Note

Discriminating Selection on Lateral Plate Phenotype and Its Underlying Gene, *Ectodysplasin*, in Threespine Stickleback

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abstract: When a signature of natural selection is discovered on a gene that is pleiotropic or in tight linkage with other genes, it is challenging to determine which of the affected phenotypes is under selection. One way to make progress is to employ methods for analyzing natural selection on correlated traits, including both genotype and phenotype. We used this approach in threespine stickleback to estimate selection on a rapidly evolving trait, lateral armor plates, while controlling for variation at its major underlying gene, *Ectodysplasin* (*Eda*), and vice versa. This allowed for independent estimates of selection on lateral plates and on *Eda* via other traits. Previously, we demonstrated allele frequency changes at *Eda* in a pond experiment. Here we show that this resulted from selection on both plates and on *Eda*, implying additional selection on other phenotypic traits affected by the same gene. This represents the first evidence for direct selection on lateral plates independent of selection on the *Eda* locus and highlights the value of measuring selection on both phenotypes and genotypes in studies of adaptation.

Keywords: genetics of adaptation, natural selection, evolutionary ecology, pleiotropy, correlated response to selection.

Introduction

The study of adaptation seeks to establish a link between phenotypic variants, their underlying genotypes, and their fitness in a given environment. Recent advances in sequencing and genomics have enabled researchers to identify genes and genomic regions under natural selection using genome scans (e.g., Beaumont and Balding 2004; Linnen et al. 2009; Jones et al. 2012; Therkildsen et al. 2013) and experimental studies of changes in allele frequency over time (e.g., Korves et al. 2007; Barrett et al. 2008; Burke et al. 2010; Fournier-Level et al. 2011; Pespeni et al. 2013; Gompert et al. 2014). However, pleiotropy and genetic linkage complicate the effort to identify phenotypic targets of selection. Even if a focal phenotype has been identified, a signature of selection at a locus may result instead via other traits determined by the same gene or linked genes, dragging the focal trait along as a correlated response. The challenge is similar to that faced by researchers attempting to identify which trait or traits, among a correlated suite, are the direct targets of phenotypic natural selection (Lande and Arnold 1983; Price and Langen 1992). Statistical methods for estimating selection on correlated phenotypic traits (Lande and Arnold 1983) have been useful in identifying the phenotypes that are direct targets of natural selection (e.g., Grant 1985; Schluter and Smith 1986; Price and Langen 1992; Nagy 1997; Reznick et al. 1997). Here we show that the same approach can be used to help determine whether selection on a focal phenotypic trait has contributed to changes in genotype frequency at an underlying gene.

We apply the approach developed by Lande and Arnold (1983) to a study of multivariate selection on armor plates and its major underlying gene, *Ectodysplasin* (*Eda*; Colosimo et al. 2005), in threespine stickleback (*Gasterosteus aculeatus*). Freshwater populations established following colonization by the marine threespine stickleback after the last ice age (10,000–12,000 years ago) have repeatedly evolved a reduction of bony lateral armor plates (Bell and Foster 1994). Adult marine stickleback generally possess 30–36 lateral plates on each side of the body, whereas most freshwater populations founded by marine colonizers have 0–9 plates (Bell and Foster 1994). The rate of evolution of plate loss in freshwater can be rapid (Klepaker 1993; Kristjánsson et al. 2002; Bell et al. 2004; Kristjánsson 2005). In every known case, loss of plates in freshwater has taken place via replacement of the "complete" armor allele at the *Eda* locus (hereafter, the *C* allele) by a relatively ancient "low" armor allele (the *L* allele; Colosimo et al. 2005). The rapid and parallel substitution of one allele by another

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strongly suggests that natural selection is responsible (Simpson 1953; Schluter and Nagel 1995), but it does not identify the mechanism of selection. In particular, we remain uncertain whether armor plating is itself the target of selection, or whether its rapid evolution is a by-product of selection on other traits affected by the same underlying gene.

The number of lateral plates has long been regarded as the direct target of natural selection (Bell and Foster 1994), and there is some evidence in support of this hypothesis (Hagen and Gilbertson 1973; Bell et al. 2004; Raeymaekers et al. 2007; Kitano et al. 2008; Leinonen et al. 2011; DeFaveri and Merilä 2013). However, no study has distinguished selection on lateral plates from selection on other traits that may be controlled by *Eda* or other tightly linked genes. In a previous study, we detected strong selection at the *Eda* locus in marine stickleback transplanted to freshwater ponds (Barrett et al. 2008). The experiment was conducted to test the growth hypothesis for the adaptive value of low armor plating in freshwater, namely, that low armor is favored because of the high cost of mineralizing bone under reduced ion availability (Giles 1983; Bell et al. 1993; Marchinko and Schluter 2007). The experiment tested the prediction that low-armor phenotypes would have faster growth, leading to higher survival and earlier reproduction, by measuring relative fitness of genotypes at the underlying *Eda* gene. All transplanted adult marine stickleback were heterozygous at the *Eda* locus (*CL* genotype), and they bred in artificial ponds to produce a cohort of all three genotypes (*CC*, *CL*, and *LL*), whose frequencies were then tracked over the course of a year. Some of the results of the study supported the growth hypothesis. In particular, mean body length was positively correlated with the number of low alleles a fish possessed. Barrett and colleagues (2008) also found strong selection at the *Eda* locus. No such selection on *Eda* was detected in similar crosses raised in the lab (Barrett and Schluter 2010). These results are consistent with the growth hypothesis; however, the study did not directly measure selection on lateral plates. Thus, it remains to be determined whether the observed changes in *Eda* genotype frequencies were the result of selection on plates or selection on other (unknown) traits affected by the *Eda* locus.

Here we estimate the strength of selection on lateral plates while controlling for *Eda* genotype. This is possible because genetic and phenotypic variation in lateral plate number is present within *Eda* genotypes (Colosimo et al. 2005). If selection acted on lateral plates, it should be detectable even while holding *Eda* genotype constant. Conversely, if genotype frequency changes at *Eda* resulted only via its pleiotropic effects on other selected traits, then holding *Eda* genotype constant should eliminate apparent selection on plates. In this case, holding the plate number constant should have little effect on the estimated strength of selection on the *Eda* genotype.

Material and Methods

Pond Experiment

The original experiment is fully described in Barrett et al. (2008). Briefly, 45 or 46 wild adult marine threespine stickleback heterozygous at the *Eda* locus were introduced into four experimental freshwater ponds on the University of British Columbia campus. The fish were allowed to reproduce, and beginning in August 2006, 50 F_1 progeny per pond per month were destructively sampled, assayed for standard length, and genotyped at a diagnostic marker for the *Eda* locus (Colosimo et al. 2005). The allele and genotype frequencies of the four F_1 populations were then compared over the course of a year.

Here we focus on the three samples of juvenile fish taken in September, October, and November of 2006, respectively, during which the strongest changes in genotypes frequencies were detected at the *Eda* locus (fig. 1). Selec-

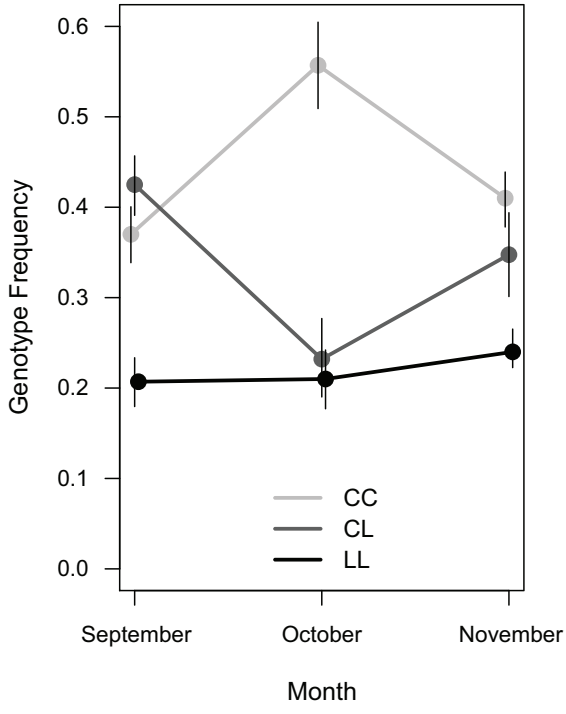

Figure 1: *Eda* genotype frequencies of marine stickleback after introduction to freshwater ponds. *CC* = carries two copies of the "complete" armor allele; *CL* = carries one complete allele and one "low" allele; *LL* = carries two copies of the low allele. Data points represent the mean of four ponds; error bars represent the standard error of the mean. Data replotted with permission from Barrett et al. (2008).

tion increased the *CC* genotype frequency by 19% between September and October and lowered the *CL* genotype frequency an equal amount. However, *C* allele frequency decreased from October to November, driven by a 15% decrease in *CC* genotype frequency and a 12% increase in *CL* genotype frequency. During both periods, the *LL* genotype frequency remained largely unchanged.

Lateral Plate Phenotype

We measured the number of lateral plates in a random sample of individuals from each of the three genotypes, yielding a total of 76–85 fish from each of the September, October, and November samples in the Barrett et al. (2008) experiment (241 fish in total). Fish specimens were fixed in 10% formalin and stained with 0.001%–0.002% w/v alizarin red S powder in a 2% w/v potassium hydroxide solution. Stained fish were then photographed under standardized conditions, and the total number of lateral plates on the left side was counted.

Lateral plate number was positively correlated with standard length, a measure of fish body size, particularly in *CC* and *CL* genotypes (*CC*: $r = 0.83$, $P = 2.07 \times 10^{-13}$; *CL*: $r = 0.53$, $P = 4.94 \times 10^{-12}$; *LL*: $r = 0.07$, $P = .45$), indicating that the number of plates had not completed development in many of the juvenile individuals in the samples. We used a breakpoint regression method (fig. A1; figs. A1, A2 are available online) to size adjust plate number as follows: we fit a model in which the logarithm of plate number Y increased linearly with fish length x up to a threshold value, x^*, beyond which no further change took place:

$$Y = a + bx \quad \text{for } x < x^*,$$
$$Y = a + bx^* \quad \text{for } x \geq x^*.$$

The constants a, b, and x^* were estimated from the data using nonlinear least squares (using the nls function in R, ver. 2.15.0; R Development Core Team 2012). Log plate counts were used in this analysis to reduce the skew of the data. The intercept and slope, a and b, were free to vary between *Eda* genotypes. We assumed that the threshold size x^* was the same for all three *Eda* genotypes, since more complex models that also varied the threshold x^* between genotypes did not improve the fit, as judged by Akaike Information Criterion scores. Incorporating separate coefficients for each of the four ponds also did not improve the fit, and the analyses we present here do not include pond effects. The threshold x^* value was estimated to be 34.0 ± 1.3 mm SE (fig. A1). In our selection analyses, the log plate number for each fish was size adjusted to a body length of 34.0 mm. Adjusted log plate counts were then back transformed to the original and more intuitive non–log scale. Adjusted plate counts exceeding the value 32 were reduced to 32 to ensure that the range did not exceed the natural maximum seen in our data set. Data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.dg82p (Rennison et al. 2014).

Selection Analysis

We estimated selection coefficients (standardized partial regression coefficients) using the method for cross-sectional data (Lande and Arnold 1983)

$$\hat{\boldsymbol{\beta}} = \mathbf{P}^{-1}[\bar{\mathbf{x}}_{\text{after}} - \bar{\mathbf{x}}_{\text{before}}],$$

where $\hat{\boldsymbol{\beta}}$ is the vector of estimated selection coefficients, $\bar{\mathbf{x}}$ is the vector of means of the focal phenotype trait and genotype scores (hereafter, traits) before and after selection, and $\mathbf{P}$ is the matrix of variances and covariances of the traits before selection. Separate analyses were carried out for the two episodes of selection, one between September and October 2006 and the other between October and November 2006 (fig. 1).

The vectors of trait means included the size-adjusted number of plates as the focal phenotypic trait. Genotype was scored using two genotype indicator variables. The first genotype variable (additive) coded the *LL* genotype as −1, the *CL* genotype as 0, and the *CC* genotype as 1. The second genotype variable (dominance) coded homozygous genotypes (*LL* and *CC*) as 0 and the heterozygotes (*CL*) as 1. All selection coefficients were standardized by multiplying each partial regression coefficient by the standard deviation of the trait before selection to allow comparison between trait and genotype scores measured on different scales (Lande and Arnold 1983). Since *Eda* genotype is categorical and lateral plate number is numerical, the method used here is an analysis of covariance to tease apart the contributions of genotype and plates to relative survival. Note that $\hat{\boldsymbol{\beta}}$ is not strictly a selection gradient because the traits do not have a multivariate normal distribution.

Our analysis is within a single generation and hence requires no assumptions about the probability distribution of breeding values of the numeric trait. Lande (1983) presents the theory for predicting evolutionary response to selection on a quantitative trait influenced by a major locus and having an otherwise additive genetic basis. We do not predict evolutionary response here because our focus is on selection.

We also calculated standardized univariate selection intensities (s') separately for lateral plates and the genotype variables as

$$s' = \frac{\bar{x}_{\text{after}} - \bar{x}_{\text{before}}}{\hat{\sigma}_{\text{before}}},$$

where $\bar{x}_{\text{before}}$ and $\bar{x}_{\text{after}}$ are the phenotype or genotype trait means (coded in the same way as the dominance variable) before and after selection, and $\hat{\sigma}_{\text{before}}$ is the estimated standard deviation of the trait before selection.

For simplicity, our analysis used size-adjusted lateral plates. However, we carried out an additional analysis in which size (standard length) was included as a trait along with unadjusted lateral plate number and *Eda* genotype. To accommodate the nonlinear relationship between plate number and size, it was necessary to adjust the standard length of the largest individuals to a maximum of 34.0 mm, the estimated breakpoint (fig. A1). The results of this analysis were not quantitatively different from the simpler analysis using size-adjusted plates, and so we do not present it.

To simplify further, our first analysis excluded the *LL* genotype and retained the *CL* and *CC* genotypes (table 1). In this case, there is only one genotype variable in the linear model (*CL* is scored as 1, and *CC* is scored as 0), which incorporates the dominance component of the *Eda* genotype but also half the additive component. This simplification is justified because the *CC* and *CL* genotypes have high variance (table A2) and overlap in plate number, which is required to disentangle the separate effects of plates and genotypes on fitness. The variance-covariance matrices for the one-genotype analysis are given in tables A3 and A4 (one for each pair of months). In contrast, there is little plate number variation within the *LL* genotype and little overlap in plate number with the other genotypes. A second analysis that included the *LL* genotype and the dominance variable is reported in the online appendix (table A1; see tables A5, A6 for the variance-covariance matrices).

We generated 95% confidence intervals for partial regression coefficients for each pair of months using a bootstrap resampling procedure. Each bootstrap replicate involved resampling with replacement, for a given month, the genotypes of n individuals from the corresponding Barrett et al. (2008) data set, where n is the number of individuals measured in our sample (the number for which we have lateral plate measurements). Next, for each genotype i, we resampled n_i phenotypes from the distribution of lateral plates corresponding to each genotype in the data. Resampling was done 10,000 times. Standardized partial selection coefficients were then calculated on each bootstrap replicate. The 0.025 and 0.975 quantiles of the coefficients were used to calculate the 95% confidence intervals of the parameters. The 95% confidence intervals of the selection intensities were estimated in the same way.

All our analyses incorporate pond as a fixed effect, essentially treating individual fish as the unit of replication. This is in contrast to the Barrett et al. (2008) study, which treated pond as a random effect and the unit of replication. Our approach here is justified because our goal is not to test for selection generally, as in the case of Barrett et al. (2008), but rather to investigate more narrowly the targets of selection within an experiment that has already demonstrated selection. All of the ponds responded similarly, and including pond in the analysis did not affect the results. For simplicity, we present only the results for the analyses without the pond variables. All statistical analyses were conducted in R (ver. 2.15.0; R Development Core Team 2012).

Table 1: Standardized partial selection coefficients for lateral plate phenotype and *Eda* genotype for the September–October 2006 and October–November 2006 episodes

	September–October $\hat{\beta}$	October–November $\hat{\beta}$
Lateral plates	.34 (−.04, .74)	−.21 (−.66, .22)
Eda genotype	−.42 (−.07, −.79)	.26 (.77, −.23)

Note: Values in parentheses are 95% confidence intervals.

Results

We detected strong selection on both *Eda* genotype and lateral plates (tables 1, A1) using multivariate methods. Between September and October, selection favored the *Eda CC* genotype over the *CL* genotype: point estimates of selection for the genotype variable and phenotypic trait, lateral plates, were similar in magnitude (table 1), although the 95% confidence interval for lateral plates narrowly spanned zero. Between October and November, the direction of selection changed, now favoring the *CL* genotype. During this period, selection on lateral plates and genotype was slightly weakened, and the confidence intervals for both variables spanned zero (table 1). Thus, selection on plates and genotype could not be disentangled in this episode. These analyses include only two genotypes and, hence, a single genotype variable that lumped additive and dominance components. When we analyzed all three genotypes in the September–October episode by including two genotype variables, one for each of the additive and dominance components, we detected strong selection on the dominance component of genotype (table A1). The *CL* genotype was disfavored, in agreement with the findings of Barrett et al. (2008), who detected heterozygote underdominance for fitness. The point estimate of selection on plates was positive, but the confidence interval (barely) spanned zero. All confidence intervals for selection between October and November spanned zero, again indicating that selection on plates and genotype could not be disentangled in this episode (table A1).

If we had limited our analyses to univariate estimates of selection (s'), which by definition do not account for selection on correlated characters, the picture would have appeared differently. The mean number of adjusted plates changed only slightly between September (18.7 ± 0.91 SE), October (19.01 ± 1.04 SE), and November (18.65 ± 0.89 SE; fig. A2). Consequently, the estimates of selection intensity indicated much weaker selection on lateral plates relative to selection on *Eda* genotype, which was at least an order of magnitude greater (table 2). Additionally, unlike the selection intensity estimates for *Eda* genotype, the selection intensity estimates for lateral plates were near zero. Correspondingly, if selection estimates on lateral plates were only univariate (table 2), the selection on lateral plates that was detected in the multivariate analysis (table 1) would have been missed.

Table 2: Standardized univariate selection intensities for *Eda* genotype and lateral plate phenotype for the September–October 2006 and October–November 2006 episodes

	September–October s'	October–November s'
Lateral plates	.04 (−.29, .38)	−.04 (−.33, .25)
Eda genotype	−.39 (−.21, −.56)	.28 (.07, .52)

Note: Values in parentheses are 95% confidence intervals.

Discussion

We compared the magnitude of selection on a trait and its major underlying gene to investigate the targets of selection during two episodes. Univariate estimates of selection indicated strong selection on *Eda* genotype and only weak selection on lateral plate phenotype (table 2). Our aim was to determine whether changes in *Eda* genotype frequency within a cohort in an experiment were due to selection on lateral plates themselves, as has been repeatedly suggested (Hagen and Gilbertson 1973; Reimchen 1992; Bell et al. 2004; Kitano et al. 2008), or the result of selection on some other unmeasured traits affected by the same underlying gene. Using Lande-Arnold methods for correlated characters, we found that selection on lateral plates was of similar strength as selection on *Eda* genotype in one of two episodes (tables 1, A1), although barely nonsignificant. In the second episode, confidence limits for both selection coefficients spanned zero, and thus selection on plates and genotype could not be disentangled (tables 1, A1). The three-genotype analysis incorporating a dominance variable (table A1) indicates that the selection on *Eda* genotype (table 1) was largely the result of selection against heterozygotes, rather than on the additive component of genotype; this heterozygote underdominance for fitness was previously suggested by Barrett et al. (2008).

These results suggest that the rapid changes in genotype frequency at the *Eda* locus during experimental freshwater introduction were partially due to selection on lateral plate phenotype and partially due to selection on additional, unmeasured traits controlled by *Eda* or a tightly linked gene. However, genotype and phenotype are strongly correlated ($r = 0.698$), and their separate effects on fitness could not be separated in the second episode (October–November). While the analysis of selection on correlated traits has its limitations (discussed by Mitchell-Olds and Shaw 1987), we suggest these methods may help to disentangle whether selection is directly or indirectly influencing a phenotypic trait of interest when genotype at its major underlying locus is known.

Direct selection on lateral plate phenotype has long been thought to be the main factor driving the repeated evolution of reduced lateral plate armor in freshwater populations (Hagen and Gilbertson 1973; Reimchen 1992; Bell et al. 2004; Kitano et al. 2008; Leinonen et al. 2011). A number of studies have suggested that plate reduction has been favored in freshwater due to an increased cost of mineralizing bone in freshwater due to reduced ion availability relative to marine environments (Giles 1983; Bell et al. 1993). This hypothesis has some empirical support; Marchinko and Schluter (2007) and Barrett et al. (2008) found that low-plated genotypes grew faster in freshwater. Other work suggests that reduced predation in freshwater environments relative to the marine environment is responsible for lateral plate reduction (Moodie et al. 1973; Reimchen 1992, 2000). Lateral plate reduction has also been suggested to improve swimming ability by increasing burst speed and buoyancy, which may aid in predation avoidance (Bergstrom 2002; Myhre and Klepacker 2009). However, until now, no study had disentangled the direct effect of plates on growth and survival from the effects of *Eda* via other unmeasured phenotypic traits.

Two previous studies of geographic variation in lateral plates and *Eda* genotype frequencies found that genetic variance among populations (F_{ST}) at *Eda* was lower than phenotypic variance in lateral plates (Q_{ST}/P_{ST}; Raeymaekers et al. 2007; DeFaveri and Merilä 2013), relative to total variance. This suggested stronger divergent selection among populations on lateral plates than on *Eda* frequencies. These results are consistent with our findings of selection on plates in a pond experiment. However, when we estimated the direct contribution of lateral plates to changes in *Eda* genotype frequencies, we found that lateral plates only partially explain these changes. Statistically controlling for number of lateral plates did not eliminate the signal of strong selection on *Eda*. These results highlight the value of measuring selection on a trait and its major underlying gene to help identify targets of selection.

On the basis of these results, we suggest that the rapid

and repeated evolution of low-plated armor in freshwater may be the result of both selection on lateral plates and a correlated response to selection on other unmeasured traits affected by *Eda*. *Eda* has been suggested to have diverse pleiotropic effects (Barrett et al. 2009*a*; Sadier et al. 2014). For example, *Eda* has been shown to affect the number of neuromasts along the lateral line (Wark et al. 2012; Mills et al. 2014). It is currently unclear whether *Eda*'s effect on neuromast distribution is direct or mediated indirectly through lateral plate development (Mills et al. 2014). Thus, there is potential that some of the selection we detect at the *Eda* locus and/or lateral plates is due to selection on the lateral line sensory system. In addition to its role in lateral plate and neuromast development, variation at *Eda* is associated with variation in schooling behavior (Greenwood et al. 2013) and propensity to switch between water conditions of varying salinity (Barrett et al. 2009*b*). Alternatively, selection might be acting on traits controlled by genes in linkage disequilibrium with *Eda* (Colosimo et al. 2005), which lies in a region of low recombination (Hohenlohe et al. 2011).

This study serves as a reminder that although genetic and genomic studies are informative about the evolution of traits, alone they provide insufficient evidence for selection on those traits, even when the link between a particular genotype and phenotype appears clear. Correspondingly, estimates of natural selection on phenotypes will remain an important component in genomic studies of adaptation (Travisano and Shaw 2013) and are required to indicate whether mechanisms such as correlated selection are at work.

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Literature Cited

Barrett, R. D. H., S. M. Rogers, and D. Schluter. 2008. Natural selection on a major armor gene in threespine stickleback. Science 322:255–257.

———. 2009*a*. Environment specific pleiotropy facilitates divergence at the *Ectodysplasin* locus in threespine stickleback. Evolution 63: 2831–2837.

Barrett, R. D. H., and D. Schluter. 2010. Clarifying mechanisms of evolution in stickleback using field studies of natural selection on genes. Pages 332–346 *in* P. Grant and R. Grant, eds. In search of the causes of evolution: from field observations to mechanisms. Princeton University Press, Princeton, NJ.

Barrett, R. D. H., T. H. Vines, J. S. Bystriansky, and P. M. Schulte. 2009*b*. Should I stay or should I go? the *Ectodysplasin* locus is associated with behavioural differences in threespine stickleback. Biology Letters 5:788–791.

Beaumont, M. A., and D. J. Balding. 2004. Identifying adaptive genetic divergence among populations from genome scans. Molecular Ecology 13:969–980.

Bell, M. A., W. E. Aguirre, and N. J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58:814–824.

Bell, M. A., and S. A. Foster, eds. 1994. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell, M. A., G. Ortí, J. A. Walker, and J. P. Koenings. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. Evolution 47:906–914.

Bergstrom, C. A. 2002. Fast-start performance and reduction in lateral plate number in threespine stickleback. Canadian Journal of Zoology 80:207–213.

Burke, M. K., J. P. Dunham, P. Shahrestani, K. R. Thornton, M. R. Rose, and A. D. Long. 2010. Genome-wide analysis of a long-term evolution experiment with *Drosophila*. Nature 467:587–590.

Colosimo, P. F., K. E. Hosemann, S. Balabhadra, G. Villarreal, M. Dickson, J. Grimwood, J. Schmutz, R. M. Myers, D. Schluter, and D. M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of *Ectodysplasin* alleles. Science 307:1928–1933.

DeFaveri, J., and J. Merilä. 2013. Evidence for adaptive phenotypic differentiation in Baltic Sea sticklebacks. Journal of Evolutionary Biology 26:1700–1715.

Fournier-Level, A., A. Korte, M. D. Cooper, M. Nordborg, J. Schmitt, and A. M. Wilczek. 2011. A map of local adaptation in *Arabidopsis thaliana*. Science 334:86–89.

Giles, N. 1983. The possible role of environmental calcium levels during the evolution of phenotypic diversity in Outer Hebridean populations of the three-spined stickleback, *Gasterosteus aculeatus*. Journal of Zoology 199:535–544.

Gompert, Z., A. A. Comeault, T. E. Farkas, T. L. Parchman, C. A. Buerkle, and P. Nosil. 2014. Experimental evidence for ecological selection on genome variation in the wild. Ecology Letters 17:369–379.

Grant, B. R. 1985. Selection on bill characters in a population of Darwin's finches: *Geospiza conirostris* on Isla Genovesa, Galapagos. Evolution 39:523–532.

Greenwood, A. K., A. R. Wark, K. Yoshida, and C. L. Peichel. 2013. Genetic and neural modularity underlie the evolution of schooling behavior in threespine stickleback. Current Biology 23:1884–1888.

Hagen, D. W., and L. G. Gilbertson. 1973. Selective predation and the intensity of selection acting upon the lateral plates of threespine sticklebacks. Heredity 30:273–287.

Hohenlohe, P. A., S. Bassham, M. C. Currey, and W. A. Cresko. 2011. Extensive linkage disequilibrium and parallel adaptive divergence across threespine stickleback genome. Philosophical Transactions of the Royal Society B: Biological Sciences 5:395–408.

Jones, F. C., M. G. Garbherr, Y. F. Chan, P. Russel, E. Mauceli, J. Johnson, R. Swofford, et al. 2012. The genomic basis of adaptive evolution in threespine sticklebacks. Nature 484:55–61.

Kitano, J., D. I. Bolnick, D. A. Beauchamp, M. M. Mazur, S. Mori, T. Nakano, and C. L. Peichel. 2008. Reverse evolution of armor plates in threespine stickleback. Current Biology 18:769–774.

Klepaker, T. 1993. Morphological changes in a marine population of threespined stickleback, *Gasterosteus aculeatus*, recently isolated in fresh water. Canadian Journal of Zoology 71:1251–1258.

Korves, T., K. J. Schmid, A. L. Caicedo, C. Mays, J. R. Stinchcombe, M. D. Purugganan, and J. Schmitt. 2007. Fitness effects associated with the major flowering time gene *FRIGIDA* in *Arabidopsis thaliana* in the field. American Naturalist 169:141–157.

Kristjánsson, B. K. 2005. Rapid morphological changes in threespine stickleback, *Gasterosteus aculeatus*, in freshwater. Environmental Biology of Fishes 74:357–363.

Kristjánsson, B. K., S. Skulason, and D. L., Noakes. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus L.*). Evolutionary Ecology Research 4:659–672.

Lande, R. 1983. The response to selection on major and minor mutations affecting a metrical trait. Heredity 50:47–65.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Leinonen, T., G. Herczeg, J. M. Cano, and J. Merilä. 2011. Predation-imposed selection on threespine stickleback (*Gasterosteus aculeatus*) morphology: a test of the refuge use hypothesis. Evolution 65: 2916–2926.

Linnen, C. R., E. P. Kingsley, J. D. Jensen, and H. E. Hoekstra. 2009. On the origin and spread of an adaptive allele in deer mice. Science 235:1095–1098.

Marchinko, K. B., and D. Schluter. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution 61:1084–1090.

Mills, M. G., A. K. Greenwood, and C. L. Peichel. 2014. Pleiotropic effects of a single gene on skeletal development and sensory system patterning in sticklebacks. EvoDevo 5:5.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149–1161.

Moodie, G. E. E., J. D. McPhail, and D. W. Hagen. 1973. Experimental demonstration of selective predation on *Gasterosteus aculeatus*. Behaviour 47:95–15.

Myhre, F., and T. Klepaker. 2009. Body armour and lateral-plate reduction in freshwater three-spined stickleback, *Gasterosteus aculeatus*: adaptations to a different buoyancy regime? Journal of Fish Biology 75:2062–2074.

Nagy, E. S. 1997. Selection for native characters in hybrids between two locally adapted plant subspecies. Evolution 51:1469–1480.

Pespeni, M. H., E. Sanford, B. Gaylord, T. M. Hill, J. D. Hosfelt, H. K. Jaris, M. LaVigne, et al. 2013. Evolutionary change during experimental ocean acidification. Proceedings of the National Academy of Sciences of the USA 110:6937–6942.

Price, T., and T. Langen. 1992. Evolution of correlated characters. Trends in Ecology and Evolution 7:307–310.

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Raeymaekers, J. A., J. K. Van Houdt, M. H. Larmuseau, S. Geldof, and F. A. Volckaert. 2007. Divergent selection as revealed by P_{ST} and QTL-based F_{ST} in three-spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-inland gradient. Molecular Ecology 16:891–905.

Reimchen, T. E. 1992. Injuries on stickleback from attacks by a toothed predator (*Oncorhynchus*) and implications for the evolution of lateral plates. Evolution 46:1224–1230.

———. 2000. Predator handling failures of lateral plate morphs in *Gasterosteus aculeatus*: functional implications for the ancestral plate condition. Behaviour 137:1081–1096.

Rennison, D. J., K. Heilbron, R. D. H. Barrett, and D. Schluter. 2014. Data from: Discriminating selection on lateral plate phenotype and its underlying gene, *Ectodysplasin*, in threespine stickleback. American Naturalist. Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.dg82p.

Reznick, D. N., F. H. Shaw, H. F. Rodd, and R. G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934–1937.

Sadier, A., L. Viriot, S. Pantalacci, and V. Laudet. 2014. The *Ectodysplasin* pathway: from diseases to adaptations. Trends in Genetics 30:24–31.

Schluter, D., and L. M. Nagel. 1995. Parallel speciation by natural selection. American Naturalist 146:292–301.

Schluter, D., and J. Smith. 1986. Natural selection on beak and body size in the song sparrow. Evolution 40:221–231.

Simpson, G. G. 1953. The major features of evolution. Columbia University Press, New York.

Therkildsen, N. O., J. Hemmer-Hansen, T. D. Als, D. P. Swain, M. J. Morgan, E. A. Trippel, S. R. Palumbi, D. Meldrup, and E. E. Nielsen. 2013. Microevolution in time and space: SNP analysis of historical DNA reveals dynamic signatures of selection in Atlantic cod. Molecular Ecology 22:2424–2440.

Travisano, M., and R. G. Shaw. 2013. Lost in the map. Evolution 67: 305–314.

Wark, A. R., M. G. Mills, L.-H. Dang, Y. F. Chan, F. C. Jones, S. D. Brady, D. M. Absher, et al. 2012. Genetic architecture of variation in the lateral line sensory system of threespine sticklebacks. Genes, Genomes, Genetics 2:1047–1056.

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