.826	0.226	0.206
	PPV		2.1	1.3	2.6	0.0	0.7	0.7
Diet $\times$ Morph	<i>F</i> (df)	10.6 (6, 201)	0.2 (1, 206)	42.5	2.9	1.4	12.5	0.6
	<i>P</i>	< 0.001	0.688	< 0.001	0.093	0.23	0.001	0.443
	PPV		0.1	15.7	1.2	0.6	5.5	0.3
PPV total		11.9	18.8	18.8	14.2	14.1	6.9	9.7
SS total/df		3.58	7.50	7.50	5.67	5.36	8.08	0.47

Arctic charr from Vatnshlidarvatn (silver and brown morphs) or Thingvallavatn (planktivorous and large benthic morph) are analyzed separately. Degrees of freedom provided for the univariate *F*-tests under UHL apply for all other traits. Percentage of explained phenotypic variation (PPV) for each factor and trait combination, as well as totalled over factors for each trait (expressed as the total sum of squares) standardized by degrees of freedom enables a comparison between lakes. UHL, upper head length; LHL, lower head length; HD, head depth; LJJ, lower jaw length; UJJ, upper jaw length; AH, articular height.

through interactions with morph. Morphs differed in their reaction norms to diet for LHL in each lake population (diet  $\times$  morph interaction;  $P < 0.006$  in both lakes), although the interaction effect size was larger in Thingvallavatn (15.7%) compared to Vatnshlidarvatn (1.9%). In charr from Thingvallavatn, the

benthic diet treatment shortened the LHL in the TPL morph compared to those reared on the limnetic diet, whereas this pattern was reversed for the TLB morph (Fig. 3). In Vatnshlidarvatn charr, the interaction between diet and morph for LHL arose because one family of the VS morph showed no response to diet

compared to the other six families where mean LHL was shortened in fish reared under the benthic compared to limnetic diet (Table 3). Developmental responses by UJL also varied between morphs in Thingvallavatn (but not in Vatnshlidarvatn) with a moderate effect size of 5.5% because members of one family of the TPL morph developed longer upper jaws on the benthic diet compared to the limnetic diet, which is opposite to the responses to these diets by two other families (Table 3).

The relative importance of the family and morph factors reversed between the two lake populations. In charr from Thingvallavatn, a greater proportion of the variation in craniofacial traits was partitioned between morphs compared to among families nested within morphs (Table 3), whereas, in Vatnshlidarvatn, the morph effect accounted for less variation than the family effect (Table 3). We also observed that the benthic charr morphs were more phenotypically specialized within each lake (TLB and VB) because they exhibited deeper heads compared to their alternate local morph (respectively, TPL and VS; Fig. 3).

## DISCUSSION

We evaluated the plasticity of craniofacial traits to benthic- and pelagic-like diet treatments in two populations of polymorphic Arctic charr from Iceland that vary in phenotypic and ecological diversity. Craniofacial traits respond to dietary cues and genetic variation in diet responses ( $G \times E$ ) exists among families, morphs, and lake populations. By contrast to the hypothesis of genetic assimilation, we found no evidence that plastic responses to diet declined as morphs or lake populations exhibited greater ecological or phenotypic specialization, indicating that ecological specialization is not consistently related to greater developmental canalization of these six craniofacial traits. Considerable variation in head morphology exists among families in both lake populations that may be available to selection. The interactions between diet treatment and family, morph, and population complicate simple interpretations of head shape diversification between morphs and populations of Arctic charr in Iceland.

As Arctic charr adaptively diverge among lake habitats and resources, genetic variation initially partitioned among families should be converted into variation between different morphs. Our results are generally consistent with adaptive diversification driving polymorphism. Families of both the brown and silver morphs from Vatnshlidarvatn were generally more morphologically distinct within morphs, as shown by a large family effect relative to the

small morph effect. Conversely, in Thingvallavatn, the planktivorous and large benthic morph were more distinct than families within morphs. Our results would be strengthened by greater replication at a number of different levels, including families within morphs, additional morphs from Thingvallavatn (small benthic and piscivorous morphs), and, ideally, more polymorphic lake populations from Iceland and elsewhere. Nonetheless, our results are consistent with other evolutionary ecological studies of Arctic charr in Thingvallavatn and Vatnshlidarvatn that indicate adaptive polymorphism (Sandlund *et al.*, 1992; Jonsson & Skulason, 2000).

Craniofacial traits of Arctic charr progeny respond to relatively simple spatial cues involving positioning larger food particles on the bottom versus in the water column as found elsewhere (Robinson & Wilson, 1995; Parsons *et al.*, 2010, 2011). The planktivorous morph from Thingvallavatn had thinner, more elongated heads compared to the sympatric large benthic morphs, which is consistent with other studies of head form in Thingvallavatn charr (Skulason *et al.*, 1989; Snorrason *et al.*, 1994), as well as in other Arctic charr populations (Johnson, 1980; Jonsson & Jonsson, 2001; Klemetsen *et al.*, 2003; Knudsen *et al.*, 2007). Rearing of TPL morphs on a benthic-like diet induced deeper heads than rearing on a limnetic diet, driving the TPL charr reared on the benthic diet to resemble more the TLB morph. The congruence of the plastic response to diet in the TPL morph with the phenotypic differences between TLB and TPL morphs suggests that plastic responses may contribute to their evolutionary divergence (Day, Pritchard & Schluter, 1994; Losos *et al.*, 2000; West-Eberhard, 2003). However, various uncertainties remain with respect to craniofacial plasticity in these charr. First, we do not know whether the cue for developmental responses reflects subtle differences in swimming behaviour, feeding behaviour, or both, because both may change with feeding position. Detailed studies of swimming and feeding behaviours at slow speed may reveal which behaviours change the most between different feeding orientations (Higham, Day & Wainwright, 2006). Second, the position, texture, and size diet treatments may induce less developmental responses than in nature where many more cues are available, such as the prey type and nutritional value that may also affect development (Wimberger, 1993). If this is the case, then our diet location treatments may underestimate developmental responses to diet differences in nature. In addition, we do not know whether any aspect of Arctic charr morphology responds to predation cues as in other fish (Bronmark & Miner, 1992). Third, developmental responses may be influenced by maternal effects, as found in other Arctic charr (Skulason *et al.*,

1989; Pakkasmaa, Penttinen & Piironen, 2006; Janhunen *et al.*, 2011). Maternal effects on morph differentiation would then represent a transgeneration form of plastic responses (Mousseau & Fox, 1998). Controlling for maternal effects requires rearing parents under common conditions. Nonetheless, the present study demonstrates that hard skeletal tissues can respond to simple diet cues in these charr, resolving one uncertainty about the proximal cause of plastic responses in prior studies of these same populations (Parsons *et al.*, 2010, 2011).

By contrast to predictions, we found no difference in the magnitude of plastic responses of craniofacial traits between the more specialized Thingvallavatn (Jonsson *et al.*, 1988; Sandlund *et al.*, 1992) and the less specialized Vatnshlidarvatn charr morphs, nor differences in plasticity between morphs within either lake population. Thus, there is no evidence that the genetic assimilation of skeletal head form differs among these Arctic charr. This is in contrast to differences in the developmental responses to diet cues of the external whole body form, which was more developmentally canalized in the more ecologically specialized morphs in these same fish (Parsons *et al.*, 2011). It is possible that hard and soft tissues in the head of Arctic charr respond to diet in different ways than that of external body form. Alternatively, selection on juvenile head morphology may differ from that acting on overall body form and so favour different levels of plasticity between these traits (Pigliucci *et al.*, 2006). Age-0 charr of the planktivorous morph in Thingvallavatn move from the littoral to the deeper open water pelagic habitat (Sandlund *et al.*, 1988), suggesting that selection could favour more canalized body form traits functionally related to caudal swimming at an earlier ontogenetic stage than head shape traits in planktivorous charr. A better understanding of habitat use and diet of juvenile charr from both populations may clarify how selection could shape these traits.

We also found evidence of genetic variation in the plastic developmental responses to diet cues among charr morphs, especially for the horizontal position of the pectoral fin (LHL). Variation in LHL is consistent with a number of functional hypotheses. The position, size, and form of the pectoral fin are variable within and among Arctic charr populations (Snorrason *et al.*, 1994), possibly because this functionally influences manoeuvrability (Westneat *et al.*, 2004). However, a longer LHL may also be correlated with greater buccal volume, facilitating feeding on larger and harder macroinvertebrate prey (Wainwright & Richard, 1995; Mittelbach, Osenberg & Wainwright, 1999) or generating greater suction force for planktivorous feeding (Wainwright *et al.*, 2004). Thus, the greater response to diet by LHL relative to the five

other craniofacial traits may be related to variation in diet or swimming conditions.

The development of head form is a complex process in fish because the proportions of many features change markedly over ontogeny (Gregory, 1959). Thus, the evolution of divergent morphology generally involves evolutionary changes in trait allometries (Shingleton *et al.*, 2009). In the present study, variation in UHL and LHL, HD, and AH exists between lake populations, although this was a result of a smaller head size in fish from Thingvallavatn compared to Vatnshlidarvatn. Variation in head proportions between lakes controlling for size could reflect genetic variation in allometry because Arctic charr display strong family effects in growth and maturation schedules (Küttner *et al.*, 2011). Significant interactions of size with morph and family effects were present for both the upper and lower jaw length, indicating that, as fish grew, their jaws did not grow in the same proportions across families, morphs or lakes. Similar variation in the allometry of upper and lower jaw lengths occurs among all four Thingvallavatn morphs (Snorrason *et al.*, 1994). Thus, our results confirm that jaw allometry has diverged among morphs in the Thingvallavatn population and between charr from Thingvallavatn and Vatnshlidarvatn. Selection may be stronger on jaw length than other head traits because it may functionally reflect a primary ecological axis of divergence among charr, or jaw length may be more evolvable than the other craniofacial traits. Variation in jaw length is a major axis of divergence in cichlid radiations involving suction and biting feeding (Cooper *et al.*, 2010; Parsons, Márquez & Albertson, 2011), and this may apply to the adaptive diversification of other fishes like Arctic charr.

Our comparative study is consistent with the idea of adaptive polymorphism in lake populations of Arctic charr in Iceland because genetic variation is partitioned more strongly among morphs than families in the more ecologically diversified lake population. The less ecologically diversified lake Vatnshlidarvatn morphs were more similar to each other than morphs from the more diversified Thingvallavatn population. Little difference in the magnitude of plastic responses by these craniofacial traits to diet treatment indicates either little genetic assimilation or no differences in the genetic assimilation of these traits between lake populations. Some plastic responses of head features appeared to be adaptive in the charr morphs from Thingvallavatn, such as a deepening head in the TPL morphs forced to feed benthically compared to those in the water column, although we do not know whether these induced changes influence feeding performance. A better understanding of feeding ecology and juvenile

habitat use by Arctic charr in both populations may provide insights into how developmental responses to variation in diet shape phenotypic variation and influence adaptive diversification.

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## SUPPORTING INFORMATION

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

**Table S1.** Results of three separate analysis of covariance evaluating the effects of family, morph and lake (factors) for each of the six linear measurements.