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Epigenetic regulation of trophic morphology through feeding behaviour in Arctic charr, *Salvelinus alpinus*

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Several models of speciation suggest that in species that are phenotypically plastic, selection can act on phenotypic variation that is environmentally induced in the earliest stages of divergence. One trait that could be subject to this process is foraging behaviour, where discrete foraging strategies are common. One species which is highly plastic in the expression of phenotype, the Arctic charr, *Salvelinus alpinus* (L.), is characterized by discrete variation in the anatomy of the head and mouthparts. These traits have been shown to have a functional significance, but the expression of which is thought to be at least partly phenotypically plastic. Here we test the hypothesis that foraging behaviour may regulate the anatomy of the head and mouthparts in Arctic charr. In a dyad experiment, size-matched pairs of fish from a mixed family group were fed a diet of either *Mysis* (a hard-bodied shrimp) or Chironomid larvae. Nine morphometric measures of head dimensions that describe wild trophic morphs were measured at the start of the experiment and 24 weeks later. Principal component scores of size-corrected morphometric measures showed highly significant differences between fish exposed to the two diets. Univariate ANOVA analysis of the head morphometric variables showed that fish fed on Chironomids developed longer, wider jaws, longer heads and a larger eye for a given body length than did those fish fed upon *Mysis*. We conclude that foraging anatomy in Arctic charr is phenotypically plastic and that variation in foraging behaviour that results in feeding specialization in the wild could induce variation in head anatomy. This in turn could reinforce foraging specialization. Very rapid epigenetic divergence into distinct feeding morphs (as demonstrated here) would allow selection to act at more than one mode and thus could promote rapid evolutionary divergence, initially prior to genetic segregation, in species which are highly plastic. *Biological Journal of the Linnean Society*, 2003, **78**, 43–49.

ADDITIONAL KEYWORDS: environmental regulation – evolution – phenotypic plasticity – speciation.

INTRODUCTION

Several models have shown that diversifying evolutionary forces operating at the very earliest stages of speciation could act on phenotypic variation that is environmentally induced in a single gene pool, i.e. epigenetic variation (West-Eberhard, 1989, 1998; Wimberger, 1994, Skúlason, Snorrason & Jónsson, 1999). This occurs when the gene pool has the ability to produce more than one discrete, alternative phenotype for a given characteristic; that is, where the population is phenotypically plastic. When this occurs, selection may be able to operate on two or more discrete phenotypes simultaneously resulting in divergence (West-Eberhard, 1989). An important element of these models is that phenotypic divergence may occur prior to any genetic segregation (West-Eberhard, 1986, 1989, 1998; Wimberger, 1994; Skúlason *et al.*, 1999). The type of phenotypic variation on which these epigenetic evolutionary mechanisms are most likely to act initially is variation in behaviour. A likely candidate for selection to act upon is foraging behaviour, because of the possibility of alternative strategies for successful foraging and its potential effects on fitness (see West-Eberhard, 1989; Wimberger, 1994; Skúlason *et al.*, 1999).

In nature, the existence of more than one discrete

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alternative phenotype in a population (polymorphism) is relatively common in a wide range of animal groups (see reviews by Skúlason & Smith, 1995 and Smith & Skúlason, 1996). Although the functional significance of many polymorphisms remain to be tested, a large number of described polymorphisms are the result of variation in the anatomy of feeding apparatus (trophic polymorphisms) clearly indicating a functional role in foraging (Skúlason & Smith, 1995; Adams & Huntingford, 2002a).

Amongst the fishes, the clearest example of a trophic polymorphism is in the parasitic scale-eating cichlid *Perissodus microlepis* (Boulenger). This species shows discrete polymorphism in the handedness of its jaw morphology with left- and right-handed morphs specializing in feeding on alternative sides of its host (Hori, 1993). The functional significance of this polymorphism is intuitively clear, and although this trophic specialism may appear to be the result of the unusual foraging habit of this species, trophic polymorphisms in fish appear to be common. In a review, Skúlason & Smith (1995) listed 15 fish species within which a discrete polymorphism with a presumed foraging link had been described.

One freshwater fish species which exhibits a very high degree of phenotypic plasticity is the Arctic charr, *Salvelinus alpinus* (L.). In addition to the expression of broad phenotypic variation between populations (Alexander & Adams, 2000), there have been a large number of reported cases of polymorphic populations living in sympatry. These often take the form of feeding behaviour specializations, frequently associated with discrete body size variations (Savvaitova, 1969; Nyman, Hammar & Gydemo, 1981; Nordeng, 1983; Klemetsen *et al*., 1985; Hindar & Jonsson, 1993), in some cases with discrete variations in foraging anatomy (head and mouth parts; Snorrason *et al*., 1989; Adams *et al*., 1998; Fraser, Adams & Huntingford, 1999). For many sympatric Arctic charr polymorphisms, the relative role of genetic and environmental factors is not known. However, for two systems where the trophic polymorphisms show discrete variation in trophic anatomy, laboratory rearing experiments have been conducted.

In common-environment rearing experiments, Arctic charr from four morphs from Thingvalavatn in Iceland showed that there was a strong genetic component controlling the ultimate morphological form of these fish (Skúlason, Noakes & Snorrason, 1989). Similar experiments on sympatric forms from Loch Rannoch, Scotland and Fjellfrosvatn, Norway showed both genetic component and environmental components to the expressed phenotype in the wild (Adams & Huntingford, 2002b; Klemetsen *et al*., 2002).

In a model of the early diverging processes in incipient species, presented by Skúlason and his coworkers (Skúlason *et al.*, 1999), they hypothesize that stable alternative feeding strategies could result in epigenetic variation in morphological features seen in some polymorphic species. The specific objective of the present study was to test the hypothesis that differential prey consumption can create variation in the morphology of the feeding apparatus in Arctic charr from a single gene pool.

METHODS

Ova stripped from female Arctic charr collected by gillnet from Loch Tay, Perthshire, Scotland, were fertilized immediately with milt from males and allowed to hydrate. Fertilized eggs from approximately ten families were combined and transported within 12 hours to standard incubation facilities at the University Field Station, Loch Lomondside.

At first feeding, alevins were transferred to a 1 m tangential flow, through-flow tank and fed a standard powdered salmon diet (BOCM – Pauls). When fry reached a mean fork-length of 78 mm, 35 pairs of individuals of equal length (± 1 mm) were selected from across the size range. These individuals were anaesthetized, marked with a unique alcian blue tattoo mark then weighed, measured (fork-length) and, for subsequent measures of head morphology, photographed on an appropriate scale in lateral view, with a 35 mm SLR camera, using extension tubes to increase magnification and slave flashguns to allow even illumination. To create a matched pair, dyad experiment, one individual of each pair was allocated to each of two 60 cm diameter, tangential through-flow circular tanks, with a water capacity of 60 L and an exchange rate of *c*. 6 litres min^{-1}. To increase density to an appropriate level for this species, a further 15 individual charr, selected at random from the stock tank were added to each group. Fish in each tank were then exposed to one of two feeding conditions. One group was fed only *Mysis*, a hard-bodied crustacean, similar to species commonly found in the diet of charr. The second group was fed only Chronomid larvae, a long, approximately cylindrical shaped, soft bodied prey of charr (Adams *et al*., 1998). Fish were fed with moist prey at the rate of 2% per day (dry weight) of total biomass in each tank. Total daily food allocation was presented as two meals at approximately 08:30 and 15:00 h each day and adjusted for fish biomass changes every seven weeks as fish grew. After 24 weeks, the mean fork-length of the experimental fish had reached 123 mm and all fish were again anaesthetized, measured (fork-length), weighed and photographed in lateral view on a scale to enable head morphometric measures to be made.

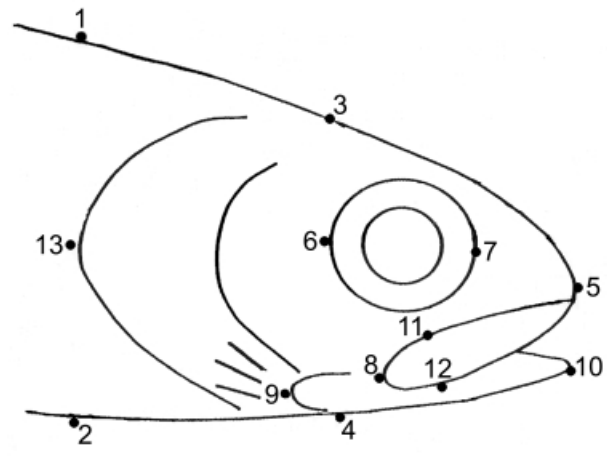

Figure 1. Nine morphometric measures of head anatomy used in this study. HDO – head depth at the operculum (landmark 1–2); HDE – head depth at the eye (landmark 3–4); HL – head length (landmark 13–5); ED – eye diameter (landmark 6–7); ML – maxillary bone length (landmark 8–5); LJL – lower jaw length (landmark 9–10); MW – maxillary bone width (landmark 11–12); SL – snout length (landmark 3–5); SW – snout width: maximum tangent length from line 3–5 to the top edge of the head.

Measurements of head morphometric parameters

To determine head morphology, nine separate measures of the head and mouth, known to define trophic polymorphism in some populations (Adams *et al*., 1998) were made from photographs at the beginning (week 0) and end (week 24) of this experiment. Photographic prints were made at around eight times magnification and nine linear measurements of head morphology made between easily ascribed landmarks directly from the prints using a dial gauge micrometer (Fig. 1). Each photograph was calibrated for scale separately.

Statistical Techniques

As measured head morphology variables were dependent on body size, size independent measures of head anatomy were derived as the residuals of each head anatomy character regressed on fork-length (Reist, 1986). To determine the effect of diet on the overall head shape, a single measure of head shape was derived using multivariate principal component analysis. Principal component factor scores for each fish were then analysed using ANOVA. Paired *t*-tests were used to compare univariate, size-corrected head anatomy measures. To reduce the risk of Type I errors associated with multiple comparisons, Bonferroni corrections were made to critical probability values. For clarity *P*-values are expressed as the conventional unadjusted equivalents (Sokal & Rolf, 1995).

Table 1. Factor loadings for PC1 and PC2 derived from a principal component analysis of the residuals of each of nine morphometric head anatomy characters regressed on fork-length in fish exposed to two feeding treatment groups at the beginning (week 0) and end (week 24) of the experiment (all groups pooled). See Fig. 1 for definition of characters

	Factor loadings	
Character	PC1	PC2
HDO	0.095	0.704
HDE	0.629	0.539
HL	0.798	−0.173
ED	0.834	−0.261
ML	0.903	0.163
LJL	0.828	0.179
SL	0.899	−0.208
SW	0.226	−0.718
MW	0.492	0.005

RESULTS

The first and second principal components (PC1 and PC2) derived from Principal Components Analysis of all nine head morphometric variables explained 48.2% and 16.6% of the variance, respectively. All successive principal components explained less than 10% of the variance and are not considered further here. Factor loadings show that PC1 is a general measure of head anatomy robustness (all loadings are positive and the range of weightings small; Table 1). PC2 in contrast, weights measures of head depth (HDO and HDE) positively and snout width (SW; Fig. 1) negatively (Table 1).

There was no difference in the component scores for fish from each of the two diet treatments at the beginning of the experiment for either PC1 ($F_{1,64} = 1.24$; $P = 0.268$; Fig. 2a) or PC2 ($F_{1,64} = 1.27$; $P = 0.263$; Fig. 2b).

In contrast, at the end of the experiment principal component scores for both PC1 and PC2 showed highly significant differences between treatments (PC1: $F_{1,64} = 37.6$; $P < 0.000001$; PC2: $F_{1,64} = 6.63$; $P = 0.012$).

Paired *t*-tests on the size-corrected residuals of each morphometric variable regressed on fork-length for each size-matched pair showed that six of the nine head size morphometric variables were significantly

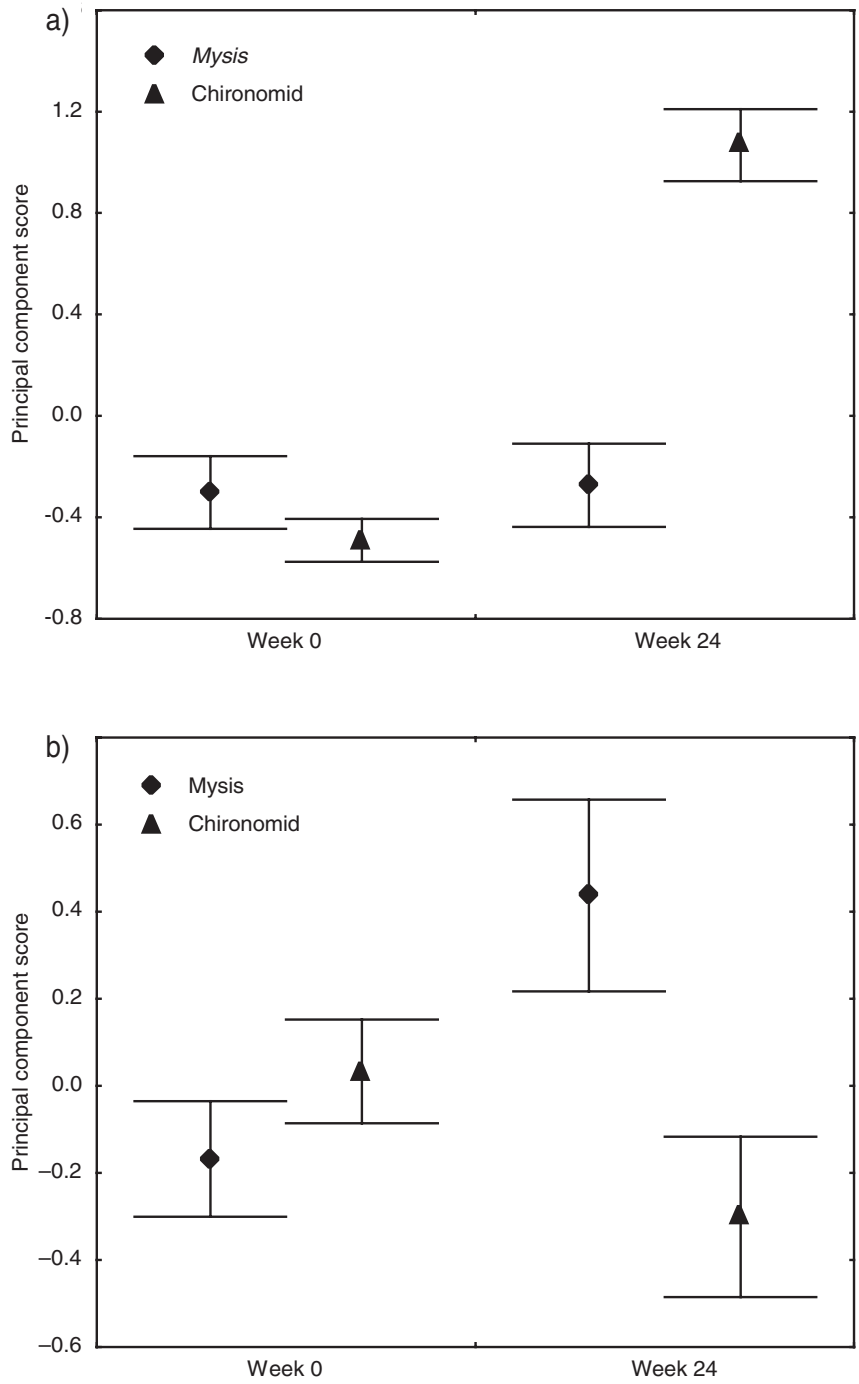

Figure 2. The mean and standard error of the principal component scores at the beginning and the end (week 24) of the experiment derived from all nine head morphometric characters measured for fish exposed to a diet of *Mysis* or Chironomid. (a) PC1: week 0, no significant difference; week 24, $P < 0.000001$. (b) PC2: week 0, no significant difference; week 24, $P < 0.012$.

different after 24 weeks exposure to the treatment groups (Fig. 3). In all of these, fish fed upon *Mysis* had a smaller size-corrected measure of head anatomy than those fish fed upon Chironomid larvae.

DISCUSSION

Our results demonstrate that a relatively short period of enforced feeding of juvenile Arctic charr from the

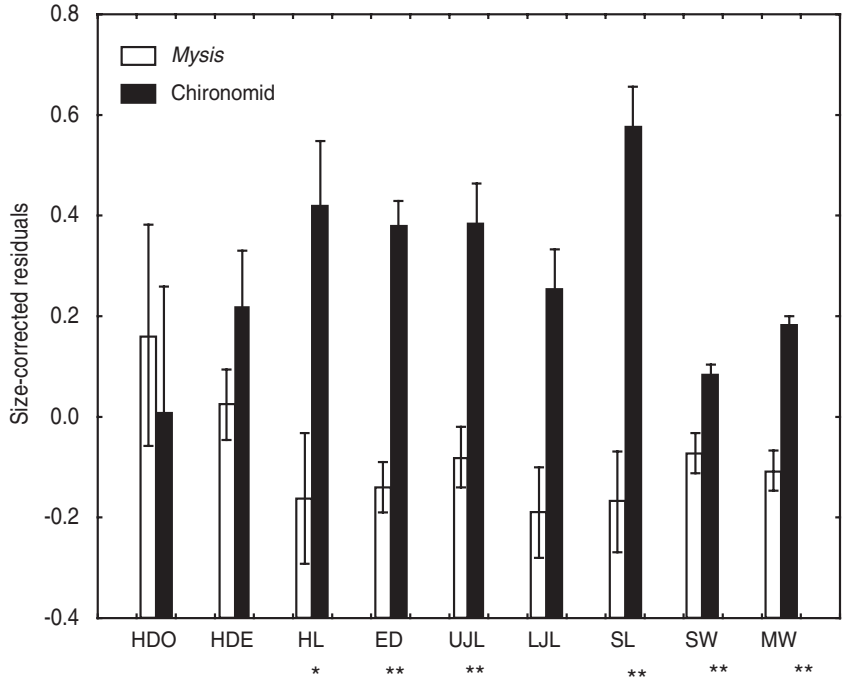

Figure 3. The mean and standard error of size-corrected residuals of each head morphometric character for charr fed either a *Mysis* or Chironomid diet, measured in week 24. (*$P < 0.05$; **$P < 0.001$).

same genetic group on different diets can cause significant divergence in the anatomy of the head and mouthparts. Thus we show that head anatomy in this species can be modified by the environment and is phenotypically plastic (*sensu* West-Eberhard, 1989). In the experiment presented here, the feeding environment to which charr were exposed resulted in significant differences in six of nine body-size-corrected morphometric measures, four related to jaw shape, plus head length and eye diameter. These same variables are known to segregate sympatric morphs of Arctic charr from some sites where they are found in the wild, with a more robust head morphology for a given size typical of morphs feeding upon zoobenthos compared with morphs feeding upon plankton (Snorrason *et al*., 1989; Adams *et al*., 1998; Fraser *et al.*, 1999). Here charr forced to feed upon a zoobenthos prey item common in their natural diet developed a more robust head morphology (longer head, longer upper jaw, etc.) than individuals foraging upon a zooplankton prey. Previously it has been shown that head and mouth size is one of the constraints upon prey choice in morphs of charr (Adams & Huntingford, 2002a).

Thus the hypothesis that feeding behaviour can modify morphology within a single animal's lifetime (Skúlason *et al.*, 1999) is supported. In this study the exposure to food was enforced (animals were not given the choice of diet). However, alternative foraging strategies that result in significant diet differences within a single population have been commonly reported in a wide range of animals and are thought to arise stochastically or though behavioural traits that govern prey choice, such as search image formation and the development of prey handling skills (Guilford & Dawkins, 1987).

Dietary influences on morphology have been described in other fish species. In the cichlids *Cichlasoma* spp. and *Geophagus* spp. diets have been shown to have an influence on trophic morphology (Meyer, 1987; Wimberger, 1991). In three-spined sticklebacks *Gasterosteus aculeatus* L. exposure to larger prey resulted in the development of deeper heads and larger mouth gape (Day & McPhail, 1996).

The experiment presented here does not provide any evidence for the mechanism through which the variation in head morphology arose. This has, however, been examined experimentally in charr by Erikson and his co-workers (Eriksson, Skúlason & Snorrason, 1999) and in cichlids by Meyer, 1987), both concluding that heterochronic growth (variation in allometric growth relationships) resulted in the observable differences in trophic morphology. The charr used here approximately doubled in length over the experimental period and although this was not specifically tested, the diet-induced variation in trophic morphol-

ogy demonstrated in this study is consistent with heterochronic growth of these characters.

The fact that epigenetic factors, such as choice of prey, can influence trophic morphology in charr has a number of general and specific evolutionary consequences.

Firstly, as behaviour is highly labile and as expressed behaviour plays a significant role in the ultimate fitness of an animal, there can be significant selection pressures influencing behavioural systems. If, as we have shown here, behavioural processes can modify anatomical characteristics in rapidly developing pre-maturation individuals within a single lifetime, this provides a mechanism through which selection forces may act upon morphological characteristics at an epigenetic level. Secondly, as available prey in relatively simple high latitude freshwater systems such as those inhabited by Arctic charr, often fall into 2–3 very discrete prey types (such as macrobenthos and plankton; Wimberger, 1994), this may further drive rapid divergence into discrete feeding modes, with different feeding behaviour characteristics, which in turn may lead to more than one morphological phenotype mode upon which selection can act. Possibly, at least initially, this may occur without reproductive isolation, and thus could act at a speed that is much greater than that predicted by selection acting upon trait variations that are genetically controlled (West-Eberhard, 1989).

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