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SYMPOSIUM

Quantitative Genetic Variation in Static Allometry in the Threespine Stickleback

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Synopsis The common pattern of replicated evolution of a consistent shape—environment relationship might reflect selection acting in similar ways within each environment, but divergently among environments. However, phenotypic evolution depends on the availability of additive genetic variation as well as on the direction of selection, implicating a bias in the distribution of genetic variance as a potential contributor to replicated evolution. Allometry, the relationship between shape and size, is a potential source of genetic bias that is poorly understood. The threespine stickleback, *Gasterosteus aculeatus*, provides an ideal system for exploring the contribution of genetic variance in body shape allometry to evolutionary patterns. The stickleback system comprises marine populations that exhibit limited phenotypic variation, and young freshwater populations which, following independent colonization events, have often evolved similar phenotypes in similar environments. In particular, stickleback diversification has involved changes in both total body size and relative size of body regions (i.e., shape). In a laboratory-reared cohort derived from an oceanic Alaskan population that is phenotypically and genetically representative of the ancestor of the diverse freshwater populations in this region, we determined the phenotypic static allometry, and estimated the additive genetic variation about these population-level allometric functions. We detected significant allometry, with larger fish having relatively smaller heads, a longer base to their second dorsal fin, and longer, shallower caudal peduncles. There was additive genetic variance in body size and in size-independent body shape (i.e., allometric elevation), but typically not in allometric slopes. These results suggest that the parallel evolution of body shape in threespine stickleback is not likely to have been a correlated response to selection on body size, or vice versa. Although allometry is common in fishes, this study highlights the need for additional data on genetic variation in allometric functions to determine how allometry evolves and how it influences phenotypic evolution.

Introduction

Locomotor performance is a major determinant of fitness in fishes, affecting success in feeding, dispersal, courtship, and avoidance of predators (Videler 1993; Plaut 2001; Walker et al. 2005). Variation in swimming performance is correlated with variation in morphology (Hanson et al. 2007; Higham 2007; Rincon et al. 2007; Langerhans 2008; Blake et al. 2009), suggesting selection on morphology through locomotor function. Replicated evolution of the same body shape-environment relationship in independently evolving taxa (Klingenberg and Ekau 1996; Lu and Bernatchez 1999; Taylor 1999; Langerhans and DeWitt 2004; Schluter et al. 2004) provides further evidence of selection on fish body shape. Replicated phenotypic evolution is often attributed to the deterministic role of directional selection (Endler 1986; Schluter 2000). However, evolution depends on the relative distribution of additive genetic variance and covariance, as well as the direction of selection (Lande 1979; Arnold et al. 2001; Hansen and Houle 2008; Kirkpatrick 2009; Walsh and Blows 2009). Therefore, repeated phenotypic evolution might also be a consequence of a bias in the

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distribution of genetic variance (Schluter 1996). The role of genetic bias in the repeated evolution of fish body shape is not known.

Pervasive pleiotropy, where the same genetic variant affects multiple traits, suggests that organismal traits are not independent entities in a genetic or evolutionary sense, and the number of independent traits is far fewer than the number of traits measured (Dickerson 1955; Johnson and Barton 2005; Walsh and Blows 2009). There is empirical support for this expectation; although all individual traits are associated with additive genetic variation, certain multivariate combinations of those traits are associated with little genetic variation (Blows et al. 2004; Hunt et al. 2007; Kirkpatrick 2009). To understand how a biased distribution of genetic variation contributes to patterns such as repeated evolution, empirical studies need to focus on the extent to which genetic variation is unevenly distributed across different multivariate combinations of traits (Kirkpatrick 2009), and the genetic independence of individual traits (Hansen and Houle 2008).

Allometry, the scaling relationship between overall size and the size of particular body parts, is a potential source of pleiotropic genetic bias that has been inadequately studied but which might have a substantial affect on evolutionary trajectories. Allometric functions can be described by two parameters, elevation and slope, and three types of allometry are generally recognized: ontogenetic, static and evolutionary (Gould 1966; Cheverud 1982; Shingleton et al. 2007). Ontogenetic allometries are growth curves, describing the change in shape as a function of age or size, and measured either longitudinally (repeated measures of individuals as they grow) or in cross-section [sampling individuals of different ages (or sizes) from a population]. In contrast, evolutionary allometry describes the variation among taxa in the shape–size relationship of individuals of the same age or stage. Finally, static allometry describes the variation in shape as a function of size among individuals of the same age or stage within one population. These types of allometry are related hierarchically: evolutionary allometry is caused by variation in static allometry among taxa, and static allometry is caused by variation in ontogenetic allometry within a population (Shingleton et al. 2007). Here, we were particularly interested in static allometry because most studies of selection or divergence compare fitness or phenotypes of individuals of the same age or stage.

Despite the potential importance of allometry for understanding patterns of replicated evolution, there is little information on the genetic variation in static allometry. Genetic correlations between size and shape have been estimated in several studies (Hlusko et al. 2006). Similarly, artificial selection experiments have provided evidence of genetic correlation between size and shape at a given age or size (Wilkinson 1993; Frankino et al. 2005; Okada and Miyatake 2009), although no such studies have been conducted in fish. Importantly, no studies have provided estimates of additive genetic variances in the allometric parameters of elevation and slope.

As recently discussed by Hansen and Houle (2008), the approach taken to characterize genetic variation must explicitly relate to the evolutionary theory used to predict phenotypic evolution. Although a range of approaches have been utilized to characterize allometry, these studies have typically focused on the mean relationship. Furthermore, the analytical approaches employed in these studies are not amenable to estimating the phenotypic, environmental or genetic variation about that mean relationship. In contrast, the function-valued approach is a particularly useful method for studying allometry (Kirkpatrick and Heckman 1989; Gomulkiewicz and Kirkpatrick 1992; Meyer and Kirkpatrick 2005; Dieckmann et al. 2006). Shape can be described as some function of the continuously-distributed variables of size or age, and is therefore a function-valued trait (Kingsolver et al. 2001). Random coefficient modeling, which is conceptually equivalent to analysis of covariance (ANCOVA), is a statistically efficient and informative approach for analyzing genetic variation in function-valued traits (Meyer and Kirkpatrick 2005; Wilson et al. 2005; McGuigan et al. 2008). This approach has recently been applied in evolutionary quantitative genetic studies of ontogenetic allometry (Ragland and Carter 2004; Wilson et al. 2005), but has yet to be extended to static allometry.

The threespine stickleback (*Gasterosteus aculeatus*) is an ideal organism with which to investigate the genetic variation in allometry of body shape. This small fish is holarctically distributed as two life-history forms, an ancestral oceanic type and a derived freshwater form. Although the oceanic form is phenotypically similar throughout its range, and has been so for millions of years, freshwater stickleback have undergone rapid diversification after colonization of new freshwater habitats that appeared following the retreat of glaciers from the last glacial maximum ~18,000 years ago (Bell and Foster 1994; Taylor and McPhail 2000; Cresko et al. 2007; Wootton 2009). This large natural experiment has made stickleback the focus of considerable research. Diversification of freshwater stickleback has involved

the evolution of many aspects of phenotype, including swimming performance (Taylor and McPhail 1986; Law and Blake 1996; Bergstrom 2002; Schaarschmidt and Jurss 2003; Blake et al. 2005; Tudorache et al. 2007), size (McPhail 1977; Snyder 1991; Nagel and Schluter 1998; Wright et al. 2004), and body shape (Walker 1997; Walker and Bell 2000; Leinonen et al. 2006; Sharpe et al. 2008; Aguirre 2009).

Relative to contemporary marine populations, freshwater fish are smaller (McPhail 1977; Snyder 1991), have relatively large heads, shorter bases to the median fins, and longer caudal peduncles (Walker and Bell 2000; Schluter et al. 2004; Leinonen et al. 2006; Aguirre 2009). They also tend to perform better at burst (anaerobic) swimming, but worse at sustained (aerobic) swimming (Taylor and McPhail 1986; Schaarschmidt and Jurss 2003; Blake et al. 2005). Within freshwater environments, two major axes of phenotypic diversity have been identified and related to variation in predators and in diet. Freshwater populations sympatric with predatory fish and feeding on planktonic prey are more similar to the marine (ancestral) phenotype than are populations with a benthic diet in the absence of predatory fish (Schluter 1993; Cresko and Baker 1996; Walker 1997; Foster et al. 1998; Walker and Bell 2000; Wootton 2009). The relative contribution of parallel selection versus genetic bias to these patterns of repeated evolution of body shape in stickleback is unknown.

Stickleback body size appears to be under both natural and sexual selection (McPhail 1977; Nagel and Schluter 1998; McKinnon et al. 2004), raising the potential for correlated changes in shape if size and shape are genetically correlated. Separate analyses of size (Snyder and Dingle 1989; Snyder 1991) and shape (Baumgartner 1995) indicate genetic variance in these traits, but provide no insight into how they co-vary. In contrast to the lack of information on the genetic basis of allometry, several phenotypic-level studies have considered ontogenetic or evolutionary allometry of threespine stickleback (Walker 1993; Walker 1997; Wright et al. 2004). The head and the posterior region of the body appear to have particularly strong allometry. Walker (1993) found that within a population, larger fish had relatively larger heads with longer snouts, and posterior-shifted insertions of the median fins. Across populations, larger fish also had relatively long bases to their median fins and short caudal peduncles, but had relatively small heads and short snouts (Walker 1997).

In this article, we describe the static allometry of body shape in a population of laboratory-reared fish bred from wild-caught anadromous parents from Rabbit Slough in south-central Alaska. This population has been intensively studied, yielding ample morphological and genetic data that it is representative of the marine ancestor from which local freshwater populations were derived (Walker and Bell 2000; Aguirre et al. 2008; Hohenlohe et al. 2010). Applying random coefficient modeling to data from a paternal half-sib breeding design, we estimated the additive genetic variation for allometry of body shape. This experiment was designed to address the general question of whether strong genetic covariation of body shape and size in this putative ancestral population could have contributed to the repeated pattern of co-evolution of size and shape in derived freshwater populations.

Materials and methods

Breeding design and sample collection

Adult males and females in breeding condition were collected in minnow traps from Rabbit Slough (61°32'N, 149°15'W, elev. 5 m) in the Matanuska-Susitna Valley of south-central Alaska in June 2004. Live fish were immediately transported to the University of Alaska, Anchorage and held in large outdoor tanks (1 m deep by 1.5 m diameter) supplied with local well water. All fish were processed within 3 days of collection. Females were stripped of their egg clutches; males were euthanized, and subsequently dissected to remove testes. Eggs and macerated testes were combined in a paternal half-sib breeding design in which one male was used to fertilize two randomly chosen females. Eggs and macerated testes were placed in a Petri dish 45 mm in diameter and left for 5–10 min. Eggs were then washed with stickleback-embryo media (Cresko et al. 2004) and clutches disassociated. A total of 104 half-sib families (52 sires mated to 104 dams) were generated. Fertilized clutches were maintained in Petri dishes at 20°C and washed daily with embryo media until 3 days postfertilization (dpf). At this point, major organogenesis has occurred. Embryos were then transferred to 50 ml tubes of embryo media, cooled on ice, and transported the same day to the University of Oregon, Eugene.

Upon arrival, clutches were transferred to 90-mm Petri dishes and placed at 20°C in a thermally stable refrigerating incubator. Each day, dead embryos and (when hatching commenced) chorion remnants were removed, and the embryo medium was replaced. At 20°C stickleback hatch at 7 dpf and finish

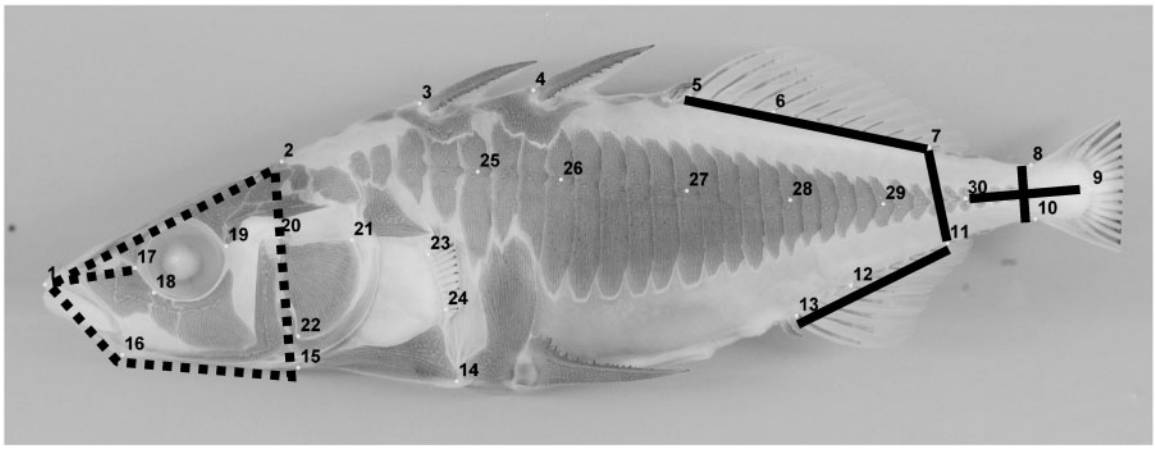

Fig. 1 The position of the 30 landmarks used in to characterise body shape and size of threespine stickleback in this study. These landmarks were: (1) anterior tip of upper lip; (2) supraoccipital notch immediately lateral to dorsal midline (DML); (3) anterior junction of first dorsal spine at DML; (4) anterior junction of second dorsal spine at DML; (5) anterior base of first dorsal fin ray at DML; (6) anterior base of the fifth dorsal fin ray at DML; (7) anterior base of the last dorsal fin ray at DML; (8) origin of caudal fin membrane on the DML; (9) caudal border of hypural plate at the lateral midline; (10) origin of caudal fin membrane at the ventral midline (VML); (11) posterior base of last anal fin ray at the VML; (12) anterior base of the fifth dorsal fin ray at the VML; (13) anterior base of first anal fin ray on the VML; (14) posterior and; (15) anterior tip of the ectocoracoid; (16) posterior tip of the angular; (17) ventral tip of the lateral ethmoid at the eye socket; (18) intersection of ventral tip of the lachrymal bone and the dorsal tip of the second orbital at the eye socket; (19) ventral tip of the sphenotic at the eye socket; (20) dorsal joint of the opercle bone; (21) dorsal/posterior vertex of the opercle bone; (22) ventral/anterior vertex of the opercle bone; (23) anterior base of the most dorsal pectoral fin ray; (24) anterior base of the most ventral pectoral fin ray; (25) midpoint of the first supporting plate; (26) midpoint of the last supporting plate; (27) midpoint of the fifth posterior lateral plate (LP); (28) midpoint of the 10th posterior LP; (29) midpoint of the 15th posterior LP and; (30) the 20th posterior LP. Linear measures used in analyses, defined by their terminal landmarks, were: dorsal length of the head (L1.2), length of the jaw (L1.16), length of the snout (L1.17), depth of the head (L2.15) ventral length of the head (L15.16), the length of the base of the second dorsal (L5.7) and anal (L11.13) fins, depth of the caudal peduncle at the insertion of the median fins (L7.11), depth of the caudal peduncle at the insertion of the caudal fin (L8.10), and length of the caudal peduncle measured on the midline (L9.30). Traits analysed in the multivariate analysis of head allometry are shown in dashed lines and traits included in analysis of the posterior region of the body are shown in solid lines.

absorbing their yolks by 10 dpf. At 10 dpf fish were transferred to 4 l tanks with ~500 ml of water, and fed on nauplii of brine shrimp. Each clutch was split between two tanks, with all tanks in the same room, arranged on five racks. Families were randomly assigned to tanks throughout the room to minimize common environment effects on full sibs. At 13 dpf the volume of water in the tanks was increased to 4 l over 8 h. Fish continued to be fed once a day on brine shrimp, which was supplemented with flake food at 2 months. A proportion (10%) of the water was changed daily by the addition of water that was purified by reverse osmosis followed by addition of a small amount of Instant Ocean® Sea Salt to produce water with salinity ~1 ppt. Fish were reared in this relatively low salinity to mimic colonization of freshwater lakes by anadromous fish. For all of the crossing and rearing methods presented above, detailed protocols are available at: http://stickleback.uoregon.edu/index.php/.

At 20 dpf, the density of fish was reduced to 15 fry per tank (30 per family). Further density controls were implemented at 1 (10 fish per tank) and 2 months (5 fish per tank). At 3 months, the experiment was terminated and 5 fish per tank were sampled. There was low natural mortality throughout the experiment, and a total of 1023 fish from the 104 families (52 sires) were available to phenotype. Sampled fish had a mean standard length [SL; the distance between Landmark 1 (LM1) and Landmark 9 (LM9) in Fig. 1] of 27 mm, a size at which the development of bony elements is complete (Hagen 1973; Bell 1981), making it easy to identify landmarks and measure body shape.

Fish were euthanized with an overdose of a tricaine methane sulphonate solution, following University of Oregon approved IACUC protocols, and preserved in 10% formalin. To visualize bony structures, fixed fish were cleared using trypsin and stained with Alizarin Red (Potthoff 1984; Cresko et al. 2004). Fish were photographed by a single technician using a tripod-mounted Nikon D70 camera with a Nikor macro lens. Sampling order was random with respect to rearing tank. Fish were immobilized and imaged on a common background in left lateral view. From these images, the positions

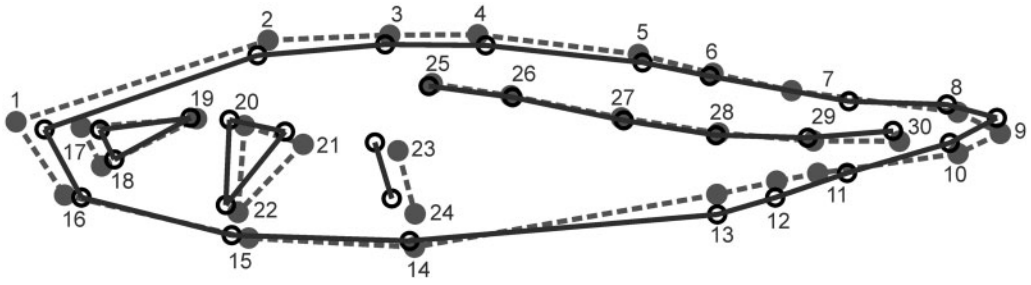

Fig. 2 An illustration of the phenotypic allometric variation in threespine stickleback in this study. This figure is based on a discriminant function analysis between the 10 largest (black outline) and 10 smallest (gray outline) fish in the study, applied to the aligned landmark coordinates using MorphoJ (Klingenberg 2008). The figure is scaled to 1.5× divergence. Note that this figure is for illustrative purposes only.

of 30 landmarks (Fig. 1) were recorded using tpsDig2 (Rohlf 2005). Landmarks were aligned, and centroid size (CS) was calculated using the default settings in tpsRelW (Rohlf 2005). Following alignment, linear distances between aligned landmarks were estimated using Morpheus (Slice 1998).

Our analyses focused on the two body regions that were the most variable in previous studies: head and the posterior of the fish (Fig. 1). In our study population, much of the phenotypic variation among fish of different sizes also appeared to occur in these body regions (Fig. 2). Head and the posterior region were each representing by five linear traits for analysis (Fig. 1). The limit of five traits per body region was dictated by computational constraints. Random coefficient modeling involves estimating the elevation (i.e., the shape at a given size, which is the size-independent shape variation), slope (i.e., the rate of change in shape with size), and covariation between these two parameters. Five traits require a 10×10 covariance matrix to be estimated, which is computationally demanding. All traits were standardized (mean = 0, standard deviation = 1) prior to analysis to aid convergence of models, which perform poorly if traits are on different scales. In preliminary analyses, we considered the consequences of our choice of traits, the use of CS (rather than SL) as the measure of size, linear rather than nonlinear allometry, and the standardization approach. Conclusions were robust to these analytical choices. Log transformation is used in ontogenetic or evolutionary allometry studies to render relationships linear, but was not needed here as the relationship was already linear over the relatively limited range of sizes represented in the data.

Data analysis

Genetic parameters were estimated using random coefficient modeling implemented in Proc Mixed within a restricted maximum likelihood framework using SAS (v. 9.02). The model:

$$\mathbf{y}_{jkl} = \alpha + \mathrm{X}_{jkl} b + \mathbf{Z}^{(\mathrm{t})}_{jkl}\boldsymbol{\delta}^{(\mathrm{t})}_{jkl} + \mathbf{Z}^{(\mathrm{d})}_{jkl}\boldsymbol{\delta}^{(\mathrm{d})}_{kl} + \mathbf{Z}^{(\mathrm{s})}_{jkl}\boldsymbol{\delta}^{(\mathrm{s})}_{l} + \varepsilon \quad (1)$$

fit a population-level elevation (α) and slope (b) for CS (the only fixed effect, as described in the design matrix, $\mathbf{X}_{jkl}$). Variation about these population-level parameters is then estimated by the other, random, terms in the model. The population-level slope corresponds to the regression slope estimated in a regression analysis. In this study, the population-level elevation was set at zero by the mean standardisation of the data, and the variance about this population-mean elevation is centred on zero.

Sire, dam nested within sire, and tank nested within sire and dam were fit as random effects. Described by their respective design matrices, $\mathbf{Z}^{(\mathrm{t})}$, $\mathbf{Z}^{(\mathrm{d})}$ and $\mathbf{Z}^{(\mathrm{s})}$, the covariance matrices, $\boldsymbol{\delta}^{(\mathrm{t})}_{jkl}$, $\boldsymbol{\delta}^{(\mathrm{d})}_{kl}$ and $\boldsymbol{\delta}^{(\mathrm{s})}_{l}$, give the departure of regression slopes and elevations of the j-th tank within each k-th dam, and l-th sire, the k-th dam within each l-th sire and the l-th sire, respectively. Allometric parameters could not be estimated for individuals in this design because each individual was represented by only one measure of size and of shape. Residual variance was therefore modelled for each trait; all traits were observed to have similar levels of residual variance (see Meyer 1991 for a discussion of error structures within random coefficient modelling). Within this experimental design, the residual variance captures random environmental (biotic and abiotic) variation among siblings sharing a tank, as well as measurement error.

Our analytical approach had several steps. First, we applied the univariate form of Equation (1) to each shape trait separately. This estimated the population-level slope, as well as the 2×2 covariance matrix of elevation and slope for each random effect, and the residual variance. At the sire (additive

genetic) level, we applied separate statistical tests of the variance in elevation and in slope using log-likelihood ratio tests (LRT) to compare two hierarchical models, one in which the parameter was estimated and one in which it was held to zero (Littell et al. 1996). Covariance at the dam and tank levels were modelled with covariance structure constrained to be of full rank (2×2). This use of constrained covariance structure was necessary for model convergence, and ensured matrices at dam and tank levels were positive definite. It is likely that problems with model convergence when dam and tank covariances were unconstrained were caused by the low levels of variation in slope at these levels in the analysis.

Next, we estimated the additive genetic variance–covariance matrix, **G**, for each of the two 5-trait sets using the multivariate form of Equation (1), and applied factor analytic modelling to determine statistical support for the estimated **G** (Kirkpatrick and Meyer 2004; Hine and Blows 2006; McGuigan and Blows 2010). Factor analytic modelling involves fitting covariance structures of different rank to the data, and comparing the fit of hierarchical models using LRTs. Statistically supported matrix rank reflects power (Meyer and Kirkpatrick 2008; Kirkpatrick 2009), as well as the amount of variance in individual traits and covariation among traits; increasing covariation of traits decreases the rank of the matrix. We extended the standard-factor analytic approach (Kirkpatrick and Meyer 2004; Hine and Blows 2006) by also applying covariance structures that estimated trait-specific variance (Smith et al. 2001; Meyer 2009; McGuigan and Blows 2010). These two types of covariance structure, with and without trait-specific variances, correspond to factor analysis and principal components analysis, respectively. The trait-specific variance is analogous to the conditional variance of Hansen (Hansen et al. 2003; Hansen and Houle 2008) and is considered critical for the independent evolution of traits. Again, it was necessary to constrain the covariance structure at the other levels in the model (i.e., dam and tank) to achieve model convergence. All analyses were applied with both tank and dam covariance matrices constrained to a rank of four. A rank of four at dam and tank was not a worse fit of the data than higher-rank models (data not shown), and was the highest rank at which all models at the sire-level converged.

Results

Phenotypic and genetic variation in size and univariate shape

Fish in this experiment ranged in SL from 21.12 to 36.44 mm (mean = 27.47 mm). This was a substantial variation in size among fish of the same age, reared under constant laboratory conditions. Most (81%) of the variation in size was among full-sib individuals reared in the same tank, indicating substantial micro-environmental (biotic and abiotic) effects on size. Nonetheless, there was statistically detectable additive genetic variation in CS ($V_A = 0.314$; one-tailed LRT: $\chi^2 = 6.91$, d.f. = 1, $P = 0.004$).

For most traits there was statistically significant allometry (Table 1; Fig. 2), indicating that fish of different sizes also differed in shape. Generally,

Table 1 The phenotypic allometry of body shape for the threespine stickleback in this study

	Slope	r^2	Elevation V_A (h^2)	Slope V_A (h^2)	r_G
L1.2	−0.505*	0.203	0.265* (0.22)	0.024 (0.02)	−0.500
L1.16	−0.223*	0.257	0.498* (0.40)	0.074 (0.06)	−0.361
L1.17	−0.186	0.055	0.326* (0.25)	0.000 (0.00)	−1.000
L2.15	−0.280*	0.057	0.397* (0.27)	0.054 (0.04)	−0.714
L15.16	−0.225*	0.064	1.148* (0.91)	0.045* (0.04)	−0.217
L5.7	0.299*	0.141	0.456* (0.43)	0.062 (0.06)	−0.233
L7.11	−0.167*	0.081	0.470* (0.44)	0.024 (0.02)	−0.312
L8.10	0.002	0.001	0.016 (0.01)	0.139* (0.09)	−1.000
L11.13	−0.018	0.000	0.488* (0.35)	0.000 (0.00)	0.089
L9.30	0.267*	0.117	0.522* (0.47)	0.023 (0.02)	−0.485

The regression slope, and the variance explained by the model, r^2- are given, along with the additive genetic variance (V_A) (and heritability, h^2) about that population mean elevation[a] and slope, and the additive genetic correlation (r_G) between elevation and slope. See Fig. 1 for definitions of traits.

[a]The population mean elevation is not shown because it was zero for all traits due to the standardization of data prior to analysis.

*$P < 0.05$.

larger fish had relatively smaller heads and more posterior insertions of their median fins, similar to the evolutionary allometry pattern observed among Alaskan populations of threespine stickleback (Walker 1997). Although allometric slopes were significant for most traits, the amount of variance in shape explained by size was typically small to moderate, ranging from 2% to 25% (Table 1). This indicates that fish of the same size varied considerably in shape.

Additive genetic variance existed for allometric elevation for most traits in this study (Table 1), indicating the presence of genetic variance for shape that was independent of the genetic variance for size. In contrast, there was little additive genetic variation in allometric slopes (Table 1), suggesting that for most traits there was little genetic variance for the rate at which shape changed with size (Fig. 3A). In contrast to this general pattern, there was significant genetic variance in the allometric slope of the posterior measure of caudal depth (L8. 10) (Table 1; Fig. 3B), suggesting segregation of alleles that increased and that decreased caudal depth with size in this population.

Genetic variation in multivariate allometry

Allometry of the head

The univariate analyses of head traits provided statistical support for genetic variance in all elevations but not in the slopes, suggesting a head-trait variance–covariance matrix, **G**, with a rank of five or less. In fact, we had statistical support for only two of the ten dimensions of the head-trait **G** (LRT of model fit from two to one dimensions: $\chi^2 = 15.79$, d.f. = 9, $P = 0.071$). These two dimensions accounted for 80% of the total additive genetic variation in allometry of the head, with a further two dimensions accounting for the remaining 20% of the genetic variance (Table 3). Kirkpatrick (2009) discussed the problems with statistical estimation of matrix rank, and proposed the effective number of dimensions (the sum of eigenvalues divided by the largest eigenvalue) as a metric for describing matrix rank. The effective dimensionality of the head-trait **G** was 1.8, within the range of values observed across datasets surveyed by Kirkpatrick (2009) albeit somewhat high. The relatively low dimensionality of the head-trait **G** (far less than the 10 traits measured) reflected both the lack of variation in allometric slopes, and also the covariation among allometric elevations of different traits.

Most (56%) of the additive genetic variance in the head was associated with $\boldsymbol{g}_1$, which described increased/decreased head length, with the strongest contribution from the ventral measure of head length (L15.16) (Table 3). The variance in this first genetic factor (1.58) was greater than for any individual trait (Table 1), consistent with contributions from multiple traits due to genetic covariance among them. Although contributing little to $\boldsymbol{g}_1$, slopes were opposite to the elevations for most traits (Table 3), consistent with the observation from the univariate analyses (Table 1). Variance in the second genetic factor ($\boldsymbol{g}_2$) was also larger than in individual traits (Tables 2 and 3), further evidence of prevalent covariation among traits. $\boldsymbol{g}_2$ described negative covariation between the ventral measure of the length of

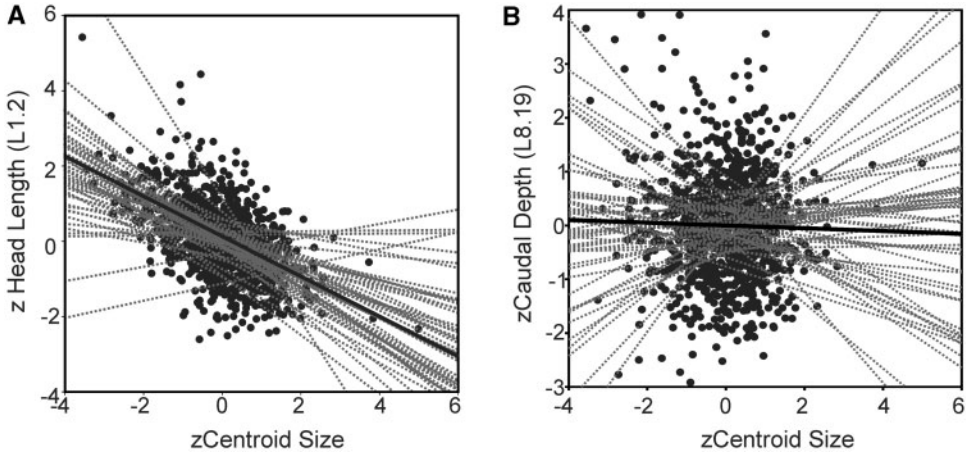

Fig. 3 Example of allometric patterns in the threespine stickleback. Allometry of (**A**) the dorsal measure of head length (L1.2) and (**B**) the posterior measure of caudal depth (L8.10). Points represent each of the 1023 individuals in the analyses. The solid dark line represents the population-level mean slope [*b* in Equation (1)]. Dashed grey lines are slopes for each of the 52 sires, illustrating variation in allometric slope about the (black line) population mean.

Table 2 The additive genetic variance (in bold on diagonal) and covariance (off-diagonal entries) matrix, **G**[a], for allometry (described by the elevation and slope parameters) of head shape in threespine stickleback

	Elevation					Slope				
	L1.2	L1.16	L1.17	L2.15	L15.16	L1.2	L1.16	L1.17	L2.15	L15.16
L1.2	**0.261**	0.130	0.281	0.221	0.363	−0.039	−0.092	−0.091	−0.006	0.048
L1.16		**0.513**	0.286	0.155	−0.027	0.056	−0.038	0.049	0.061	0.012
L1.17			**0.356**	0.217	0.347	−0.018	−0.094	−0.065	0.027	0.042
L2.15				**0.447**	0.123	−0.020	−0.094	−0.081	−0.086	0.081
L15.16					**0.964**	−0.201	−0.264	−0.224	−0.091	−0.023
L1.2						**0.064**	0.075	0.055	0.056	0.020
L1.16							**0.114**	0.069	0.070	0.018
L1.17								**0.067**	0.047	−0.005
L2.15									**0.086**	0.011
L15.16										**0.034**

See Fig. 1 for definitions of traits.
[a]Estimated from a model with the covariance matrices at all levels constrained to a rank of four.

Table 3 Eigenanalysis of **G** (Table 2) of allometry of head shape in threespine stickleback

	g_1	g_2	g_3	g_4
V_A	1.580	0.794	0.368	0.163
Total V_A (%)	55.8	28.1	13.0	5.7
L1.2	0.38	−0.12	0.07	0.39
L1.16	0.17	−0.69	−0.41	−0.43
L1.17	0.40	−0.31	−0.18	0.26
L2.15	0.29	−0.35	0.77	−0.04
L15.16	0.70	0.46	−0.24	−0.02
L1.2	−0.12	−0.18	−0.05	0.28
L1.16	−0.22	−0.08	−0.05	0.46
L1.17	−0.17	−0.13	−0.14	0.00
L2.15	−0.07	−0.12	−0.30	0.46
L15.16	0.02	−0.09	0.16	0.31

The additive genetic variance (V_A) and the proportion of the total V_A associated with each eigenvector ($\boldsymbol{g_1}$ to $\boldsymbol{g_4}$) are shown, along with the trait loadings on each eigenvector. Eigenvectors were normalised to unit length ($\boldsymbol{g'g} = 1$). See Fig. 1 for definitions of the traits.

the head (L15.16) and the length of the jaw (L1.16), consistent with these two traits being based on the same landmark. Shifts in this landmark will affect both traits antagonistically.

The Akaike Information Criteria (AIC) supported the model that estimated two common genetic factors ($\boldsymbol{g_1}$ and $\boldsymbol{g_2}$ described above) plus trait-specific variances as a better fit of the data than a model without trait-specific variances (two dimensional model AIC for no specific variance: 11965.6 versus specific variance: 11964.9). The only trait for which trait-specific variance was greater than zero was depth of the head (L2.15), for which specific additive genetic variance (0.111) was 28% of the total additive genetic variance in that trait. This independent genetic variation in the depth of the head was captured by $\boldsymbol{g_3}$ of the four common (no trait-specific variance) factor model (Table 3).

A potential statistical benefit of analysing allometry in a multivariate framework is that, if there are common genetic factors, the increased variance associated with these composite traits will be easier to detect. There was little indication that this was the case for allometric slopes, but to gain a better understanding of the possible genetic variance in slopes we divided **G** (Table 2) into two 5×5 sub-matrices, one associated only with elevation and the other associated only with slopes, and subjected these sub-matrices to eigenanalyses. The eigenanalysis of elevation closely reflected that of the total **G**. This was expected because most variance was in elevation, and we do not re-interpret it here. The first eigenvector of the slope sub-**G** explained 76% of the variance in slopes, and 10% of the total **G**. This factor described positive contributions from all slopes. This pattern of trait contributions was observed in the contribution of slopes to $\boldsymbol{g_4}$ (Table 2). The similar contribution from slopes of different traits was suggestive of a latent genetic factor with a general effect on allometry of the head, causing all measured aspects of the head to change simultaneously with size. Nonetheless, we cannot demonstrate statistically that such a factor exists.

Allometry of the posterior part of the body

Similar to the allometric analysis of head shape, only four factors were required to explain the total

Table 4 The additive genetic variance (in bold on diagonal) and covariance (off-diagonal entries) matrix, **G**[a], for allometry (described by the parameters elevation and slope) of the posterior part of the body in threespine stickleback

	Elevation					Slope				
	L5.7	L7.11	L8.10	L11.13	L9.30	L5.7	L7.11	L8.10	L11.13	L9.30
L5.7	**0.492**	−0.400	0.120	0.176	0.414	−0.035	0.014	−0.167	−0.046	−0.022
L7.11		**0.477**	−0.091	−0.095	−0.348	0.031	−0.064	0.036	0.026	0.018
L8.10			**0.047**	0.126	0.127	−0.010	−0.003	−0.050	−0.013	−0.002
L11.13				**0.459**	0.268	−0.021	−0.032	−0.115	−0.025	0.008
L9.30					**0.447**	−0.079	0.058	−0.149	−0.065	−0.061
L5.7						**0.041**	−0.041	0.017	0.024	0.040
L7.11							**0.062**	0.024	−0.019	−0.042
L8.10								**0.126**	0.027	0.013
L11.13									**0.017**	0.023
L9.30										**0.041**

See Fig. 1 for definitions of traits.
[a]Estimated from a model with the covariance matrices at all levels constrained to a rank of four.

Table 5 Eigenanalysis of **G** (Table 4) of allometry of the posterior part of the body in threespine stickleback

	g_1	g_2	g_3	g_4
V_A	1.456	0.432	0.162	0.159
Total V_A (%)	66.1	19.6	7.4	7.2
L5.7	0.55	−0.19	−0.17	0.46
L7.11	−0.48	0.48	0.32	0.42
L8.10	0.16	0.13	−0.06	−0.02
L11.13	0.33	0.80	−0.15	−0.36
L9.30	0.54	0.05	0.37	−0.04
L5.7	−0.07	0.01	−0.45	0.09
L7.11	0.04	−0.17	0.43	−0.31
L8.10	−0.18	−0.20	−0.14	−0.61
L11.13	−0.06	−0.01	−0.26	−0.04
L9.30	−0.04	0.05	−0.48	0.04

The additive genetic variance (V_A) and the proportion of the total V_A associated with each eigenvector ($\boldsymbol{g}_1$ to $\boldsymbol{g}_4$) are shown, along with the trait loadings on each eigenvector. Eigenvectors were normalised to unit length ($\boldsymbol{g}'\boldsymbol{g} = 1$). See Fig. 1 for definitions of the traits.

additive genetic variation in the posterior region of the fish (Tables 4 and 5). Again, we had statistical support for just two dimensions (LRT of model fit from two to one dimension: $\chi^2 = 18.54$, d.f. = 9, $P = 0.029$), which explained 75% of the total genetic variance. The effective dimensionality of this set of traits (1.5) was again considerably less than the number of measured traits, but within the range observed by Kirkpatrick (2009).

The first eigenvector of the four-dimension **G** accounted for 66% of the total variance, and was again associated with considerably greater variance than for any individual trait (Tables 4 and 5). $\boldsymbol{g}_1$ described anterior-posterior variation in the position of landmarks 7 and 11 (Table 5). The second factor of **G** described positive covariation between elevations of the anterior measure of caudal peduncle depth (L7.11) and of caudal peduncle length (L9.30) (Table 5).

AIC supported a model with one common factor and trait-specific variances over a model with two common factors (AIC for no specific variance: 12743.9; AIC for specific variance: 12740.1). Trait-specific genetic variance existed in allometric elevation of the anterior measure of the depth of the caudal peduncle and caudal length (29 and 55% of the variance in these traits, respectively). There was also independent genetic variance in the slope of the posterior measure of the depth of the caudal peduncle (66% of the variance).

We again explored the genetic variance in slopes further by subdividing **G** into two sub-matrices corresponding to elevation and slope. Because of the similarity of the elevation sub-**G** to the full **G** (as with the head result) we do not re-interpret it here. The eigenanalysis of the slope sub-**G** identified two factors that explained 7 and 6% of the total variance in **G**, respectively. The first of these mirrored the first eigenvector of the total (elevation) **G**, contrasting the anterior measure of the depth of the caudal peduncle with the other traits (see the slope portion of $\boldsymbol{g}_3$ in Table 5). This pattern suggested that a latent genetic factor might contribute variation to both allometric elevation and slope. The second major axis of the slope sub-**G** was determined

by similar contributions from both measures of caudal depth, a pattern observed in $\boldsymbol{g}_4$ of the full **G** (Table 5). This suggests a latent genetic factor contributing variation to the shape of the relationship between overall size and the depth of the caudal peduncle, both anterior and posterior measures of it. Positive covariation of the two measures of caudal depth was not detected for allometric elevation.

Discussion

Allometry might be a general source of bias in the evolution of size and shape, affecting the phenotypic distribution of variation upon which selection can act, and the independent genetic variation available for an evolutionary response to that selection. However, there is relatively little information on the variation in static allometry in populations, and it is currently not known whether the theoretical biasing effects of allometry contribute significantly to observed patterns of phenotypic evolution. In a population of threespine stickleback from Alaska, we observed statistically significant, but relatively weak, phenotypic static allometry in the shape of the head and the posterior region of the body. Therefore, although fish of different size also differed in shape, there was a range of shapes associated with a given size (and vice versa), providing ample variation upon which selection could act to change the relationship between size and shape. Whether the relationship responds to selection depends on the genetic variation in allometry.

At the genetic level, the potential impact of static allometry on evolution can be considered from two perspectives. First, if much of the variation in shape is shared with size, correlated phenotypic evolution of size and shape might be expected, potentially biasing evolutionary trajectories. For example, previous work on stickleback has shown than size is under directional selection (Nagel and Schluter 1998) and may be important in sexual selection (McKinnon et al. 2004). Shape, and therefore swimming and foraging performance, may exhibit a correlated response to this selection on size. However, we found that the general pattern was for genetic variance in allometric elevation, but not in slope. Therefore, genetic variance in shape was typically independent of size, leading us to the same conclusion as Walker (1997); the observed evolutionary codiversification of size and shape in stickleback is unlikely to reflect allometric bias.

Surprisingly, despite being a model organism for evolutionary studies, genetic variance in the body shape of stickleback has been estimated in only one previous study (Baumgartner 1995). Our estimates of heritability (mean $h^2 = 0.37$) were similar to those observed in the Californian population (mean $h^2 = 0.26$) studied by Baumgartner, although we had greater power to detect these levels of variation. Baumgartner (1995) took a common approach, standardizing traits describing shape to mean size prior to estimation of genetic parameters. Although our result of a lack of genetic heterogeneity of allometric slopes supports this as an appropriate approach, further understanding of genetic variation and evolution of allometry depends on explicit estimation of the allometric relationships. Notably, for the one trait in this study with substantial genetic variance in allometric slope there was little evidence of phenotypic allometry. Heterogeneity of slopes resulted in a population-level mean slope of zero for the anterior measure of the depth of the caudal peduncle. This result suggests the further caution in using allometry at the phenotypic level as the criterion for determining when allometry might bias the direction of evolution (Lande 1979; Atchley and Rutledge 1980).

The distribution of size-independent shape in both the head and the posterior region of the body indicated that some traits likely shared genetic variation. In the head, all five allometric elevations were associated with additive genetic variance, but only four dimensions were required to capture all of the genetic variance in the shape of the head. Similarly, in the posterior region of the body, there was strong covariation between the length of the second dorsal and anal fins, and caudal peduncle length. These results suggest the potential for genetic covariation among traits to lead to correlated responses to selection, potentially contributing to patterns of replicated evolution. In contrast, other body-shape traits were associated with genetic variance that was relatively independent of other traits, for example, the depth of the head. Our finding of both genetic covariation and independence is consistent with a recent quantitative trait locus (QTL) mapping study of divergence in body shape between a marine and a freshwater population of stickleback (Albert et al. 2008). Most of the QTL mapped between these populations affected the position of only one landmark, but several had effects on a number of landmarks. Further information on the distribution of genetic variance in the shape of the body relative to the directions of selection or divergence are required to understand the contribution of genetic covariance to replicated evolution in threespine stickleback.

The second perspective on the genetics of static allometry is that the evolution of the allometric

relationship is dependent on genetic variance in allometric elevation, allometric slope, or both. This perspective leads to the realization that allometry might be characterized by two general, and apparently contradictory, observations: considerable variation in allometry among taxa, as described by evolutionary allometry, but limited variation in ontogenetic or static allometry within populations (Frankino et al. 2007; Shingleton et al. 2007). Many studies in fishes have estimated body-shape allometry, but there is limited data on the genetic variance in allometry parameters from which to assess whether there is typically variance in static allometry.

We detected additive genetic variation for the evolution of allometric elevations but not for allometric slopes. This pattern of genetic variance in elevation might be common; artificial selection experiments have demonstrated genetic variance in allometric elevation (Emlen 1996; Frankino et al. 2005, 2007; Okada and Miyatake 2009). We detected no genetic variance in allometric slopes, and we are not aware of any experimental attempts explicitly aimed at evolving allometric slopes. In contrast, several studies (albeit not in fishes) have detected genetic variance in ontogenetic allometric slopes (Atchley et al. 1984; Long et al. 2006; Li et al. 2007). Given this, we expected to detect additive genetic variance for static allometric slopes in this study.

There is some evidence that the genetic variation in allometry has different sources at different ages (Cheverud et al. 1996). It is possible that we would have detected genetic variance in allometric slopes had we sampled fish at a different age. There is also some evidence of differences in the genetic variance in allometry among sexes (Carreira et al. 2009). We did not determine sex in this experiment. Differences among sexes in allometric slope (but not allometric elevation) might have contributed to the lack of variance among sires. The generality of our result can only be assessed through further studies, particularly across different ages, and taking sex into consideration.

In general, explanations for limited additive genetic variance are either the depletion of variation through strong selection, or a failure to generate new variation through mutation. Our data cannot distinguish whether selection or reduced mutational replenishment might have caused the low levels of genetic variance in allometric slope. Nonadditive genetic variance might be important for allometric slopes (Pavlicev et al. 2008), but this source of variance could not be estimated in our experimental design. Importantly, quantitative genetic approaches, as utilized in this study, aim to estimate genetic variation segregating among individuals within populations. A lack of genetic variation does not imply that there is no genetic basis to the expression of the phenotype, only that all individuals in the population carry the same alleles—or alleles with equivalent effects on the phenotype of interest—at contributing loci.

Despite the strong overall trend in our study for little additive genetic variation in allometric slope, this trend was not universal. The anterior measure of caudal depth was associated with substantial additive genetic variation for allometric slope. We have no expectation for this trait to have a different developmental basis or effect on fitness such that greater variation is generated or that variation is eroded more slowly through selection. It is, therefore, difficult to explain this variation in behaviour among our traits. Further studies in other species will identify general patterns.

Information on the consequences of allometry for fitness is sparse. Following phenotypic evolution in response to artificial selection on allometric elevation, Frankino et al. (2007) demonstrated that individuals with artificially shifted allometric elevation had lower fitness than did individuals with the ancestral allometry. Although body shape divergence has been well characterised in stickleback (Walker and Bell 2000; Leinonen et al. 2006; Aguirre 2009), there is limited information on contemporary selection on shape. Similarly, size affects fitness (Nagel and Schluter 1998; McKinnon et al. 2004), there is no estimate of the selection gradient for the match of shape to size. Difficulties in estimating selection in natural populations were highlighted by a recent study on stickleback, demonstrating that the choice of microhabitat by individuals was phenotype-specific (Bolnick et al. 2009). This study suggests that the ecological scale on which the fitness consequences of phenotype are meaningful might be very small, and therefore difficult to accurately estimate. Nonetheless, estimates of selection constitute a missing link in studies of the evolution of body shape in fish, and further studies are required.

In summary, although allometry has been demonstrated previously in the threespine stickleback system, we observed no evidence of phenotypic or genetic bias in the distribution of body shape in a population that is representative of the colonists of fresh water habitats in the region. Our data therefore provide little support for a role of allometric bias in the well-documented parallel phenotypic evolution of size and shape in stickleback populations occupying similar freshwater habitats, and instead point to the role of consistent correlational selection. Further

estimates of the genetic and environmental variation in allometric elevations and slopes from a variety of taxa are required before any general conclusions can be drawn about the relative importance of allometric bias and consistent selection for replicated evolution in the wild.

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