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CRYPTIC GENETIC VARIATION AND BODY SIZE EVOLUTION IN THREESPINE STICKLEBACK

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The role of environment as a selective agent is well-established. Environment might also influence evolution by altering the expression of genetic variation associated with phenotypes under selection. Far less is known about this phenomenon, particularly its contribution to evolution in novel environments. We investigated how environment affected the evolvability of body size in the threespine stickleback (*Gasterosteus aculeatus*). *Gasterosteus aculeatus* is well suited to addressing this question due to the rapid evolution of smaller size in the numerous freshwater populations established following the colonization of new freshwater habitats by an oceanic ancestor. The repeated, rapid evolution of size following colonization contrasts with the general observation of low phenotypic variation in oceanic stickleback. We reared an oceanic population of stickleback under high and low salinity conditions, mimicking a key component of the ancestral environment, and freshwater colonization, respectively. There was low genetic variation for body size under high salinity, but this variance increased significantly when fish were reared under low salinity. We therefore conclude that oceanic populations harbor the standing genetic variation necessary for the evolution of body size, but that this variation only becomes available to selection upon colonization of a new habitat.

KEY WORDS: **Buffering, colonization, *Gasterosteus aculeatus*, heritability, salinity.**

Adaptive phenotypic evolution can be predicted from estimates of the strength and direction of selection, and the additive genetic variation associated with the phenotype under selection (Lande 1979; Hansen and Houle 2008). These quantitative genetic parameters are effective at predicting phenotypic evolution over the short term (Grant and Grant 1995; Hill 2010), but over longer time periods the evolution of allele frequencies causes predictions to become less accurate (Barton and Turelli 1987; Reeve 2000). Considerable empirical and theoretical attention has focused on understanding the circumstances and time frame of the evolution of genetic variation associated with phenotypes (Steppan et al. 2002; Johnson and Barton 2005; Arnold et al. 2008). Equally crucial, but less often considered, the predictive power of quantitative genetics will be reduced if the environment not only determines selection, but also directly affects the additive genetic variation available for the response to that selection (Turelli 1988; Schlichting 2008; McGuigan and Sgro 2009). A direct effect of environment on the expression of genetic variation, and thus on evolvability, has been suggested to contribute to patterns of evolutionary stasis in stable environments and rapid evolution in novel environments (Waddington 1957; Rutherford and Lindquist 1998; Gibson and Dworkin 2004; Badyaev 2005; Wagner 2005; Schlichting 2008; Masel and Siegal 2009). Although the expression of additive genetic variation sometimes increases in novel or stressful environments (Hoffmann and Merila 1999), the opposite pattern has also been observed (Charmantier and Garant 2005), and it remains to be determined how environmental effects on the expression of variation contribute to evolutionary diversification (McGuigan and Sgro 2009).

Environment might also affect phenotypic evolution by altering the distribution of phenotypes (Ghalambor et al. 2007; Pfennig et al. 2010). Change in the environment might increase

the overall phenotypic variation, increasing the phenotypic space that could be explored during adaptation to novel conditions, and potentially facilitating rapid phenotypic evolution toward a new adaptive peak (Kirkpatrick 1982; Price et al. 2003). Effects of the environment on phenotype might also reduce the mismatch between mean and optimal phenotype, reducing the strength of selection (and potentially the rate of evolution), and therefore facilitating population persistence through larger population size (Fear and Price 1998; Pigliucci and Murren 2003; Price et al. 2003; Badyaev 2005; Ghalambor et al. 2007; Pfennig et al. 2010). However, there are few empirical examples of phenotypic plasticity promoting phenotypic evolution in this manner (Pfennig et al. 2010; Scoville and Pfrender 2010).

The threespine stickleback, *Gasterosteus aculeatus*, has an evolutionary history that makes it well suited to studying evolutionary processes (Bell and Foster 1994; Taylor and McPhail 2000; Cresko et al. 2007; Wootton 2009), including environmental effects on evolvability. These small fish are holarctically distributed in coastal regions and are represented by oceanic and freshwater life-history forms. Freshwater populations, which live and breed in freshwater streams and lakes, evolved repeatedly from oceanic ancestors that colonized the freshwater habitats opened up by the retreat of glaciers from the last glacial maximum less than 20,000 years ago. Independently evolving freshwater populations have undergone parallel evolution affecting many aspects of phenotype (Bell and Foster 1994; Taylor and McPhail 2000; Cresko et al. 2007; Wootton 2009). A notable pattern of parallel phenotypic evolution in stickleback involves rapid changes in overall body size; freshwater fish are smaller than oceanic fish (Snyder and Dingle 1989; Mori 1990; McKinnon et al. 2004; Kristjansson 2005).

In many taxa, size is a determinant of both sexual (Hunt et al. 2009) and nonsexual (Bystrom et al. 2006; Carlson et al. 2009) fitness (see Kingsolver and Pfennig 2004), and a trait that evolves rapidly (Herczeg et al. 2009; Moen and Wiens 2009; Thomas et al. 2009). Although explicit estimates of selection gradients for stickleback size are generally lacking (but see Schluter 1993; Barrett et al. 2008), there is evidence that size is associated with a variety of fitness components, including survival and reproduction (Schluter 1993; McKinnon et al. 2004; Baker et al. 2008; Marchinko 2009). In addition to direct effects on fitness, it has been suggested that genetic correlation of size with other traits could have contributed to diversification of these other traits through correlated selection responses. In particular, direct selection on growth rate might have driven the evolution of reduced armor in freshwater habitats (Giles 1983; Bell et al. 1993; Marchinko and Schluter 2007; Barrett et al. 2009). Furthermore, size might be involved in generating reproductive isolation, promoting further phenotypic diversification; at least some stickleback populations exhibit size-assortative mating (Nagel and Schluter 1998; Ishikawa and Mori 2000; McKinnon et al. 2004). These observations suggest that understanding how environment determines selection on and variation in body size might be key to understanding the stickleback radiation.

Despite the irrefutable evidence of rapid (sometimes within a few decades) phenotypic diversification following freshwater colonization (Bell and Foster 1994; Bell et al. 2004; Kristjansson 2005; Gelmond et al. 2009), oceanic populations of threespine stickleback are characterized by little spatial or temporal phenotypic variation (Bell 1994; Walker and Bell 2000). The probability of a beneficial allele contributing to adaptation rather than being lost from a population due to sampling depends on its frequency (Haldane 1927; see Barrett and Schluter 2008). The pattern of rapid, repeated diversification in freshwater stickleback suggests substantial standing genetic variance in oceanic stickleback, but the observed low phenotypic variation in these populations appears to refute this. Little is known about the distribution of standing additive genetic variance of size in stickleback. Several studies have provided evidence that body size is heritable (McPhail 1977; Snyder and Dingle 1989; Snyder 1991), but there are no explicit estimates of additive genetic variation in an oceanic (ancestral) population. It therefore remains to be determined whether there is sufficient standing genetic variation in oceanic (ancestral) populations of threespine stickleback to account for the rapid evolution of freshwater colonists, and whether the tempo and direction of phenotypic evolution in these novel habitats might have been affected by environmentally induced changes in the expression of genetic variation occurring immediately after colonization.

In this paper, we used a paternal half-sib breeding design to estimate the additive genetic variance (and evolvability) of body size in an oceanic population of threespine stickleback. Parental fish were collected from Rabbit Slough in the Matanuska-Susitna Valley of south central Alaska. This population has been the focus of considerable study, with phenotypic and genetic evidence that it is representative of the oceanic ancestor from which local freshwater populations were derived (Walker and Bell 2000; Aguirre et al. 2008; Hohenlohe et al. 2010). Elaborating the experimental design through a split-brood approach, where fish were reared under salinity treatments that represented the oceanic (high salinity, 35 ppt) and freshwater (low salinity, 1 ppt) environments, allowed us to further determine whether the environment affected the phenotype, or the expression of additive genetic variation. Specifically, we tested whether the standing genetic variation in body size in this oceanic population was consistent with rapid body-size evolution following freshwater colonization, and whether the release of cryptic genetic variation (i.e., genetic variation that did not contribute phenotypic variation in the ancestral environment) could have contributed to this evolution.

Materials and Methods

FISH HUSBANDRY

Crossing, rearing, and sampling of stickleback followed University of Oregon approved animal use protocols. Adults were captured from Rabbit Slough (61°32′N, 149°15′W, elev. 5 m) in June 2004. Clutches were stripped from 104 females, and testes of 52 males were dissected out and macerated. Gametes were combined in a paternal half-sib breeding design (Falconer and Mackay 1996; Lynch and Walsh 1998), with sperm from one male fertilizing egg clutches from two females. Detailed fertilization and rearing protocols are available at: http://stickleback.uoregon.edu/index.php/. This breeding design was partly described in McGuigan et al. (2010), where the genetic basis of body shape allometry was determined for fish reared in low salinity. Here, we addressed a different question, whether the environment affected the expression of additive genetic variation. We analyzed body size of fish reared in low salinity, as well as their siblings reared in high salinity; the sample size in the current study is consequently approximately twice that reported in McGuigan et al. (2010).

Fertilizations were conducted in embryo media, and embryos and hatchlings were maintained in embryo media at 20°C. At 10 days post fertilization (dpf), fish were transferred to 4 L tanks with ∼500 mL of reverse osmosis purified water to which Instant Ocean® Sea Salt was added to a salinity of ∼1 ppt. Each clutch was split in four, and randomly assigned to two tanks in each of two treatments (a total of 416 tanks). Random assignment of families to tanks was used to minimize the common environment effects on replicate tanks of siblings. Fry were fed on newly hatched brine shrimp nauplii. From 2 months onwards, we supplemented the brine shrimp diet with dried flake food.

At 13 dpf, once fish had begun to actively forage, the volume of water in the tanks was increased to 4 L over 8 h, and the high salinity treatment was introduced. Fish in the low salinity treatment were maintained in 4 L at ∼1 ppt salinity, while Instant Ocean® Sea Salt was added to reverse osmosis purified water to ∼35 ppt in the high salinity treatment. No recirculating system was used, rather 10% of the water was manually replaced daily to maintain water quality. Cohorts were density controlled at 20 dpf (culled from ∼20 to 30 down to 15 individuals per tank), 1 month (10 fish), and 2 months (five fish). Culled fish were randomly chosen, a dip net the width of the tank was used to remove all fish and the desired number returned to the tank from the net.

At 3 months, the experiment was terminated and the remaining five fish per tank were sampled. Survival was not explicitly recorded during the experiment, but the low natural mortality was evidenced by the relatively few missing data points in the analyses; a total of 2050 (of a possible 2080) (1027 [of 1040] high and 1023 [of 1040] low salinity) fish from 104 families (52 sires) were sampled. Three tanks (15 fish) were lost due to handling errors during sampling. Standard length (SL; the distance between landmarks [LMs] 1 and 9 in Fig. 1) averaged 27 mm, a size at which the development of lateral plate (LP) and pelvic armor is complete (Hagen 1973; Bell 1981). These bony structures provide consistent LMs with which to characterize fish shape and size (Fig. 1). We considered this an informative age at which to study size; a similar age was considered in other studies of stickleback size phenotypes (Marchinko and Schluter 2007; Barrett et al. 2009), and it is the age at which growth rates slow (Wright et al. 2004).

Fish were euthanized with an overdose of a tricaine methane sulphonate solution, 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, in a random order with respect to rearing tank. Fish were immobilized and imaged on a common background in left lateral view. From these images, the positions of 30 LMs (Fig. 1) were recorded using tpsDig2 (Rohlf 2005). LMs were aligned, and centroid size (CS) calculated using the default settings in tpsRelW (Rohlf 2005). CS is the square root of the sum of squared distances of each of the 30 LMs to their centroid, and a metric commonly used to describe size (Rohlf

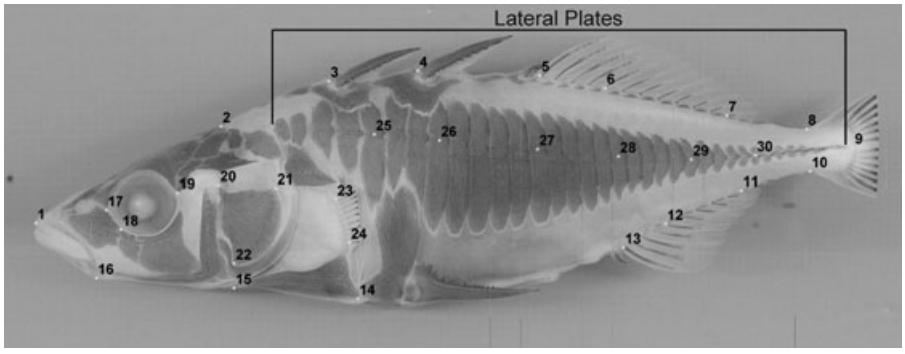

Figure 1. The landmarks (LMs) recorded and used to estimate centroid size (all LMs) and standard length (distance between LM 1 and 9). A full description of landmarks is given in McGuigan et al. (2010).

1999). From the digital images, we also measured SL, the distance between LMs 1 and 9 (Fig. 1), using Morpheus (Slice 1998). Qualitatively similar results were obtained from both metrics of size, and here we present only the analyses of CS. The total number of LPs was also recorded from these images (Fig. 1).

DATA ANALYSES

Data were analyzed using mixed modeling within a restricted maximum likelihood framework implemented in Proc Mixed in SAS (version 9.2. SAS Institute Inc., Cary, NC) with the model:

$$Y_{ijklm} = \mu + E_i + S_j + D_{k(j)} + T_{l(ijk)} + \varepsilon_{ijklm}.$$

Salinity (E) was fit as a fixed effect, and three random effects were fit: sire (S), dam nested within sire (D), and rearing tank nested within salinity, sire, and dam (T). The residual (error: ε) variance in this model was the variation among full sibs within a single rearing tank, and captures measurement error as well as microenvironmental variation within tanks, including effects of the social environment.

We had two general questions in this study. First, was there additive genetic variation for body size in this population, and did the estimates of this variation differ between the two salinity treatments? In our breeding design, the sire variance is one-fourth of the additive genetic variance (Falconer and Mackay 1996). We ran the above model separately for the two salinity treatments (without fixed effects), and applied log-likelihood ratio tests (LRTs) comparing hierarchical models (with vs. without sire) to test for additive genetic (sire) variance within each environment. These LRTs were one-tailed to account for the fact that variance components cannot be negative. We further implemented Bayesian analyses within Proc Mixed to estimate 95% credibility intervals (CIs) for sire-level variance within each salinity environment. We generated 10,000 pseudorandom samples from the joint posterior density of the variance components using a noninformative reference version of Jeffreys' prior, and an independence chain algorithm. Negative variance components were allowed, ensuring that CI could include zero. In the high salinity treatment, the estimated variance between replicate tanks of full sibs was zero; to implement the Bayesian analysis in this treatment, it was necessary to omit tank from the model. We note both that the median values of the variance component estimates from the Bayesian analysis were very similar to the estimates from the mixed model analysis, and that Bayesian analysis using MCMCglmm (Hadfield 2010) gave similar estimates of the median and 95% CI for the variance components (data not shown).

We used model fit criteria and LRTs to determine the statistical support for differences in sire variance between the two treatments. We considered 16 models, ranging from a single variance component at each level in the model (sire, dam, tank, and residual) through to salinity-specific estimates for all four random effects (Table S1). Analyses were implemented using a GROUP statement in Proc Mixed, and the Akaike Information Criterion (AIC) was interpreted to identify the model that best explained the data. We complimented this AIC model-fit approach with LRTs comparing hierarchical models to provide further statistical support for salinity-specific sire (genetic) variance component estimates.

The second general question posed in this study was whether the salinity treatment had an effect on either mean body size, or the phenotypic variance in size. To determine whether there was any difference in mean size between salinities, we interpreted the F-test of the fixed effect of salinity on size. The main effect of salinity was significant in all models; we only report the results of the F-test within the best model. We addressed the question of phenotypic variation in body size in two ways. First, model fit support for salinity-specific versus common estimates of variance components provided evidence of differences in phenotypic variance between salinities (Table S1). Second, we estimated the total phenotypic variation in each environment, and again used LRT to determine if estimating salinity-specific variance improved model fit. To estimate the total phenotypic variance, we applied a model that fit salinity as a fixed effect, and the total phenotypic variation as the residual (i.e., no sire, dam, or tank terms). Fixed effects can greatly reduce estimates of phenotypic variance (Wilson 2008), but here resulted in only a slight decrease in estimates (10.6 vs. 10.5 and 12.2 vs. 12.0 in low and high salinity, respectively), and did not alter the significance of the LRT or therefore the interpretation that total phenotypic variance differed between treatments.

Results

EFFECTS OF SALINITY ON ADDITIVE GENETIC VARIANCE

The model that best described the variance in size was one that allowed salinity-specific estimates of variance at the sire, tank, and residual levels (AIC: 10,697.6), with the second-best model having salinity-specific sire and residual estimates (AIC: 10,705.2) (Table S1). Fitting salinity-specific sire variance improved model fit over other models (vs. only salinity-specific tank and residual LRT: $X^2 = 7.73$, df $= 1$, $P = 0.005$; vs. only salinity-specific residual LRT: $X^2 = 13.00$, df $= 1$, $P < 0.001$; vs. no salinity-specific effects LRT: $X^2 = 11.58$, df $= 1$, $P = 0.001$). These results suggest differences in the additive genetic variance expressed in the different salinity environments.

There was no statistically detectable additive genetic variance in size under high salinity ($V_A = 0.085$ [95% CI: -1.830, 2.016], $X^2 = 0.010$, df $= 1$, $P = 0.461$; $h^2 = 0.007$ [-0.144, 0.165]). In contrast, under low salinity, there was significant additive genetic

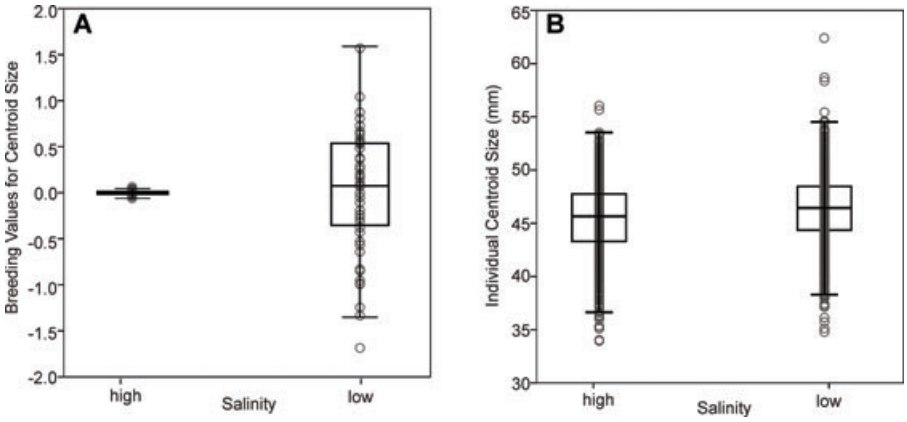

Figure 2. **(A) Genetic and (B) phenotypic variance in centroid size in high (ancestral) and low (derived) salinity rearing environments. (A) Sire breeding values (best linear unbiased predictors [BLUPs]) for each of the 52 sires, estimated separately in each salinity. (B) Size of each individual reared in each salinity. In (A) BLUPs and in (B) individual values (in gray) are overlaid with boxplots indicating the lower and upper quartiles and median (central line in box), with whiskers corresponding to 1.5 times the interquartile range. BLUPs were mean-centered (i.e., are deviations of each sire from the population mean). Several individuals with extreme values of centroid size are evident in B). We performed all analyses with and without these individuals; results were very similar in both cases, and only the analyses including all individuals are presented.**

variance ($V_A = 3.289$ [0.829, 6.847], $X^2 = 6.906$, df = 1, $P = 0.004$), corresponding to a h^2 of 0.313 (0.079, 0.603). This study therefore provides strong evidence of release of cryptic genetic variance under the environmental conditions associated with rapid evolution in natural populations (Fig. 2A).

EFFECTS OF SALINITY ON PHENOTYPIC VARIANCE AND MEAN

Model fit indicated statistical support for a difference in phenotypic variance between the salinity treatments (AIC were 10,778.6 and 10,775.9 for shared vs. specific variances, respectively; LRT: $X^2 = 4.674$, df = 1, $P = 0.030$). However, this difference was in the opposite direction to that observed at the additive genetic level (above), or the direction expected if phenotypic plasticity contributed to evolution of body size following freshwater colonization by increasing the phenotypic space a population could explore during adaptation. Specifically, there was greater phenotypic variation under high ($V_P = 11.972$ [11.008 – 13.065]) versus low ($V_P = 10.458$ [9.597 – 11.430]) salinity (Fig. 2B).

We detected a statistically significant ($F_{1,102} = 23.01$, $P < 0.0001$ from best model) difference in mean size between treatments. Low salinity fish (mean SL = 27.47 mm [27.47, 27.48]; mean CS = 46.29 mm [46.27, 46.31]) were, on average, larger than fish from high salinity (mean SL = 26.93 mm [26.92, 26.94]; mean CS = 45.37 mm [45.35, 45.40]). This difference corresponded to an increase in average size of 2%. However, as freshwater stickleback are smaller than their oceanic conspecifics (Snyder and Dingle 1989; Mori 1990; McKinnon et al. 2004), the salinity effect on mean body size observed here was again contrary to expectations if phenotypic plasticity had contributed to size evolution by shifting the population mean toward the new phenotypic optimum following freshwater colonization.

Discussion

If the environment affects the genetic variation that is available to selection, predicting evolutionary responses following environmental change becomes more challenging (Turelli 1988; McGuigan and Sgro 2009). Nonetheless, environment-driven changes in the expression of genetic variation might be an important evolutionary process, generating macroevolutionary patterns (Waddington 1957; Rutherford and Lindquist 1998; Gibson and Dworkin 2004; Badyaev 2005; Wagner 2005; Schlichting 2008; Masel and Siegal 2009). In this study, we manipulated the environment along one axis of natural habitat variation (salinity) and demonstrated that the environment substantially affected the estimate of additive genetic variation, and thus the potential to evolve, of a trait that has undergone natural evolution following colonization of a new habitat.

Our results suggest that body size is likely to change only slowly, if at all, in response to novel selection under oceanic conditions. Non-zero additive genetic variance is the rule (Lynch and Walsh 1998; Futuyma 2010), and the high salinity treatment in this study represents one of a few published examples of a lack of additive genetic variance for a univariate trait (Blows and Hoffmann 2005; Kellermann et al. 2006). Evolutionary stasis, such as seen in oceanic threespine stickleback (Bell 1994; Walker and Bell 2000), is thought to reflect long-term stabilizing selection, rather than a lack of genetic variation (Estes and Arnold 2007). Nonetheless, persistent stabilizing selection might reduce genetic variation through effects on allele frequencies of loci

contributing to variation in the trait (Barton and Turelli 1989; Hunt et al. 2007). Stabilizing selection might also contribute to the evolution of mechanisms that reduce variation in allelic effects at these loci (de Visser et al. 2003). The relationship between body size and fitness in oceanic threespine stickleback, and the processes responsible for the low level of additive genetic variation estimated in this study remain to be determined.

In contrast to the low genetic variance under high salinity, there was substantial additive genetic variation when our experimental population was reared under simulated freshwater conditions, suggesting the genetic potential to respond to selection toward a new body size optimum following freshwater colonization. Two processes could account for the difference in the additive genetic variance between high and low salinity treatments: a change in allele frequencies (i.e., selection), or a change in allelic effects (i.e., release of cryptic genetic variance) (Lande 1979; Lynch and Walsh 1998).

Increased additive genetic variance is expected under selection due to previously rare alleles increasing toward intermediate frequencies (Falconer and Mackay 1996). The observed differences in estimated additive genetic variation could reflect selection against rare alleles in high salinity and against common alleles in low salinity, a pattern that might be expected if allele frequencies reflected the selection history of the oceanic population. If allele frequencies are highly asymmetric (i.e., common alleles at very high frequency), then little selection (mortality) might be needed to cause the low estimate of additive genetic variation in high salinity. However, such highly symmetrical allele frequencies would require considerable mortality across a large population to elevate the frequency of rare alleles toward intermediate frequencies, and thus increase the estimate of additive genetic variance in the low salinity rearing environment. We did not record survival in this experiment but in daily inspection of tanks observed few dead fish, and suggest that relatively low mortality is indicated by the fact that 99% of the target offspring survived to be sampled in both treatments. We therefore do not expect that changes in allele frequencies underlie the difference in the estimates of additive genetic variation in the two rearing environments. Other stickleback studies manipulating salinity reported reduced hatching success of freshwater populations reared in saltwater (Marchinko and Schluter 2007) and decreased larval survival of saltwater populations reared in freshwater (McCairns and Bernatchez 2010), but it is not known whether these survival differences affected allele frequencies in those studies.

It has been suggested that effects of most alleles will be context dependent to some extent (Wagner 2005). A recent study in threespine stickleback demonstrated variation between high and low salinity environments in the expression of certain loci, candidates for osmoregulation, suggesting the opportunity for salinity-specific allelic effects (McCairns and Bernatchez 2010). Further support for environment-specific allelic effects comes from two recent studies reporting salinity-specific effects on growth of the Ectodysplasin-A (*Eda*) locus in stickleback (Marchinko and Schluter 2007; Barrett et al. 2009), a locus within a region of LGIV that is strongly associated with divergence in LP phenotypes between oceanic and freshwater populations (Colosimo et al. 2004; Cresko et al. 2004; Colosimo et al. 2005). These studies suggest that changes in allelic effects might be a more likely explanation for our observed difference in genetic variation than changes in allele frequency. Although broadly consistent in terms of the inference that environment determines the effect on body size of genetic variants, the current study differs from these previous studies in two key points.

First, both Marchinko and Schluter (2007) and Barrett et al. (2009) manipulated the genetic composition of their experimental populations to generate intermediate (0.5) allelic frequencies. The two identified alleles of *Eda* naturally occur at disparate frequencies, with one rare and one common allele ($q = 0.002$ and 0.038, respectively, for the low frequency allele in two oceanic populations: Colosimo et al. 2005). The crossing design also necessarily generated considerable linkage disequilibrium between loci (confounding asignment of causality to a particular locus within a linkage group: Cresko 2008). The manipulation of the genetic composition of the populations makes it difficult to infer how the observed salinity-specific effects translate into differences in additive genetic variation in the wild, the information needed to predict natural phenotypic evolution. Our study extends the evidence of environment-specific allelic effects to demonstrate that they can alter the estimation of additive genetic variation of stickleback size in a random sample from a natural population. If allelic effects vary considerably among environments, as suggested by the results of the current and previous studies, then alleles contributing to genetic variance (and potentially evolution) in the novel freshwater conditions might be at appreciable frequencies (i.e., not rare) in oceanic populations, providing a general explanation for the rapid evolutionary responses observed in stickleback.

The second point of difference between our study and previous work is that salinity-specific size variation was associated with LP phenotype in the two previous studies, but not in the current study. Both Marchinko and Schluter (2007) and Barrett et al. (2009) observed that the environmental effects on size differed between complete and reduced LP morphs. In the current experiment, there were 2031 fish with the complete LP morphotype ($\geq$30 LP; see Barrett et al. 2009), 19 partial morphs (10–29 LPs), and no low LP morphs ($<$10 LPs). Excluding the 19 partially plated individuals from the analyses of genetic variance in size did not alter the results; there was still over 30 times more additive genetic variation in size under low versus high salinity rearing environments ($V_A = 3.17$ vs. 0.09). The average number of LPs was 32.8 in low salinity and 32.9 in high salinity (mode

of 33 in both environments), very similar to the 33.1 reported for this population previously (Cresko et al. 2004). Consistent with the observation by Barrett et al. (2009) that LP inheritance was not salinity specific, there was no difference in partial LP morph frequency between salinities in the current experiment (11 in low and eight in high salinity: $X^2 = 0.220$, df $= 1$, $P = 0.6387$).

The current study demonstrates that environment-specific allelic effects can contribute to differences in the evolutionarily relevant additive genetic variance at naturally occurring allelic frequencies. Further, the difference in genetic variance between salinity environments occurred in individuals that were monomorphic for LP phenotype, extending previous results to suggest that loci other than *Eda* (or loci linked to *Eda*) have salinity-specific effects on stickleback body size. Continuously distributed traits, such as size, typically tend to be highly polygenic (Flint and Mackay 2009). Few loci affecting body size in stickleback have been identified, with one on LGXIII (Albert et al. 2008) and one on LGXIX (Colosimo et al. 2004; Kitano et al. 2009) (note that these loci are independent of *Eda*, which is located on LGIV: Colosimo et al. 2004, 2005). However, genome-wide identification of variants contributing to body size variation within a population has not been the focus of any study to date, and body size in stickleback might be polygenic. If the contribution of genetic variants to size is context dependent, and if size variation is due to many loci with relatively small-effect alleles, then identifying these variants using standard approaches might be difficult (Eyre-Walker 2010; Hill 2010; Houle 2010).

The effect of the salinity treatment on both phenotypic mean and variance suggested that plastic responses to the novel, low salinity conditions were unlikely to contribute to rapid evolution of body size following freshwater colonization (Kirkpatrick 1982; Price et al. 2003). Although there was a statistically significant effect of the salinity treatment on body size mean and variation, these effects were small relative to the large effect on the genetic variation and were in the opposite direction to expected if plasticity had accelerated adaptation to freshwater. A recent experimental evolution study suggested the direction of selection on growth might change over ontogeny in threespine stickleback (Barrett et al. 2008). There is also some evidence that salinity-specific effects on growth change over ontogeny (Barrett et al. 2009; McCairns and Bernatchez 2010). More information is needed on stickleback growth, and on variation in the direction of selection on size across ontogeny to determine if the observed salinity effects on size might have contributed to adaptation.

Conclusions

Further progress in understanding the subtleties of evolution depend on understanding how genetic variances evolve under selection (Barton and Turelli 1989; Walsh and Blows 2009). Similarly, there is a need to consider how genetic variation responds to the environment in which the population finds itself (Hoffmann and Merila 1999; Charmantier and Garant 2005; McGuigan and Sgro 2009). In this experiment, we manipulated the rearing environment of a population from a relatively stable (oceanic) environment, and demonstrated very low genetic variance for size in this ancestral (common) environment, but a large increase in the estimated genetic variance in a novel environment. Our results suggest that release of cryptic genetic variation could have contributed to the rapid evolution of size following freshwater colonization by stickleback. Further empirical information is required to determine the generality of this observation.

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Supporting Information

The following supporting information is available for this article:

Table S1. The model fit parameters from mixed model analyses of variance, where either a single estimate of each random effect (sire, dam, tank, and error) is estimated, or salinity-specific effects are fit.

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

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