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Convergent evolutionary processes driven by foraging opportunity in two sympatric morph pairs of Arctic charr with contrasting post-glacial origins

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The expression of two or more discrete phenotypes amongst individuals within a species (morphs) provides multiple modes upon which selection can act semi-independently, and thus may be an important stage in speciation. In the present study, we compared two sympatric morph systems aiming to address hypotheses related to their evolutionary origin. Arctic charr in sympatry in Loch Tay, Scotland, exhibit one of two discrete, alternative body size phenotypes at maturity (large or small body size). Arctic charr in Loch Awe segregate into two temporally segregated spawning groups (breeding in either spring or autumn). Mitochondrial DNA restriction fragment length polymorphism analysis showed that the morph pairs in both lakes comprise separate gene pools, although segregation of the Loch Awe morphs is more subtle than that of Loch Tay. We conclude that the Loch Awe morphs diverged *in situ* (within the lake), whereas Loch Tay morphs most likely arose through multiple invasions by different ancestral groups that segregated before post-glacial invasion (i.e. in allopatry). Both morph pairs showed clear trophic segregation between planktonic and benthic resources (measured by stable isotope analysis) but this was significantly less distinct in Loch Tay than in Loch Awe. By contrast, both inter-morph morphological and life-history differences were more subtle in Loch Awe than in Loch Tay. The strong ecological but relatively weak morphological and life-history divergence of the *in situ* derived morphs compared to morphs with allopatric origins indicates a strong link between early ecological and subsequent genetic divergence of sympatric origin emerging species pairs. The emergence of parallel specialisms despite distinct genetic origins of these morph pairs suggests that the effect of available foraging opportunities may be at least as important as genetic origin in structuring sympatric divergence in post-glacial fishes with high levels of phenotypic plasticity. *Biological Journal of the Linnean Society*, 2012, **106**, 794–806.

ADDITIONAL KEYWORDS: alternative phenotypes – discrete morphological variation – ecological segregation – foraging specialism – speciation.

INTRODUCTION

Within a single species, individuals often express one of two or more possible phenotypes for a given trait. Where these expressed phenotypes are discrete (i.e. without intermediates), they have been variously referred to as morphs, ecotypes, ecomorphs, and polyphenisms. The exact definition of each of these terms differs, and has not been consistently applied in the literature and, according to some studies, depends

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upon the underlying nature of the expressed phenotype (West-Eberhard, 1989). However, all definitions have one common attribute, a discontinuity in the spectrum of expressed phenotypes for a given trait (i.e. expression of discrete, alternative phenotypes) (Garduno-Paz & Adams, 2010). The expression of two or more discrete phenotypes allows multiple, alternative modes upon which selection can act semi-independently, providing a basis for the divergence of alternative phenotypes towards different evolutionary outcomes (West-Eberhard, 2003). This effect is particularly evident where alternative phenotypes are expressed in sympatry (Schluter & McPhail, 1992) and where the expressed phenotypes have a strong functional significance (West-Eberhard, 2005).

The coexistence of alternative phenotypes (with the term 'morph' being used to describe the group expressing alternative phenotypes), differing in foraging related traits, is relatively common in fishes inhabiting post-glacial lake systems (McPhail, 1992; Skúlason & Smith, 1995; Smith & Skúlason, 1996). There is now a robust and growing literature that demonstrates a link between the expression of two or more discrete suites of alternative phenotypic traits and alternative foraging ecology in fishes from a range of evolutionary lineages, including three-spined stickleback, *Gasterosteus aculeatus* L. (Schluter, 1993; Baker, Foster & Bell, 1995; Vamosi & Schluter, 2004), whitefish, *Coregonus lavaretus* (L.) (Bernatchez *et al*., 1996; Kahilainen & Ostbye, 2006; Harrod, Mallela & Kahilainen, 2010), Arctic charr, *Salvelinus alpinus* (L.) (Klemetsen *et al*., 2003), pumpkinseed, *Lepomis gibbosus* (L.) (Wainwright, Osenberg & Mittelbach, 1991; Robinson & Wilson, 1996; Parsons & Robinson, 2007), and brook charr *Salvelinus fontinalis* (Mitchill) (Imre, McLaughlin & Noakes, 2001).

Amongst Arctic charr, sympatric morphs showing foraging specialisms have been described from a number of post-glacial lakes throughout the species' distribution (Snorrason *et al*., 1994; Adams *et al*., 1998; Alekseyev *et al*., 2002; Klemetsen *et al*., 2002; Adams, Wilson & Ferguson, 2008). These most frequently include individuals specializing in zooplanktivory, benthivory or piscivory, accompanied by associated discrete morphological variation in functionally significant traits (Adams & Huntingford, 2002). In the present study, we compared two lake systems from different drainages supporting two discrete Arctic charr aiming to address a series of hypotheses related to their origin.

Sexually mature Arctic charr from Loch Tay, Scotland, exhibit a bimodal length-frequency, with individuals either in the fork-length range 190–290 mm or 80–160 mm (large and small body size morphs, respectively) and are found in sympatry (Adams *et al*., 2003). In Loch Awe, Arctic charr show no obvious discontinuity in body size, although they segregate into individuals that spawn in either spring or autumn (spring-spawning and autumn-spawning charr morphs, respectively) (Alexander & Adams, 2000; Kettle-White, 2001).

We compare these two contrasting sympatric, morph pairs aiming to address six hypotheses related to their status and the evolutionary processes that led to their formation. These are that the morph pairs in each lake:

(1) represent genetically distinct units;
(2) show similar genetic origin;
(3) comprise ecologically distinct units;
(4) differ in functionally significant morphological characteristics;
(5) exhibit different life-history traits;
(6) show similar patterns of evolutionary divergence.

MATERIAL AND METHODS

Study areas and sampling

Arctic charr were collected from Loch Tay, Perthshire which drains to the east (56°30′ N; 004°10′ W, 26.4 km^2 area; 102 m maximum depth; Murray & Pullar, 1910) during the spawning season, in October 2006. Charr were also collected from Loch Awe, Argyll and Bute, which drains to the west (56°20′ N, 005°05′W; 38.5 km^2 area; 93 m maximum depth; Murray & Pullar, 1910) during the spawning seasons for this population, between 8 and 15 November 2006 (autumn-spawning charr) and 21 and 26 February 2007 (spring-spawning charr). Sampling in Loch Awe was conducted at known spawning sites (56°22′21.1″N, 005°4′24.6″W; autumn) and (56°15′06.3″N, 005°16′24.1″W; spring).

Arctic charr were collected at all sites using standard benthic Nordic mono-filament survey gill-nets (Jensen & Hesthagen, 1996). Nets were set on the bottom of the lake (maximum depth in the range 2–20 m) perpendicular to the shore and fished overnight.

Collected specimens were killed immediately and taken to the laboratory within 3 h; each individual was photographed, measured (standard length ± 1 mm), weighed (±0.1 g) and their sex and maturity status determined. Otoliths were removed for age determination. The adipose fin was removed and preserved in 100% ethanol for genetic analysis.

Allocation of charr to either the small or large body size morphs was determined (for sexually mature fish only) on the basis of body size only (Adams *et al*., 2003). Charr from Loch Awe were allocated to one of the alternative morphs only by the occurrence of sexual maturity at one of the two sampling periods.

Genetic analysis

Genetic divergence between morph was explored using restriction fragment length polymorphism. To provide a context for this analysis, these data were compared with similar data obtained for a well-studied, sympatric morph system from Loch Rannoch (56°41.7′N; 004°17.6′W; River Tay catchment, Scotland), which supports a specialist piscivore, a benthivore, and a planktivore (Adams *et al*., 1998; Adams & Huntingford, 2002). The three specialist foraging groups show clear genetic segregation (Verspoor *et al*., 2010).

Composite restriction fragment length polymorphism haplotypes were defined by restriction enzyme analysis of ND1, CYTOB, and D-loop regions of the mitochondrial DNA (Verspoor *et al*., 2010). DNA was extracted from fin tissue. Polymerase chain reaction amplification and subsequent DNA digestion, fragment separation, variant scoring, and haplotype assignment followed that of Verspoor *et al*. (2010) for Loch Rannoch morphs. A minimum spanning network showing the genetic relatedness of the haplotypes based on restriction differences was generated manually. Haplotype frequencies for Loch Rannoch morphs were taken from Verspoor *et al*. (2010). Within-morph haplotype and nucleotide diversities were calculated using DA in REAP (McElroy *et al*., 1991).

Pairwise genetic divergence was calculated for the different morph groups within and among locations. F_{ST} (i.e. variance among morphs) and D_A (i.e. a standard measure of genetic divergence based solely on allele frequency differentiation) (Nei & Kumar, 2000) were estimated using POPULATIONS (Langella, 1999). Estimates of pairwise nucleotide divergence, P (Nei & Tajima, 1981), a distance based on both allele frequency differentiation and nucleotide divergence among haplotypes, were obtained using DA in REAP. Between-morph differences in haplotype frequencies were tested using the MONTE in REAP. Minimum evolution (ME) trees were constructed using MEGA4 (Tamura *et al*., 2007) and multi-dimensional scaling (MDS) plots using PAST (Hammer, Harper & Ryan, 2001).

The foraging ecology of co-existing groups

Those charr captured during the spawning period in the present study had not fed and, because it was impossible to identify morphs outside the spawning season, stomach content analysis was impossible. Hence, analysis of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes was used to examine the foraging ecology of individuals from each morph group.

Samples of dorsal muscle were removed from the left flank below the dorsal fin and above the lateral line from each charr from Loch Awe, dried at 40 °C for 7 days, ground and mixed, and approximately 0.5 mg was used for analysis. For comparison, stable isotope analysis results for charr from Loch Tay were derived from Adams *et al*. (2003). Carbon and nitrogen stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry at the Max Planck Institute for Limnology, Plön, Germany, *sensu* Harrod & Grey (2006). Typical precision for a single analysis was ±0.1‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$. Because lipids are depleted in ^{13}C, variation in lipid concentrations between samples were arithmetically lipid-normalized *sensu* Kiljunen *et al*. (2006).

Functionally significant morphological characteristics

Landmark-based geometric morphometric analyses were used to detect variation in the shape of individual charr. Fish were photographed (left side) using a Canon digital camera (EOS 350D) fixed to a camera stand. Photographs were compiled (using tpsUtil; Rohlf, 2006a) and 28 landmarks on each fish (Fig. 1) were located and digitized (using tpsDig2; Rohlf, 2006b).

Generalized least squares procrustes superimposition was used to translate, scale, and rotate raw landmarks to minimize the summed, squared, inter-landmark distances among fish (Rohlf & Slice, 1990); this procedure removes the effect of body size on the position of landmarks and produces partial warp scores for each landmark on each fish (Rohlf, 2007). Centroid size was used as a measure of overall body size (Zelditch *et al*., 2004).

Relative warp (RW) analysis (equivalent to principal component analysis) of the partial warps scores of each individual was used to reduce the number of informative variables. Goodall's F resampling test was performed using TWOGROUP6 (Sheets, 2003) to derive and compare the mean shape differences among morph pairs within lakes (Goodall, 1991; Adams, Rohlf & Slice, 2004).

Life-history characteristics

Three different, although related, elements of life history (age, growth, and maturity) were compared in

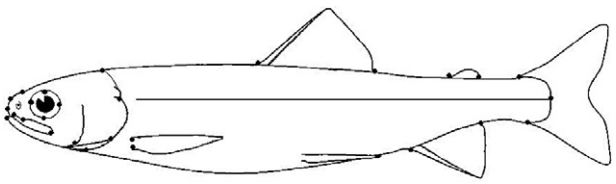

Figure 1. The location of 28 anatomical landmarks used to define fish shape.

the present study. To determine age, the surface of sagittal otoliths was ground, polished, and annuli counted *sensu* Fraser, Adams & Huntingford (1998). Three counts were performed and the final age determination was made by agreement of two independent readers. Growth of Arctic charr from each morph group was expressed using the simplified Von Bertalanffy equation (Von Bertalanffy, 1960) fitted to observed length-at-age (standard length was used throughout) using Marquardt least squares nonlinear regression:

$$L_t = L_\infty(1 - \exp^{[-k(t-t_0)]})$$

Where L_t is the length at age t (annuli number), L_∞ is the typical asymptotic length, k is the growth coefficient and t_0 is the the theoretical age at zero length (assumed to be 0). The nonlinear estimation of growth parameters was determined using FISAT II, version 1.2.2 (Food and Agriculture Organization of the United Nations). A multivariate maximum likelihood ratio method (Hesslein, Hallard & Ramlal, 1993) was used to compare growth model parameters among morphs, using SPSS, version 13.0 (SPSS Inc.) *sensu* Kimura (1980).

RESULTS

A total of 77 sexually mature fish were captured from Loch Awe: 33 males and ten females in autumn; 21 males and 13 females in spring. Thirty-four immature fish were also collected (eight in autumn and 26 in spring). A total of 159 individual charr were captured in Loch Tay, of which 120 were sexually mature. Forty-four mature fish of the small body size morph (24 males and 20 females) and 76 of the large body size morph (39 males and 37 females) were collected. Immature fish were not analyzed further.

Mitochondrial DNA analysis

Nine composite haplotypes were observed in morph groups collected across all sites including those from Loch Rannoch (Table 1). Genetic relatedness as indicated in a minimum spanning network (Fig. 2) shows two distinct higher-order clans of related haplotypes (a clan being a higher-order grouping of genetic types in an unrooted tree (Wilkinson *et al.*, 2007)). Two haplotypes were shared by charr from Lochs Tay, Awe, and Rannoch (Table 2). Loch Tay and Loch Rannoch charr had two further haplotypes in common that were absent in charr from Loch Awe. One haplotype was unique to Loch Awe charr, and four haplotypes were found only in Loch Rannoch charr.

Loch Awe charr showed the lowest haplotype diversity (number of variants), with only three haplotypes identified from a single clan. These were present in both morphs, although one haplotype was not found elsewhere (Fig. 2, Table 2). However haplotype frequencies were significantly different between the Loch Awe sympatric morph pair ($\chi^2 = 6.1$, $P = 0.03$). Additionally, haplotype and nucleotide diversities were higher in spring-spawning than in autumn-spawning charr (Table 2). F_{ST} between the two groups was 0.025 indicating 2.5% of observed genetic variation amongst charr from Loch Awe was accounted for by variation between morphs.

Table 1. Composite restriction fragment length polymorphism haplotypes observed in Lochs Tay and Awe

		CYT B		ND1		
Composite haplotypes	D-loop *Bcc1*	*Hinf I*	*Mse l*	*Bcc l*	*Dde l*	*Hae lll*
AAABBB	A	A	A	B	B	B
ACABBB	A	C	A	B	B	B
AAABAB	A	A	A	B	A	B
BBBBAB	B	B	B	B	A	B
BBBBBB	B	B	B	B	B	B

By contrast, four haplotypes, comprising two clans were found in Loch Tay charr (Table 2), with the two most common ones shared by both morphs, whereas the remaining two were only recorded from small body size charr (Table 2). The differences in haplotype frequencies between morphs from Loch Tay were substantive and highly significant ($\chi^2 = 116.2$, $P < 0.0001$). Haplotype and nucleotide diversities were both higher in small body size fish compared to the large body size charr. The F_{ST} between the morph pair in Loch Tay was 0.398, indicating that approximately 40% of genetic variation in the Tay charr is attributable to differences between morphs.

The genetic distance between the two Loch Tay charr morphs was much larger than between the two Loch Awe charr morphs (Table 3). D_A was approximately six times greater, whereas P was approximately 60 times greater. The smallest pairwise genetic distance was between morphs from different and isolated water bodies. The Loch Tay large body size morph and Loch Awe autumn-spawning morph groups returned an overall nucleotide divergence (P) of effectively zero, and a between-morphs Nei's distance (D_A) of 0.023.

There was evidence of a closer genetic relationship between the large body size charr from Loch Tay and both of the Loch Awe morphs, than between sympatric Tay morphs (pairwise D and P, Table 3; ME, Fig. 3). In addition, the dominant haplotype in the large body size charr from Loch Tay was also the

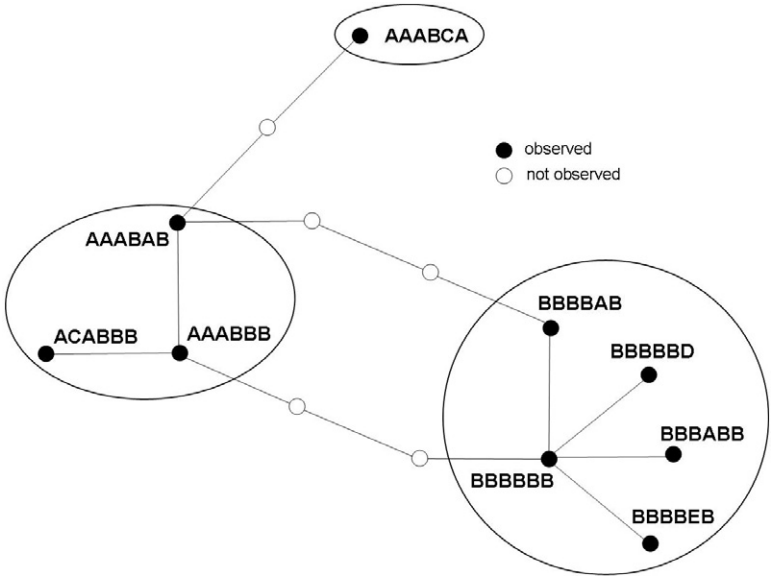

Figure 2. Minimum spanning network for restriction fragment length polymorphism haplotypes observed in charr from Lochs Tay, Awe and Rannoch showing higher-order clans (circled).

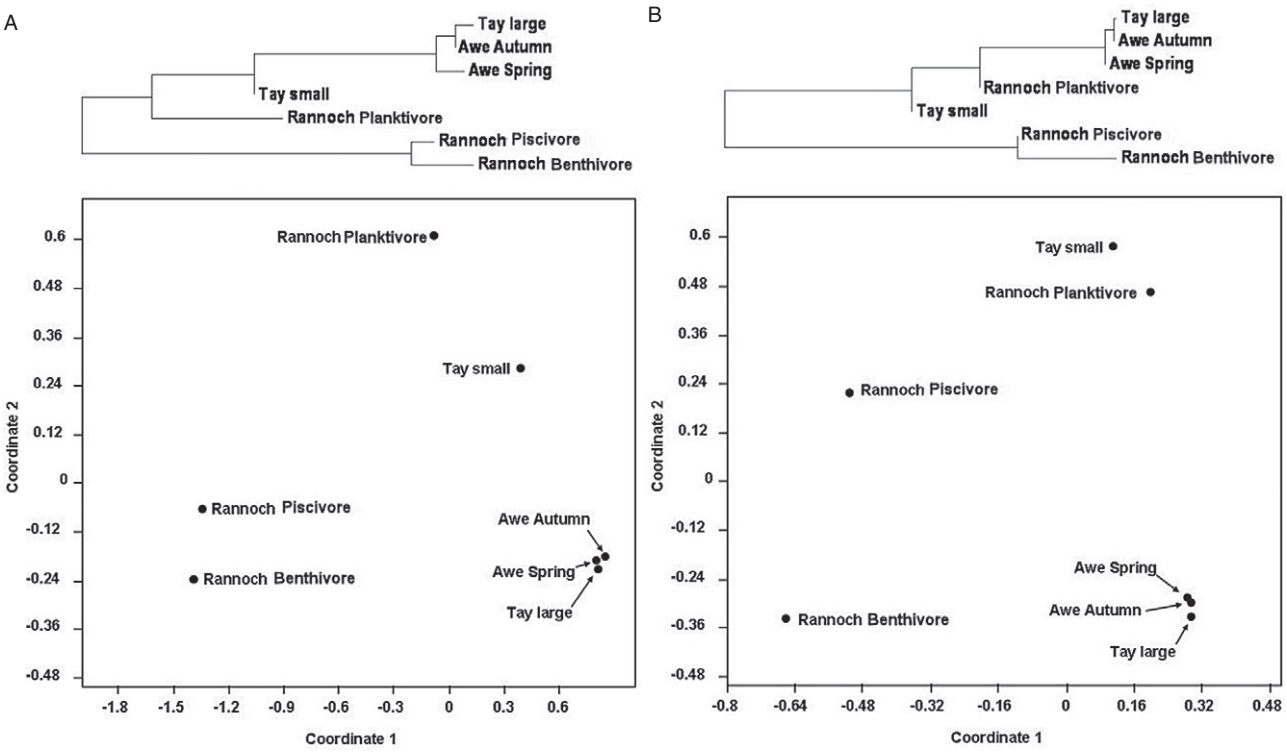

Figure 3. Genetic differentiation among lochs and morphs based on (A) haplotype frequencies using D_A and (B) haplotype frequencies and nucleotide divergence using P; in each case, the minimum evolution dendrogram is at the top and the multi-dimensional scaling plot at the bottom.

dominant haplotype in both Loch Awe morphs, although it was not recorded in the small body size charr from Loch Tay. The MDS and ME cluster analysis shows that the Loch Tay small body size morph was most closely related to the Loch Rannoch planktivorous charr of the groups analyzed in the present study (Fig. 3). The relationship between the Loch Tay small body size charr and the Loch Rannoch

Table 2. Frequencies of haplotypes observed for different phenotypes in Lochs Tay and Awe (this study), as well as in Loch Rannoch (from Verspoor *et al.*, 2010)

		Composite haplotype										
Loch	Morph	AAABBB	AAABAB	ACABBB	AAABCA	BBBBBB	BBBABB	BBBBAB	BBBBEB	BBBBBD	Haplotype diversity*	Nucleotide diversity
Tay	Small	0	29	0	0	3	0	12	0	0	0.492 (0.044)	0.0415
Tay	Large	84	4	0	0	0	0	0	0	0	0.087 (0.029)	0.0021
Awe	Autumn	45	4	2	0	0	0	0	0	0	0.216 (0.052)	0.0054
Awe	Spring	43	5	12	0	0	0	0	0	0	0.444 (0.046)	0.0113
Rannoch	Benthivore	0	0	0	0	12	11	9	0	0	0.673 (0.014)	0.0256
Rannoch	Piscivore	0	0	0	0	4	20	2	1	0	0.431 (0.076)	0.0187
Rannoch	Planktivore	1	18	0	2	6	0	0	0	1	0.543 (0.065)	0.5590

*SE given in parenthesis.

planktivorous group was closer than that between the three groups from Loch Rannoch (Fig. 3).

Stable isotope analysis

In Loch Awe, autumn-spawning charr muscle had considerably and significantly depleted $\delta^{15}N$ and $\delta^{13}C$ values compared to the spring-spawning charr (Fig. 4A). The average autumn-spawning charr was a mean of 2.9‰ more depleted in $\delta^{15}N$ ($t_{33} = -6.46$; $P < 0.0001$) and 1.5‰ more depleted in lipid-corrected $\delta^{13}C$ ($t_{33} = -3.16$; $P < 0.003$) than the spring-spawning charr (Fig. 4A).

Stable isotopes values also differed significantly between sympatric Loch Tay morphs. The muscle of large body size Arctic charr from Loch Tay was a mean of 1.2‰ more depleted in $\delta^{15}N$ ($t_{71} = 5.48$; $P = 0.00001$) and 0.6‰ more depleted in $\delta^{13}C$ ($t_{71} = 2.86$, $P = 0.006$) than the small body size charr (Fig 4B).

Morphological analysis

There was a significant difference in mean overall shape between the two charr spawning groups from Loch Awe (generalized Goodall's $F_{52,3432} = 2.65$; $P = 0.00001$). The partial Procrustes distance, indicative of overall shape difference, showed a mean ± SE difference of 0.014 ± 0.002. However, centroid size, a measure of overall body size, was not significantly different between the morph groups (autumn-spawning charr centroid size = 35.3 ± 6.5 and spring-spawning charr = 32.2 ± 6.4; $P = 0.072$). Taken together, the first three relative warps (equivalent to principal components) explained 49% of overall shape variation across both charr morphs. Both RW1 and RW3 (but not RW2) score means were significantly different between morphs. In addition, RW1 scores were significantly different between sexes (Table 4).

Compared to the spring-spawning charr, autumn-spawning charr had a shorter, deeper head, shorter jaw, smaller eye, deeper body, a more terminal mouth position, a wider caudal peduncal, and more anteriorly positioned pectoral fins (Fig. 5).

As in Loch Awe, the two charr groups from Loch Tay showed clear and significant differences in body shape (generalized Goodall's $F_{42,4956} = 71.9$; $P < 0.00001$) and a mean ± SE partial Procrustes distance of 0.054 ± 0.002. RW 1, 2 and 3 accounted for 41%, 15% and 9% of shape variation, respectively (Table 4). RW1 and RW3 scores (but not RW2) showed significant differences for both morph and sex (Table 4).

Compared to small body size charr, large body size charr, independent of the effect of body size, had a

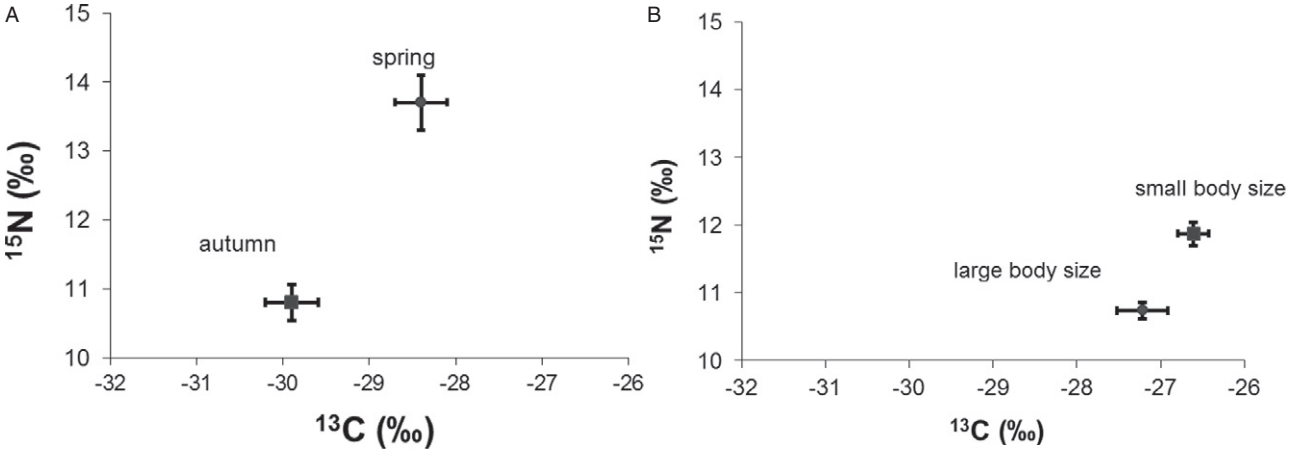

Figure 4. Variation in mean ± SE $\delta^{13}C$ and $\delta^{15}N$-values of muscle of co-existing phenotypes from (A) Loch Awe – autumn and spring-spawning morphs and (B) Loch Tay large and small body size morphs.

Table 3. Pairwise genetic distances among different phenotypes from Lochs Tay, Awe and Rannoch

Population	Tay small	Tay large	Awe Autumn	Awe Spring	Rannoch benthivore	Rannoch piscivore	Rannoch planktivore
Tay small		0.0290	0.0274	0.0275	0.0562	0.0851	0.0005
Tay large	0.2613		0.0000	0.0008	0.0918	0.1107	0.0130
Awe autumn	0.2418	0.0226		0.0004	0.0910	0.1103	0.0120
Awe spring	0.2935	0.1114	0.0354		0.0891	0.1085	0.0125
Rannoch benthivore	0.6561	1.0000	1.0000	1.0000		0.0057	0.0619
Rannoch piscivore	0.8090	1.0000	1.0000	1.0000	0.1154		0.0895
Rannoch planktivore	0.2626	0.6379	0.5906	0.6014	0.7113	0.8186	

Nei's standard distance D_A below diagnonal and nucleotide diversity, P, above diagonal.

Table 4. Multivariate tests of relative warps among phenotype, sex and their interaction for fish from Loch Awe and Loch Tay

Relative warp	Variance explained (%)	*P*		
		Phenotype	Sex	Phenotype × Sex
Loch Awe				
1	26	**0.010**	**0.0001**	**0.0001**
2	14.5	0.2	0.4	0.4
3	8.5	**0.004**	0.2	**0.004**
Loch Awe				
1	41.4	**0.0001**	**0.0001**	**0.001**
2	15.3	0.3	0.4	0.78
3	9.3	**0.04**	**0.0001**	**0.01**

P is the *F*-test significance of each relative warp. Bold values are significant.

smaller eye, shorter head, deeper body, wider caudal peduncal, with pectoral fins positioned more anteriorly (Fig. 6).

Age and growth parameters

Charr from Loch Awe ranged between 2 and 5 years of age in spring-spawning charr, and 2 and 6 years in autumn-spawning charr. Von Bertalanffy estimated asymptotic length (L_∞) did not differ between morphs ($P = 0.07$; Fig. 7A); however, the growth coefficient, k, was markedly and significantly ($P = 0.0001$) higher in spring-spawning charr (1.6) than in autumn-spawning charr (0.2) (Table 5). The mean ± SE age of sexual mature individuals was higher in autumn-spawning charr (4.7 ± 0.2 years) compared to spring-spawning charr (3.2 ± 0.3 years) ($F_{1,41} = 18.9$; $P = 0.0009$).

Large body size charr from Loch Tay ranged from 2 to 5 years in age. Small body size charr were aged between 2 and 7 years; however, only a single

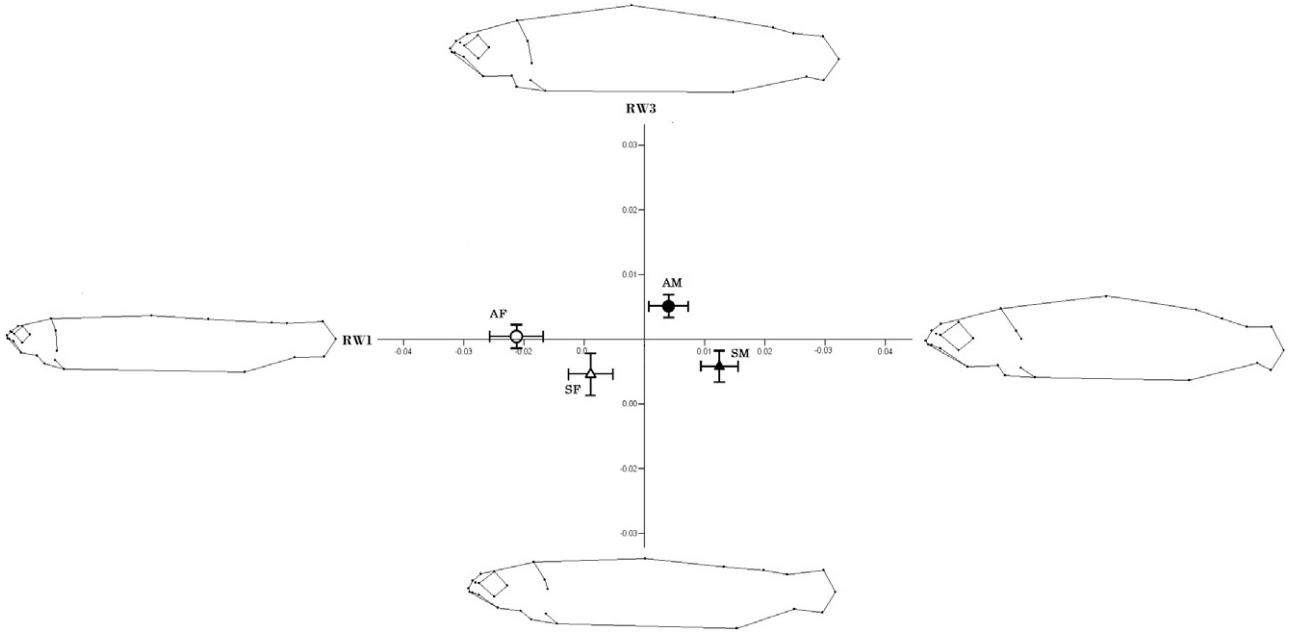

Figure 5. Relative warp (RW)1 and RW3 scores of autumn and spring-spawning male and female charr from Loch Awe, means ± SE. Graphic representations of shape variation of the most extreme negative and positive values of each axis defined as deviates from the pool mean shape (represented by the origin) (coordinates 0,0). AM, autumn-spawning males; AF, autumn-spawning females; SM, spring-spawning males; SF, spring-spawning females. Landmarks are connected by lines to facilitate the visualization of the shapes.

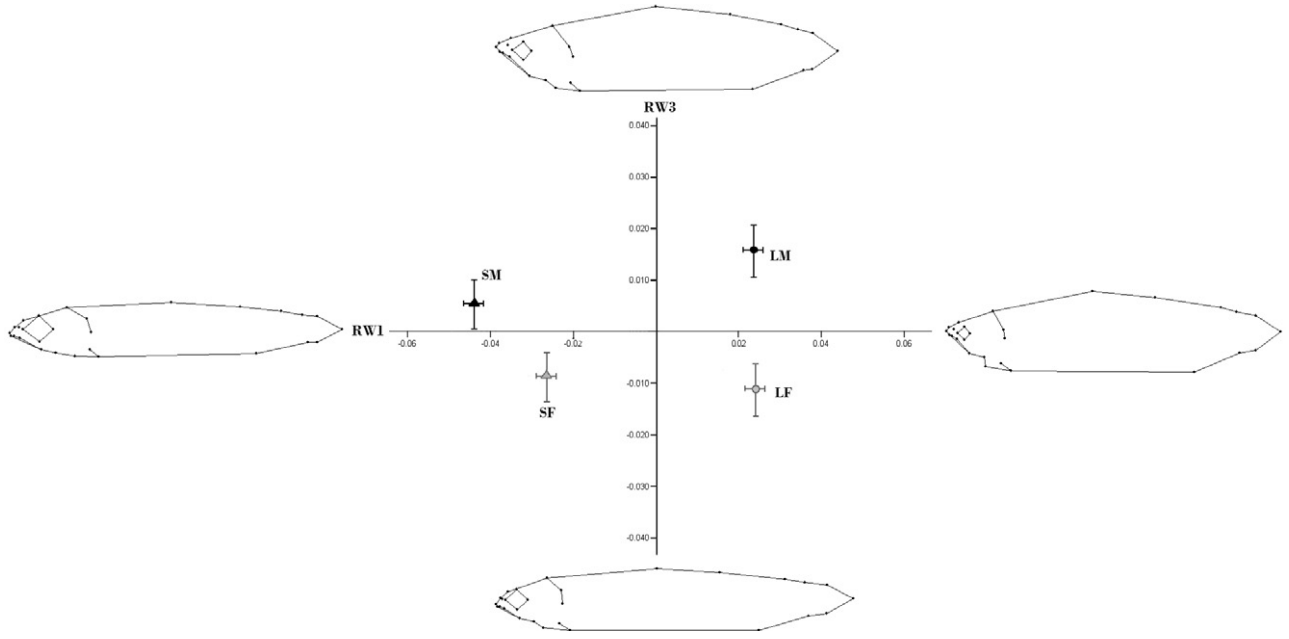

Figure 6. Relative warp (RW)1 and RW3 scores of large and small body size spawning male and female charr from Loch Tay, and their mean ± SE. Graphic representations of shape variation of the most extreme negative and positive values of each axis defined as deviates from the pool mean shape (represented by the origin) (coordinates 0,0). LM, large body size males; LF, large body size females; SM, small body size males; SF, small body size females. Landmarks are connected by lines to facilitate the visualization of the grand mean shape.

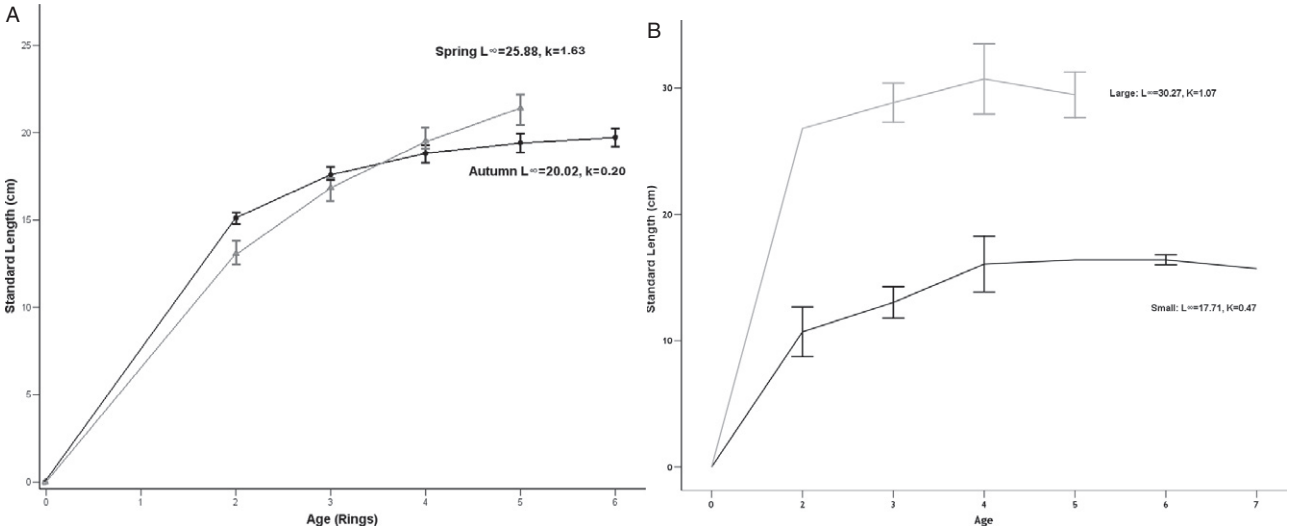

Figure 7. Growth curve of mean ± SE standard length by age obtained using a Von Bertalanffy model fitted to (A) Loch Awe; spring and autumn-spawning Arctic charr and (B) Loch Tay; small and large body size charr.

Table 5. Likelihood ratio tests comparing Von Bertalanffy parameter estimates for spring and autumn-spawning Arctic charr from Loch Awe (total number of mean length at age values = 9) and from large and small body size charr groups from Loch Tay (age values $N = 10$)

	Loch Awe			Loch Tay		
Form	Spring	Autumn	*P*	Large	Small	*P*
L_∞ (cm)	19.4	29.5	0.07	17.0	30.2	< 0.0001
k	1.6	0.2	0.05	0.53	1.1	< 0.0001

L_∞, typical asymptotic length; k, growth coefficient.

individual aged 7 years was recorded. The mean age of sexually mature individuals was not significantly different ($F_{1,38} = 0.001$; $P = 0.97$) between small body size (3.6 ± 0.9 years) and large body size (3.6 ± 1.4 years) charr. Not unexpectedly, the Von Bertalanffy model asymptotic length (L_∞) estimates were significantly higher for the large body size charr ($L_\infty = 30.3$ cm) compared to small body size charr ($L_\infty = 17.7$ cm) (Table 5). In addition, estimates of k were higher for large body size charr (1.1) than for small body size charr (0.5) (Fig. 7B).

DISCUSSION

It is clear that morph pairs in both Lochs Awe and Tay comprise separate gene pools, supporting Hypothesis 1. Although there was no evidence of temporal or spatial segregation of the large and small body size morphs from Loch Tay at spawning time (Adams *et al.*, 2006), the morph pair was clearly and highly genetically differentiated, with the large body size morph almost fixed for a haplotype absent from the small body size charr.

Conversely, the genetic separation between the sympatric morph pair in Loch Awe was much less substantive relative to Loch Tay. As might be expected from their temporal reproductive isolation, they differed significantly in haplotype frequencies, although the low F_{ST} (0.03) between the two morph groups in Loch Awe indicate a much more subtle genetic divergence than between the Tay morph pair ($F_{ST} = 0.40$) or the three morphs from Loch Rannoch (pairwise $F_{ST} = 0.16$–0.49) (Verspoor *et al.*, 2010).

The data reported in the present study strongly suggest a different post-glacial origin for each of the two sympatric morph pairs. ME cluster analysis and MDS analysis both indicate that the small body size charr morph from Loch Tay is more closely related to the Loch Rannoch planktivorous morph (and that the large body size morph from Loch Tay is more closely related to the Loch Awe morph pair) than they are to

each other. By contrast, ME and MDS analyses indicate a very close genetic relationship between the Loch Awe morph pair.

The most parsimonious interpretation of the patterns shown here is that the divergence of the two morph-pairs in the two sites arose through different routes. Specifically that the morph pair in Loch Awe arose *in situ* (in sympatry within Loch Awe) and relatively recently (post-glacial) (Wilson *et al*., 2004; Adams *et al*., 2008; Verspoor *et al*., 2010). By contrast, the results are more consistent with between-morph genetic divergence in Loch Tay resulting from two genetically different, post-glacial colonizing groups and that these groups diverged much earlier than those from Loch Awe. Thus, our second hypothesis is rejected.

This conclusion not only reinforces a small but growing body of evidence of sympatric divergence of genetically, ecologically, and morphologically discrete fishes in northern post-glacial lake systems (Gíslason *et al*., 1999; Adams *et al*., 2008; Verspoor *et al*., 2010), but also provides support for an alternative hypothesis for the origin of sympatric morphs (i.e. multiple post-glacial invasions; Behnke, 1972) and supports the conclusion of Wilson *et al*. (2004), based on microsatellite data indicating that the two populations of charr in Loch Tay are not monophyletic.

The co-existing, morph pairs in both Loch Awe and Loch Tay show clear evidence of separation in trophic ecology; $\delta^{13}C$ and $\delta^{15}N$ differed significantly between co-existing morphs at both study sites. Thus hypothesis 3 is supported.

There were small differences (3 months) in the timing of sampling of muscle tissue from the Loch Awe morphs. Muscle stable isotope values of temperate latitude salmonids in late winter have been shown to mostly reflect foraging in the previous summer feeding and growth period (Perga & Gerdeaux, 2005). In Loch Awe, the $\delta^{15}N$ of spring-spawning charr was enriched by 2.9‰ relative to that of the autumn-spawning charr. Assuming that the nitrogen isotopic baseline is similar in both foraging habitats (Post, 2002), this indicates that the spring-spawning charr consumes prey at approximately one trophic level higher than the autumn-spawning charr (Vander Zanden & Rasmussen, 1999; Post, 2002; McCarthy *et al*., 2004). Muscle of spring-spawning charr was also significantly more enriched in $\delta^{13}C$ (by 1.5‰). Although this may reflect some influence of trophic enrichment, the most likely explanation for this is that spring-spawning charr have a much greater reliance on ^{13}C-enriched littoral macro-benthic prey compared to the autumn-spawning charr, which had a greater reliance on ^{13}C-depleted pelagic zooplankton (France, 1995a, b; Vander Zanden *et al*., 2005; Harrod *et al*., 2010; Knudsen *et al*., 2011).

Between-morph segregation of foraging in Loch Tay, although evident, was not as distinct as that exhibited in Loch Awe. Small body size charr had muscle tissue $\delta^{15}N$ enriched by a mean of 1.2‰, indicating that small body size charr are feeding at a mean trophic level approximately 40% higher than large body size charr. $\delta^{13}C$ values also showed a small but significant foraging difference between morphs, with the large body size charr indicating a greater reliance on pelagic-derived carbon (i.e. more depleted in $\delta^{13}C$) than the small body size charr. However, the degree of isotopic segregation was much less marked in the Loch Tay morph pair, indicating the existence of a more discrete inter-morph foraging segregation in Loch Awe.

There was clear evidence of morphological differentiation, in both morph pairs (supporting Hypothesis 4). However, the magnitude of differences between pairs also differed between sites. In Loch Awe, autumn-spawning charr exhibited some characteristics typical of a plankton feeding specialist; a terminal mouth position, smaller eye, and deeper body, as well as some characteristics more frequently documented in a macrobenthos feeder (e.g. deeper head) (Walker, 1997; Adams *et al*., 1998; Michaud, Power & Kinnison, 2008; Harrod *et al*., 2010). In Loch Awe, the shape difference between morphs was relatively subtle, in contrast to Loch Tay, where the morphological differences between the co-existing morph pair was much more distinct (Partial Procrustes Distance 4 times larger). In Loch Tay, it was the large body size morph that exhibited characteristics more consistent with a fish specializing in feeding on plankton, a more terminal mouth position, deeper body and smaller eye (*cf.* the small body size charr) (Snorrason *et al*., 1994; Walker, 1997; Adams *et al*., 1998; Michaud *et al*., 2008).

For both study sites, there were also clear morphological differences between sexes. These shape differences were most likely the result of body shape change (principally of the abdomen), caused by egg development, and ovulation.

In both study sites, co-existing morph pairs showed clear evidence of differences in life-history traits related to growth and size, supporting Hypothesis 5. In Loch Awe, spring-spawning charr grew faster and reached older age than the autumn-spawning charr. However, these differences were relatively subtle compared to the clear and striking differences in growth and asymptotic size in the co-existing morphs from Loch Tay. Here, the large body size morph grew faster and reached a much larger asymptotic size than the small body size charr.

Although the two morph pairs most likely arose through very different routes (i.e. multiple invasions and *in situ* divergence) and, consequently, the time

since divergence is likely to differ, the charr morph pairs in Loch Tay and Loch Awe show considerable evidence of parallel evolutionary divergence. Loch Awe autumn-spawning charr and Loch Tay large body size charr both show evidence of specializing in plankton feeding (morphology and stable isotopes analysis). By contrast, the Loch Awe spring-spawning charr and the Loch Tay small body size charr show evidence of littoral benthic invertebrate foraging specialism, albeit that the within-lake differences are considerably more subtle in more recently separated morphs of Loch Awe than they are in the more ancient Loch Tay. These findings suggest that the effect of available foraging opportunities (Garduno-Paz & Adams, 2010) may be at least as important as genetic origin in structuring sympatric divergence in post glacial fishes with high levels of phenotypic plasticity (Adams, Woltering & Alexander, 2003; Adams & Huntingford, 2004). The independent evolution of similar alternative phenotypes in distinct evolutionary lineages (Loch Awe in sympatry and Loch Tay in allopatry) represent the unfettered ability of natural selection to produce optimal solutions to problems repeatedly posed by the environment.

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