

Nonlinear effects of temperature on body form and developmental canalization in the threespine stickleback

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Keywords:

body shape;
canalization;
development;
Gasterosteus aculeatus;
geometric morphometrics;
growth;
reaction norm;
temperature.

Abstract

Theoretical models predict that nonlinear environmental effects on the phenotype also affect developmental canalization, which in turn can influence the tempo and course of organismal evolution. Here, we used an oceanic population of threespine stickleback (*Gasterosteus aculeatus*) to investigate temperature-induced phenotypic plasticity of body size and shape using a paternal half-sibling, split-clutch experimental design and rearing offspring under three different temperature regimes (13, 17 and 21 °C). Body size and shape of 466 stickleback individuals were assessed by a set of 53 landmarks and analysed using geometric morphometric methods. At approximately 100 days, individuals differed significantly in both size and shape across the temperature groups. However, the temperature-induced differences between 13 and 17 °C (mainly comprising relative head and eye size) deviated considerably from those between 17 and 21 °C (involving the relative size of the ectocoracoid, the operculum and the ventral process of the pelvic girdle). Body size was largest at 17 °C. For both size and shape, phenotypic variance was significantly smaller at 17 °C than at 13 and 21 °C, indicating that development is most stable at the intermediate temperature matching the conditions encountered in the wild. Higher additive genetic variance at 13 and 21 °C indicates that the plastic response to temperature had a heritable basis. Understanding nonlinear effects of temperature on development and the underlying genetics are important for modelling evolution and for predicting outcomes of global warming, which can lead not only to shifts in average morphology but also to destabilization of development.

Introduction

Plasticity of traits can be a key factor in evolutionary responses to environmental change (e.g. West-Eberhard, 1989; Robinson & Wilson, 1994; Ghalambor *et al.*, 2007; Chevin *et al.*, 2010), especially with regard to global warming (Daufresne *et al.*, 2009; Moran *et al.*, 2010; Barrett *et al.*, 2011). Temperature is one of the most important factors driving phenotypic plasticity in fish ontogeny (Gillooly *et al.*, 2002; Löffler *et al.*, 2008; Georga & Koumoundouros, 2010). The influence of

temperature can be direct via altered metabolism (Houde, 1989; Clarke & Johnston, 1999), or indirect through changes in the physicochemical properties of water (e.g. viscosity, density, salinity and dissolved oxygen) resulting in morphological changes during ontogeny (Haas *et al.*, 2010; Sfakianakis *et al.*, 2011).

Fishes often grow faster at higher temperature, affecting maturation and reproductive output, and it has been shown that thermal history during development influences adult body shape (Pörtner *et al.*, 2001; Angilletta *et al.*, 2004; Georga & Koumoundouros, 2010). Specifically, temperature has been shown to affect overall morphology (Marcil *et al.*, 2006; Georgakopoulou *et al.*, 2007; Sfakianakis *et al.*, 2011), meristic counts such as the number of vertebrae or fin rays (Hubbs, 1922; Itazawa, 1959; Lindsey, 1962), growth rate (Allen

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& Wootton, 1982; Houde, 1989; Lefebvre *et al.*, 2011; Ott *et al.*, 2012) mortality, especially at early life stages (Hokanson *et al.*, 1977; Houde, 1989; Pepin, 1991; Löffler *et al.*, 2008), muscle development (Vieira & Johnston, 1992; Johnston & Hall, 2004; Koumoundouros *et al.*, 2009), and the occurrence of deformities (Bolla & Holmefjord, 1988; Sfakianakis *et al.*, 2004; Abdel *et al.*, 2005). However, it is still not well understood how temperature-induced variation in growth influences organismal development (Georga & Koumoundouros, 2010; Kuparinen *et al.*, 2011; Ott *et al.*, 2012).

Environmental effects on threespine stickleback development are well documented (Walker, 1997; Kitano *et al.*, 2007; Spoljaric & Reimchen, 2007; Kuparinen *et al.*, 2011; Leinonen *et al.*, 2011; Aguirre & Bell, 2012; Hendry *et al.*, 2013; Ravinet *et al.*, 2013), but detailed studies on temperature-induced variation of whole body shape are rare. Most studies focused on single traits such as behaviour, growth, maturity and survival (Craig-Bennett, 1931; Blahm & Snyder, 1975; Allen & Wootton, 1982; Bell & Stamps, 2004; Sokolowska & Kulczykowska, 2010). Recent studies have shown that oceanic populations of sticklebacks are relatively homogenous in body form, whereas freshwater populations differ considerably across a wide variety of habitats throughout the entire geographic distribution (Walker, 1997; McKinnon *et al.*, 2004; Leinonen *et al.*, 2006; Aguirre *et al.*, 2008; Spoljaric & Reimchen, 2012; Hendry *et al.*, 2013; Ravinet *et al.*, 2013). Several studies on stickleback populations found evidence that variation in body shape has a genetic basis (Lavin & McPhail, 1993; Leinonen *et al.*, 2006, 2011; Raeymaekers *et al.*, 2007; Spoljaric & Reimchen, 2007; Hendry *et al.*, 2011), whereas others reported that phenotypic plasticity acts as major driving force (Day *et al.*, 1994; McKinnon *et al.*, 2004; Spoljaric & Reimchen,

2007, 2012; Leinonen *et al.*, 2011; McCairns & Bernatchez, 2012).

The aim of this study was to investigate how temperature affects body size and shape of subadult oceanic threespine sticklebacks. We used a paternal half-sibling, split-clutch experimental design, and reared offspring under three different temperature regimes (13, 17 and 21 °C). Using geometric morphometric methods, we compared average body size and shape as well as variation of size and shape across the three temperature groups and between the two sexes. Because most previous studies compared only two temperature regimes, we were particularly interested in whether or not temperature-induced plasticity remains linear over multiple temperature regimes. Theoretical models predict that nonlinear environmental effects on the phenotype (a nonlinear reaction norm) also affect developmental canalization, and thus age-specific size and shape variation (e.g. Rice, 1998, 2002; Wolf *et al.*, 2001; Hermisson & Wagner, 2004; Pavlicev *et al.*, 2008; Mitteroecker, 2009). For example, if, for a certain temperature range, the average effects of temperature on the phenotype are small, individual variation in temperature exposure or in the developmental response to temperature will have little effect on phenotypic variation – development is canalized with respect to temperature. By contrast, if, for another temperature range, the developmental effects of temperature are more pronounced, individual variation in temperature exposure or developmental response will translate into increased phenotypic variation (Fig. 1).

Alterations in the amount and pattern of phenotypically expressed genetic variation, in turn, can influence the tempo and course of organismal evolution. For example, a release of genetic variation resulting from disrupted canalization can accelerate a population's

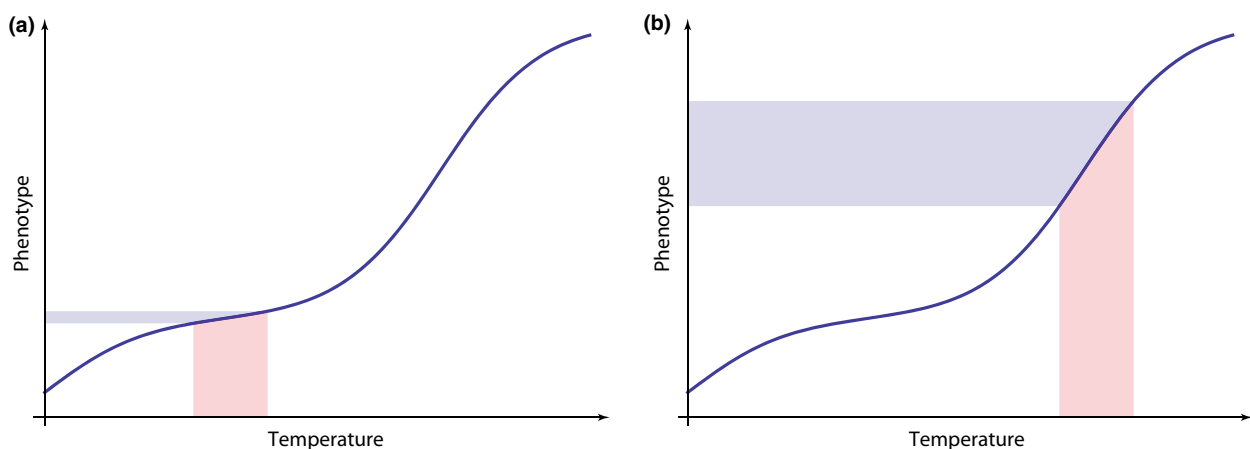


Fig. 1 Plasticity can be modelled by the reaction norm, a function (shown as the blue curve) relating the expected phenotype of a given genotype to the environment. The steepness of the curve indicates the degree of plasticity. For a nonlinear reaction norm, phenotypic variance depends on the average temperature. For the temperature range indicated by the red bar in (a), the slope of the curve is small and variation of temperature has little effect on the phenotype, whereas in (b) the same variation of temperature induces much more phenotypic variance (the phenotype is less canalized).

response to natural selection on a trait. Increased canalization, by contrast, tends to reduce the 'evolvability' of affected traits and may contribute to evolutionary stasis (e.g. Gibson & Wagner, 2000). In case of a continually changing environment, reduced genetic variation increases the risk of population extinction (Chevin *et al.*, 2010). It has also been argued that canalization can increase the potential for evolutionary divergence by allowing for the accumulation of hidden genetic variance that is expressed and subjected to selection after canalization breaks down (e.g. Rice, 1998; Gibson & Wagner, 2000). For complex phenotypes, an altered variance–covariance pattern may lead to a modification of indirect responses to selection, and hence, to a change in the direction of the evolutionary trajectory (e.g. Lande, 1979).

Materials and methods

Wild adult threespine sticklebacks were caught from an oceanic population in the Sylt-Rømø Bight (SRB) in the south-east of the North Sea (54°52'–55°10'N, 8°20'–8°40'E) (Fig. 2) and brought to the laboratory. Threespine stickleback populations from the southern North Sea represent the ancestral form of the northern European and Fennoscandian populations (Münzing, 1963; Banbura, 1994; Reusch *et al.*, 2001; Leinonen *et al.*, 2006, 2011; Mäkinen *et al.*, 2006; Mäkinen & Merilä, 2008). Adult sticklebacks were held in groups of

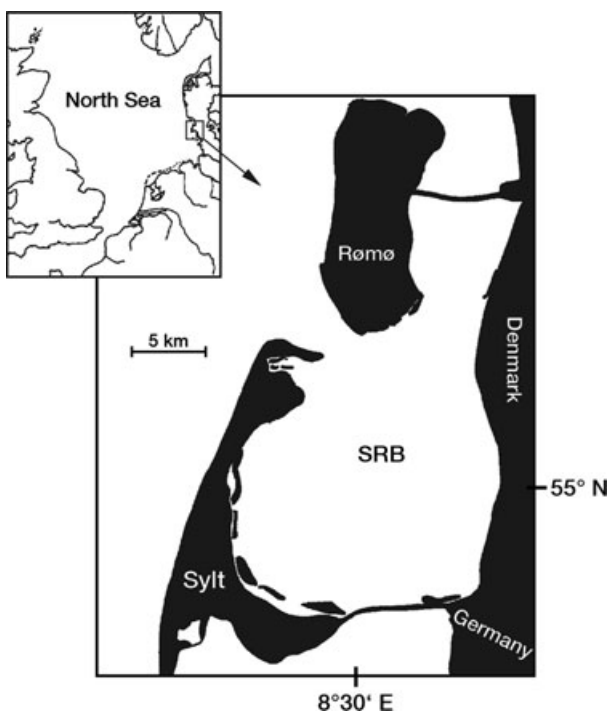


Fig. 2 Map of the Sylt-Rømø bight (SRB) modified after Asmus & Asmus (2000).

approximately 20 fish in 20-L aquaria at 17 °C (the mean ambient North Sea surface temperature during summer months).

We produced ten families following a paternal half-sibling mating design: five males were each crossed with two different females. Crosses were performed by strip-spawning eggs into a Petri dish containing paper towel soaked with filtered seawater. We killed a male in an excess of MS-222 (tricane methanesulphonate) and removed the testes, which were then crushed in an isotonic nonactivating medium (Fauvel *et al.*, 1999). The solution was applied to eggs, and eggs were left for 30 min before assigning them to treatments. Egg clutches were split into three groups and randomly assigned to one of three temperature groups (13, 17, or 21 °C). Each egg mass was placed individually in one 1-L glass beaker containing filtered seawater and an air supply. Beakers were placed into water baths heated with aquarium heaters set at either 13, 17 or 21 °C. These temperatures remained constant throughout the experiment.

Hatchlings were held in beakers for the first 30 days. Water was changed in the beakers every week. At 30 days post-hatch, ten randomly chosen offspring from each clutch were transferred to a 5-L aquarium connected to a flow-through seawater system set at either 13, 17 or 21 °C. Hatchlings were fed live *Artemia* sp. nauplii *ad libitum* for the first 30 days. After 30 days, a fixed volume of food (live *Artemia* sp.) was given to all aquaria. Throughout the experiment, a 14 h light : 10 h dark photoperiod was maintained.

Fish were approximately 100 days old when they were killed with an overdose of MS-222 and frozen at –80 °C. Sex was determined genetically using the stickleback *Idh* locus, and following the thermocycling protocol of Peichel *et al.* (2004). Whole genomic DNA was extracted from gills using DNeasy blood and tissue extraction kits (Qiagen, Hilden, Germany), and each sample was diluted to 5 ng DNA μL^{-1} prior to PCR amplification. Allele sizes were determined using the QIAXCEL electrophoresis system and Biocalculator software (Qiagen). Individuals for which sex determination failed ($N = 33$) were excluded from the analysis of sexual dimorphism but included in the other analyses.

We used a total of 466 threespine stickleback juveniles for the morphometric analysis (Table 1). Standard length (SL) ranged from 21.5 to 35.2 mm, measured as the distance between landmarks 1 and 10 (see Fig. 3). The number of individuals and the ratio of males to females were similar across all temperature groups (Table 1). Fishes were preserved in 6% buffered formalin for 30 days. After bleaching and clearing to remove skin pigmentation and make the body translucent, the bony structures were stained with Alicarin Red S, and fish were stored in glycerine (Potthoff, 1984; Darius *et al.*, 2010). The left side of each fish was then scanned with a flatbed scanner, following Herler *et al.* (2007).

	Total	Males	Females	Unknown Sex	SL	SL ^{SD}	SL ^{rg}
13 °C	159	77	74	8	29.62	2.87	21.5–35.1
17 °C	154	70	74	10	29.85	1.97	24.3–35.2
21 °C	153	71	67	15	28.06	2.28	21.5–34.2
Sum	466	218	215	33	–	–	–

Table 1 Number and sex ratio of individuals, mean standard length (SL), standard deviation of standard (SL^{SD}) and range of standard length (SL^{rg}) for each temperature group.

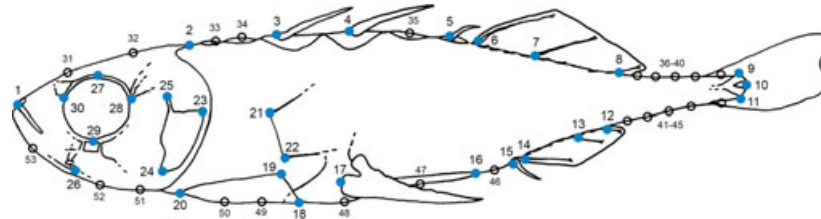


Fig. 3 The set of 30 landmarks (dots) and 23 semilandmarks (circles) used for geometric morphometric analysis. (1) anteriodorsal tip of premaxilla, (2) posterior edge of supraoccipital, (3) anterior intersection of first dorsal spine (DS) with pterygiophore (PT) III on the dorsal outline, (4) anterior intersection of second DS with PT IV on the dorsal outline, (5) anterior intersection of third DS with the PT VI on the dorsal outline, (6) anterior base of first dorsal fin ray, (7) base of fifth dorsal fin ray, (8) posterior base of last dorsal fin ray, (9) dorsal tip of hypural fan, (10) posterior midpoint of hypural fan, (11) ventral tip of hypural fan, (12) posterior base of last anal fin ray, (13) base of fifth anal fin ray, (14) anterior base of first anal fin ray, (15) anterior intersection of anal spine with ventral outline, (16) posterior tip of ventral process of the pelvic girdle, (17) anteroventral base of left pelvic spine, (18) posterior tip of ectocoracoid, (19) dorsal tip of ectocoracoid, (20) anterior tip of ectocoracoid, (21) dorsal base of first pectoral fin ray, (22) ventral base of last pectoral fin ray, (23) posteriodorsal edge of operculum, (24) anteroventral edge of operculum, (25) anteriodorsal edge of operculum, (26) posteroventral edge of articular, (27) most dorsal point of the eye outline formed by the frontal, (28) anteroventral tip of sphenotic, (29) most ventral point of the eye outline formed by the suborbitals, (30) posteroventral tip of lateral ethmoid, (31–34) dorsal outline of head, (35) dorsal outline of abdomen, (36–40) dorsal outline of caudal peduncle, (41–45) ventral outline of caudal peduncle, (46–48) ventral outline of abdomen, (49, 50) ventral outline of breast, (51, 52) ventral outline of throat, (53) ventral outline of chin.

After randomizing the images, 30 landmarks and 23 semilandmarks were digitized on every specimen using tpsDig2 (James Rohlf; see Fig. 3). Semilandmarks are points on smooth curves, for which the exact location along this curve is estimated statistically to establish geometric correspondence across the measured specimens (Bookstein, 1997; Gunz & Mitteroecker, 2013). The landmark configurations were superimposed by a generalized Procrustes analysis, standardizing for location, scale and orientation of the configurations (Rohlf & Slice, 1990; Mitteroecker & Gunz, 2009). The resulting Procrustes shape coordinates were analysed by between-group principal component (PC) analysis (Mitteroecker & Bookstein, 2011), and shape differences were visualized by thin-plate spline deformation grids (Bookstein, 1991). Allometry – the relationship between size and shape – was estimated by a multivariate, pooled, within-group regression of shape on centroid size (Bookstein, 1991; Klingenberg, 1998; Mitteroecker *et al.*, 2013). Overall shape variation of a group was measured by total variance, the trace of the corresponding covariance matrix. Statistical significance of group mean differences and of differences in total variance was estimated using Monte Carlo permutation tests with Procrustes distance and total variance as test statistics (Good, 2000). All morphometric analyses were per-

formed with Mathematica 8 (Wolfram Research Inc., Champaign, IL, USA).

We also partitioned phenotypic variance observed for SL and the first PC of shape into additive genetic variance (V_A) and residual environmental variance (V_E) using generalized linear mixed models implemented in the R package MCMCglmm (Hadfield, 2010). We fitted a character state multivariate animal model predicting SL and shape in each environment with the fixed effect 'sex' and random effect 'animal' (the pedigree of the fish describing the relatedness). Weak but informative priors of half the observed phenotypic variance were used. To account for the fact that measurements stemmed from separate individuals, we set the covariance between environment-specific measurements to 0. Markov chains were run for 500 000 iterations, and after removing 300 000 iterations of burn-in, we used a thinning interval of 100 values to generate posterior distributions of random and fixed parameters. Genetic correlations (r_G) between character states were calculated as the covariance between the trait values in two environments divided by the square root of the variance of both traits.

Results

Despite careful preparation and storage of the specimens, the first two PCs of the shape coordinates

apparently reflected lunate-like (Fig. 4, upper row) and S-like (Fig. 4, lower row) bending of the specimens, not actual variation in body shape. This was confirmed by examining the specimens with extreme scores along these two PCs. Bending of the specimens mainly results from shrinkage due to fixation and from slight differences in the scanning position (tilting) and body posture of the fish (see also Kristoffersen & Salvanes, 1998; Valentin *et al.*, 2008). This artificial shape variation, which was unrelated to both temperature and sex, was removed from the data by projecting the specimens into the subspace perpendicular to the first two PCs (Burnaby, 1966; Valentin *et al.*, 2008). All the subsequent shape analyses, including further principal component analyses, were based on these residual data.

Standard length (the length from the tip of the snout to the caudal margin of the hypuralia) differed on average among the three temperature groups but was relatively homogenous across the two sexes (Fig. 5a). SL was largest at 17 °C and smallest at 21 °C (group mean differences between 13 and 21 °C and between 17 and 21 °C were significant at $P < 0.001$). Variance of SL also differed among the temperature regimes (Fig. 5b), with the smallest variance occurring at 17 °C (13 vs. 17 °C $P < 0.001$; 17 vs. 21 °C $P = 0.041$; 13 vs. 21 °C $P = 0.003$). The decomposition of phenotypic variance of SL into additive genetic and environmental variance components (Table 2) showed that genetic variance at 13 and 21 °C was about three times larger than genetic variance at 17 °C. Environmental variance, by contrast, was relatively similar in all three temperature regimes.

Average body shape differed significantly between the sexes ($P < 0.001$) and across the three temperature groups ($P < 0.001$ for all three pairwise comparisons), despite substantial individual overlap (Fig. 6a). However, the three temperature groups did not differ in a linear way, that is, the shape differences between 13 and 17 °C differed considerably from those between 17 and 21 °C. Fish raised at 17 °C had, on average, a relatively larger head and relatively larger eyes, as well as a slightly reduced relative body depth than fish raised at 13 °C (Fig. 7a). Fish raised at 21 °C had a reduced relative size of the ectocoracoid, the operculum, and the ventral

process of the pelvic girdle as compared to fish reared at 17 °C group (Fig. 7b).

The temperature effects on body shape were very similar for males and females, and hence, sexual dimorphism remained constant across the three temperature groups (Fig. 6b). Males, on average, had deeper bodies, longer median fins, and a shorter free abdominal region than females (Fig. 7c). When dividing the sample into four size classes, shape dimorphism was already statistically significant in the smallest size class (SL < 27 mm), and the magnitude of average shape dimorphism increased with body size (Table 4).

Fish raised at 17 °C were less variable in body shape than fish raised at either 13 or 21 °C (Fig. 5c). This is also indicated by the group-wise equal frequency ellipses in the PC plot of Fig. 5a. The difference in total variance between 13 and 17 °C was significant at $P = 0.05$ and that between 17 and 21 °C at $P = 0.003$. For the first PC of shape, additive genetic variance at 13 and 21 °C was more than two times larger than additive genetic variance at 17 °C, whereas environmental variance was relatively similar in all three temperature regimes, resembling the results obtained for SL (Table 3).

Because the temperature groups differed in size, the observed shape differences could result from allometry instead of direct effects of temperature on body shape. Hence, we estimated pooled within-group allometry and removed it from the data by projecting the individuals into the subspace perpendicular to the allometry vector (Burnaby, 1966). Body size was significantly associated with body shape ($P < 0.001$) but accounted only for 2.3% of within-group shape variation. On average, larger individuals had relatively smaller eyes and a relatively larger ectocoracoid and operculum as compared to smaller individuals (Fig. 8). However, removing allometric shape variation from the data did not change the reported differences in mean shape and shape variance between temperature groups or between sexes.

Discussion

We studied temperature-induced phenotypic plasticity in threespine stickleback development using a split-

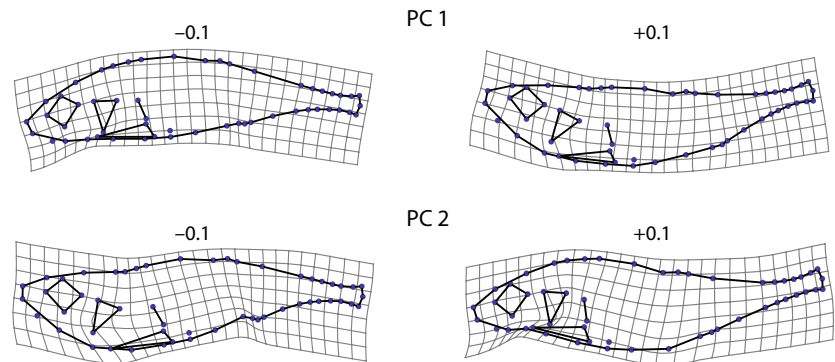


Fig. 4 Visualization of the first two principal components (PCs) of the Procrustes shape coordinates as deformation grids (deformations of the mean shape to ± 1 unit along the PC). The two components reflected bending of the specimens, and hence, were removed from the data.

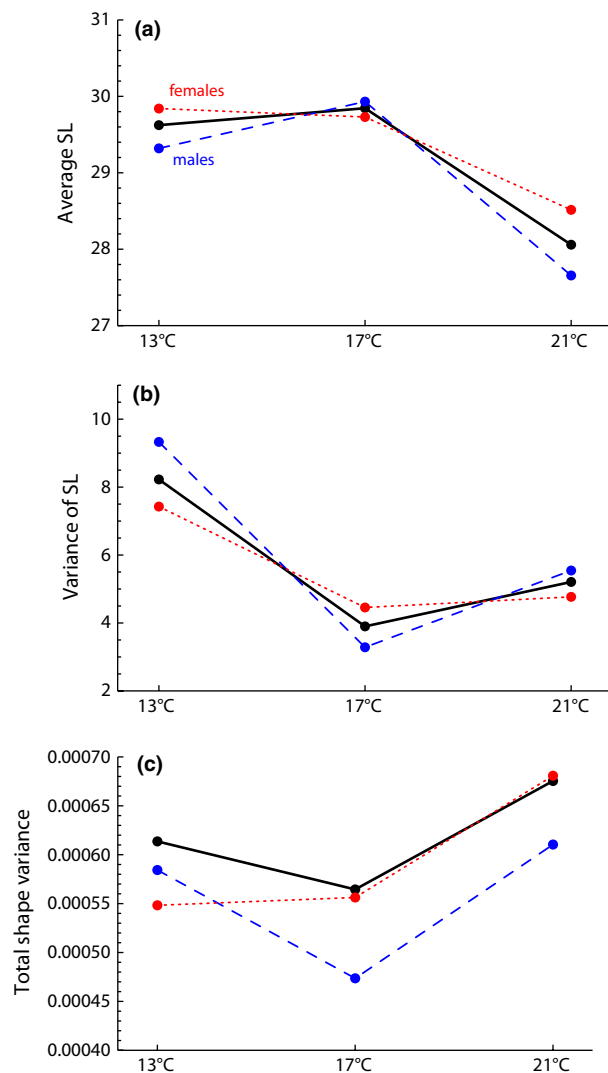


Fig. 5 (a) Total average (all individuals) and sex-specific averages of standard length (SL) for the three temperature groups. (b) Total sample variance and sex-specific variances of SL for the three temperature groups. (c) Total sample shape variance and sex-specific shape variances for the three temperature groups. Dashed line = males, dotted line = females, solid line = full sample (all individuals).

clutch experimental design, and rearing offspring under three different temperature regimes. The approximately 100-day-old individuals differed significantly in both size and shape across the temperature groups. Most important, the temperature-induced differences between 13 and 17 °C deviated considerably from those between 17 and 21 °C, indicating nonlinear effects of temperature on phenotypic development in the observed temperature range. Average shape differences between 13 and 17 °C mainly comprised relative head and eye size, whereas shape differences between 17

and 21 °C involved the relative size of the ectocoracoid, the operculum and the ventral process of the pelvic girdle. Body size was largest at the intermediate temperature of 17 °C (Figs 5–7).

If the effects of an environmental factor, such as temperature, on the phenotype are nonlinear, phenotypic variation in a population is expected to differ across the temperature regimes (e.g. Rice, 1998, 2002; Wolf *et al.*, 2001; Mitteroecker, 2009; see also Fig. 1). This is exactly what we found here: for both size and shape, variance was significantly smaller at 17 °C than at 13 and 21 °C. Hence, development of sticklebacks from the population used here appears to be most stable and canalized at 17 °C, which also seems to be reflected by the largest average body size at 17 °C.

It is tempting to speculate that this apparent temperature optimum at 17 °C is an evolutionary adaptation to the environment in the SRB, which has a mean ambient surface temperature during the summer months of approximately 17 °C (Polte & Asmus, 2006). Furthermore, parental sticklebacks were held in aquaria at 17 °C before we produced the half-sibling crosses. A recent study on the same SRB stickleback population (L. N. S. Shama & K. M. Wegner, unpublished data) demonstrated strong environment-specific maternal effects on offspring growth. During early life stages (0–30 days), offspring grew best at the temperature their mothers were acclimated to, whereas growth in later stages (30+ days) was primarily influenced by the environment, with all groups growing faster at 17 °C than 21 °C. However, conclusive insights require further experiments and comparisons with populations from other geographic regions.

We decomposed overall phenotypic variation of SL and of the first PC of shape into an additive genetic (i.e. heritable) component and a residual (nonheritable) component containing environmental variation (Tables 2 and 3). Interestingly, whereas residual/environmental variance was similar in all three temperature groups, genetic variance at 17 °C was considerably smaller than genetic variance at 13 and 21 °C. The estimates of additive genetic variance *per se* are difficult to interpret because they largely owe to variance in genotype–environment interactions and may be overestimated due to a small number of sires in the analyses and a common environment stemming from our animal husbandry. Although any such bias will affect the absolute estimates of V_A , these effects should be comparable between the environments. The clear relative differences in additive genetic variance between the temperature groups indicate that closely related fish react similarly to the different temperatures. The plastic response to temperature, hence, has a heritable genetic basis that is subject to selection. The low genetic correlations between temperature groups also indicate considerable nonlinear genotype–environment interactions (Tables 2 and 3).

Table 2 Decomposition of overall phenotypic variance of standard length into additive genetic (V_A) and environmental (V_E) variance components, separately for the three temperature groups. The table further provides narrow-sense heritabilities (h^2) and genetic correlations between character states in each environment (r_G), along with all corresponding 95% confidence intervals in parentheses.

	13 °C	17 °C	21 °C
13 °C	V_A : 4.96 (3.22–6.92) V_E : 1.29 (0.69–2.39) h^2 : 0.81 (0.61–0.90)	r_G : 0.55 (0.03–0.76)	r_G : 0.51 (0.12–0.75)
17 °C		V_A : 1.49 (0.77–4.19) V_E : 1.83 (0.71–2.75) h^2 : 0.62 (0.26–0.83)	r_G : 0.18 (–0.38 to 0.59)
21 °C			V_A : 4.53 (2.08–6.36) V_E : 1.46 (0.56–2.50) h^2 : 0.76 (0.50–0.90)

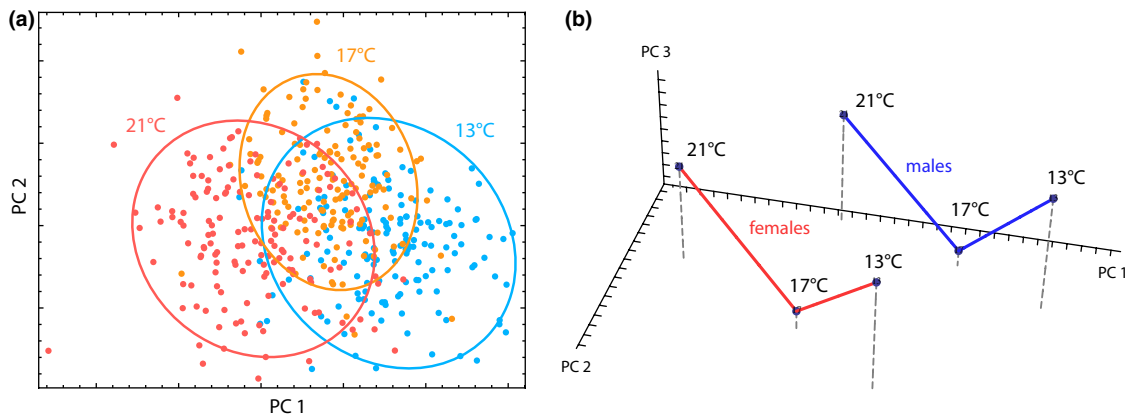


Fig. 6 (a) Scatterplot of the two between-group principal components (PCs) of body shape. The three ellipses are the 90% equal frequency ellipses for the three temperature groups. (b) First three PCs of the sex-specific mean shapes of the three temperature groups.

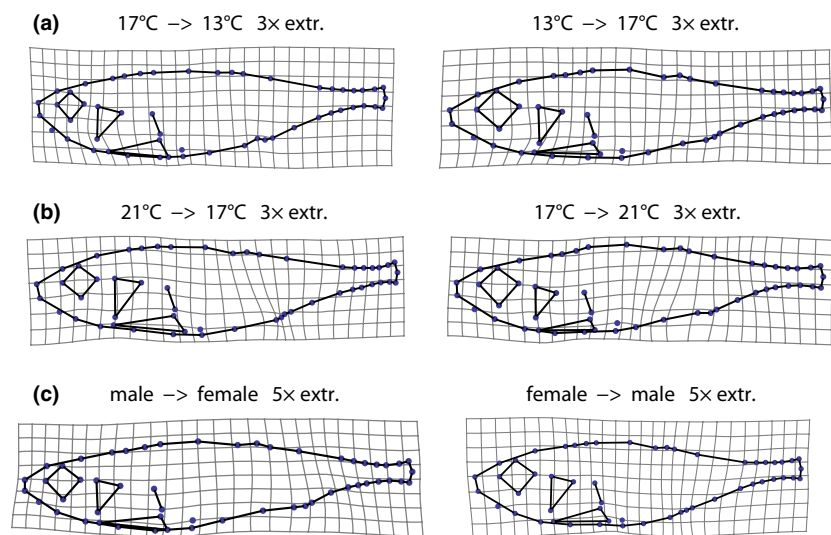


Fig. 7 (a) Average shape differences between sticklebacks raised at 13 and 17 °C. The shape differences are extrapolated by a factor of 3 to ease interpretation. (b) Average shape differences between sticklebacks raised at 17 and 21 °C, three times extrapolated. (c) Average shape differences between male and female sticklebacks, five times extrapolated.

A temperature increase of 4 °C, as in our experiment, is well within the range of predictions by the various model scenarios for global warming (e.g. IPCC, 2007). Our results indicate that global warming will – at least

in the short term – move the developmental system away from its current optimum at 17 °C and lead to a reduction in average body size as well as to an additional reduction in the relative size of several bony

	13 °C	17 °C	21 °C
13 °C	V_A : 5.92 (2.48–8.87) V_E : 2.22 (1.03–4.18) h^2 : 0.71 (0.43–0.89)	r_G : 0.52 (–0.11 to 0.77)	r_G : –0.29 (–0.62 to 0.26)
17 °C		V_A : 2.54 (1.24–5.29) V_E : 2.41 (1.19–3.68) h^2 : 0.54 (0.31–0.81)	r_G : 0.39 (–0.19 to 0.77)
21 °C			V_A : 6.71 (3.42–11.3) V_E : 2.22 (1.13–4.83) h^2 : 0.74 (0.47–0.92)

Table 3 Decomposition of overall phenotypic variance in the first principal component of shape into additive genetic (V_A) and environmental (V_E) variance components (multiplied by a factor of 10^5), separately for the three temperature groups. The table further provides narrow-sense heritabilities (h^2) and genetic correlations between character states in each environment (r_G), along with all corresponding 95% confidence intervals in parentheses.

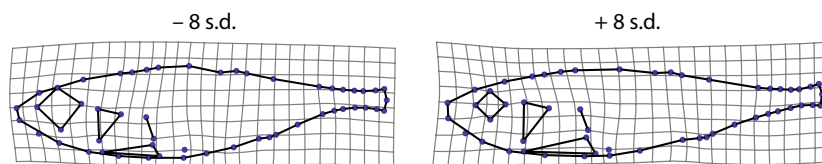


Fig. 8 Visualization of allometric shape differences. The deformation grids represent the average shape change corresponding to ± 8 standard deviations (SD) of centroid size.

structures. Smaller body sizes have been proposed as a universal ecological response to global warming (Daufrès *et al.*, 2009), but it is unclear if smaller body sizes are in fact disadvantageous for stickleback fitness. However, a reduction in bony elements involved in the defensive complex is likely to lead to a higher predation rate. The temperature-induced increase in phenotypic and genetic variation may lead to a higher mortality rate of ill-adapted stickleback individuals, but it may also accelerate evolutionary adaptation at the population level.

Temperature effects on body shape were very similar for the two sexes. Hence, sexual dimorphism remained constant across the three temperature groups (Fig. 6b), suggesting stable genetic effects accounting for sexual dimorphism of body shape (Kitano *et al.*, 2007, 2012; Aguirre *et al.*, 2008; Aguirre & Akinpelu, 2010). In our laboratory-raised population, males had relatively larger heads with larger eyes, snout and jaws, along with deeper bodies and relatively larger median fins than females. The pectoral fin had a larger base and a more posterior insertion in males. Females had a relatively longer unprotected abdominal area (LM15–LM16) along with a generally shorter pelvic girdle (Fig. 7c). These findings are in accordance with previous studies (Walker & Bell, 2000; Kitano *et al.*, 2007, 2012; Spoljaric & Reimchen, 2007, 2012; Aguirre *et al.*, 2008; Aguirre & Akinpelu, 2010; Leinonen *et al.*, 2011) and show that the dimorphic traits are consistent across wild and laboratory-reared stickleback populations.

Some studies reported that sexual dimorphism in stickleback body shape occurs only after sexual reproduction is reached (Kitano *et al.*, 2007; Leinonen *et al.*, 2011; McCairns & Bernatchez, 2012). Yet, we found significant shape dimorphism in 100-day-old individuals with a SL ranging from 21.5 to 35.2 mm (SRB stick-

lebacks reach 60 mm SL as adults; Polte & Asmus, 2006; H. Ahnelt, unpublished data). In fact, shape dimorphism was already statistically significant in the smallest size class (SL < 27 mm), but the magnitude of average shape dimorphism increased with body size (Table 4), indicating a gradual divergence of average male and female growth patterns, starting early in the life history of SRB sticklebacks. We did not find significant sexual dimorphism in body size for our subadult laboratory-reared sticklebacks, even though adult females generally are larger than adult males in the wild. Likewise, Kitano *et al.* (2007) and Leinonen *et al.* (2011) found no size-related sexual dimorphism in laboratory-reared fish before maturity.

Body size was significantly related to body shape in our sample, but this allometric relationship accounted only for a small amount of shape variation (2.3% of total within-group shape variation). Larger specimens tended to have relatively smaller eyes, a relatively larger ectocoracoid and a larger operculum as compared to smaller specimens (Fig. 8). Allometry accounted for neither the observed shape differences between the temperature groups nor for sexual shape dimorphism. Similarly, McGuigan *et al.* (2010) and McCairns & Bernatchez (2012) found that allometric effects did

Table 4 Procrustes distance between male and female mean shape for each of the four size classes of standard length (SL), along with the sample size and statistical significance of the comparisons.

	SL ≤ 27	27 < SL ≤ 29	29 < SL ≤ 31	31 < SL
Procrustes distance	0.0120	0.0106	0.0145	0.0154
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
N	87	126	145	108

not drive sexual dimorphism in stickleback body shape.

Conclusions

Temperature-induced differences between 13 and 17 °C differed considerably from those between 17 and 21 °C. In other words, the effect of temperature on body size and body shape was nonlinear over a wider temperature range. Consequently, variation of body size and shape differed across the temperature regimes as predicted by theoretical models. The plastic nonlinear response to temperature had a heritable basis because the variance released at 13 and 21 °C was mainly additive genetic variance. Apart from an altered selective regime itself, changes in environmental temperature may thus also affect the amount and pattern of exposed genetic variation, which determines the response to natural selection and the rate of adaptation. This shows the high relevance of reaction norm shape for predicting phenotypic change exerted by environmental change such as global warming. Restrictions to linear reaction norms appear to be significant shortcomings of current evolutionary models (e.g. Nussey *et al.*, 2007; Chevin *et al.*, 2010). Environmental alterations might lead not only to shifts in average morphology but also to destabilization of development with increased phenotypic variation. Environmentally induced changes of the variance–covariance pattern of complex traits can influence a populations' response to selection in many ways and alter the evolutionary trajectory.

Acknowledgments

We would like to thank Hanne Halliger for sexing the fish, René Gerrits and Reimer Magens for setting up the aquaria system and taking care of the fish. P.M. was supported by the Focus of Excellence 'Biometrics of EvoDevo' of the Faculty of Life Sciences, University of Vienna. K.M.W. and L.N.S.S. were funded by an Emmy-Noether Grant WE4641-1 given by the Deutsche Forschungsgemeinschaft (DFG).

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Received 12 August 2013; revised 26 November 2013; accepted 26 November 2013