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Materials and Methods
SOM Text
Figs. S1 to S6
Tables S1 to S3
References

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Natural Selection on a Major Armor Gene in Threespine Stickleback

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Experimental estimates of the effects of selection on genes determining adaptive traits add to our understanding of the mechanisms of evolution. We measured selection on genotypes of the *Ectodysplasin* locus, which underlie differences in lateral plates in threespine stickleback fish. A derived allele (low) causing reduced plate number has been fixed repeatedly after marine stickleback colonized freshwater from the sea, where the ancestral allele (complete) predominates. We transplanted marine sticklebacks carrying both alleles to freshwater ponds and tracked genotype frequencies over a generation. The low allele increased in frequency once lateral plates developed, most likely via a growth advantage. Opposing selection at the larval stage and changing dominance for fitness throughout life suggest either that the gene affects additional traits undergoing selection or that linked loci also are affecting fitness.

Adaptive evolution occurs when genetic variation affects phenotypes under selection. This process has been detected by the discovery of candidate genes underlying phenotypic traits whose adaptive significance is known or suspected (*1*–*7*) and by identifying statistical signatures of selection on genomic regions affecting phenotypic traits (*8*–*12*). However, field experiments evaluating the fitness consequences of allelic substitutions at candidate loci should provide estimates of the timing and strength of selection, enhance understanding of the genetics of adaptation, and yield insights into the mechanisms driving changes in gene frequency.

Freshwater threespine sticklebacks (*Gasterosteus aculeatus*) originated from marine populations that invaded newly created coastal lakes and streams throughout the Northern Hemisphere following the last ice age. Within the past 20,000 years or less, freshwater populations repeatedly underwent a loss in bony armor plating (*13*). Marine sticklebacks are typically armored with a continuous row of 30 to 36 bony lateral plates on each side (complete morph), whereas freshwater sticklebacks typically have 0 to 9 plates (low morph) or, less often, an intermediate number of plates (partial morph) (*13*–*15*) (Fig. 1). Armor reduction following colonization of freshwater evolved rapidly (*16*–*19*) from the fixation of a clade of low alleles of the *Ectodysplasin* gene (hereafter, the *Eda* low allele). This allele evolved ~2 million years ago and is rare (~1%) in the ocean (*1*). The repeated fixation of this allele implies that it undergoes positive selection in freshwater, because genetic drift alone is unlikely to produce a strong correlation between phenotype and environment (*20*–*22*).

Fish with reduced armor have a juvenile growth advantage (*23*), which may result from the higher cost of mineralizing bone in freshwater (*24*, *25*), which has low ion concentrations relative to marine environments. This increased growth rate should, in turn, reduce predation by insects (*26*), as well as increase lipid stores, which results in higher over-winter survival (*27*). Larger fish also may breed earlier (*28*) and have access to better terri-

Fig. 1. Lateral plate morphs in marine stickleback. Complete morph (top), partial morph (middle), and low morph (bottom). Fish were stained with Alizarin red to highlight bone.

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tories, an increase in mating success, and a higher reproductive output (*28*–*36*). To test this hypothesis, we tracked adaptive evolution at the *Eda* locus in replicated transplants of marine stickleback to freshwater environments. We predicted that we would observe positive selection on the low allele via advantages in growth, survival, and reproduction. We also looked for deviations from this expectation, which might suggest that *Eda* or linked genes have unexpected fitness effects.

We experimentally introduced adult wild marine fish heterozygous at the *Eda* locus to four freshwater ponds (*37*). The fish were trapped from a marine stickleback population in southwestern British Columbia. We introduced approximately equal numbers of these fish (n = 45 to 46) to each pond in the spring of 2006, initiating replicate freshwater invasions. Within 60 days, we observed larval fish in each colonized pond, indicating that the marine colonizers were breeding. Genotyping of four microsatellite markers, which were all in linkage equilibrium with *Eda*, confirmed that nearly all alleles present in the parents were at similar frequencies in the progeny (fig. S1), which suggested that founding events did not confer any sampling artifacts. Genotype frequencies at *Eda* in the F_1 generation were not significantly different from the predicted 1:2:1 ratio (Fig. 2A) [pond 1: $\chi^2(2) = 0.06$, $P = 0.97$; pond 2: $\chi^2(2) = 1.09$, $P = 0.58$; pond 3: $\chi^2(2) = 1.09$, $P = 0.58$; and pond 4: $\chi^2(2) = 1.20$, $P = 0.55$]. Subsequently, we sampled 50 fish from each pond 10 times over 1 year to monitor changes in offspring allele frequencies.

We observed strong fluctuations in *Eda* allele and genotype frequencies, with replicate ponds showing nearly parallel oscillations (Fig. 2A). We did not observe strong changes in allele frequency in the unlinked microsatellite markers, which suggested that these results are not due to demographic effects (fig. S1). Fish achieved their adult number of lateral plates after reaching a standard length of ~30 mm (*25*, *38*, *39*). Most experimental fish passed this threshold between October and November 2006 [average length in October was 27.32 mm (± 5.99 SD); average length in November was 33.14 mm (± 4.70 SD)]. In agreement with our predictions for growth, by October, juvenile fish carrying the low allele were larger than juvenile fish homozygous for the complete allele. Mean body length was positively associated with the number of low alleles per genotype in all ponds [one-tailed t test of four slopes, $t(3) = 2.53$, $P = 0.043$]. We also noted higher overwintering survival rates in fish with the low allele. From October 2006 to May 2007, the frequency of the complete allele dropped from 67 to 49%, which reflected the comparatively poor survival of individuals homozygous for the complete allele. We calculated that the selection coefficient (S) against the complete allele between these dates was 0.52 (± 0.10 SEM) (Fig. 2) (*37*).

At the start of the breeding season in May 2007, the number of low alleles carried by an individual was again positively associated with body length in all ponds [one-tailed t test of four slopes, $t(3) = 2.35$, $P = 0.050$], and sexually mature individuals were significantly larger than nonbreeding individuals (Fig. 3) [Welch two-tailed t tests, pond 1: $t(6) = 2.47$, $P = 0.049$; pond 2: $t(2) = 9.40$, $P = 0.006$; pond 3: $t(9) = 2.61$, $P = 0.027$; and pond 4: $t(13) = 4.23$, $P < 0.001$]. The genotypes of the earliest reproductive individuals were biased toward carrying the low allele compared with nonreproductive individuals, with 95% being heterozygous or homozygous low (Fig. 3) [tested by the interaction between breeding status and genotype in a log-linear model, $\chi^2(2) = 7.30$, $P = 0.026$; no effects of pond were detected, $\chi^2(6) = 2.88$, $P = 0.82$]. By July 2007, most individuals had reached sexual maturity, and we observed little difference in genotype frequencies between sexually mature individuals and the overall population (Fig. 3) [$\chi^2(2) = 2.56$, $P = 0.28$]. By this time, we also could not detect a correlation between size and *Eda* genotype [$t(3) = -0.30$, $P = 0.607$]. In all four ponds, the frequency of the low allele was greater in the first sample of F_2 offspring in June 2007 than in all F_1 adults sampled in May [June F_2: 57.0% (± 4.1% SEM), May F_1: 51.6% (± 1.4% SEM)] (Fig. 2A) [one-tailed t test, $t(3) = 2.14$, $P = 0.061$]. By July, the frequency of the low allele in F_2 juveniles had decreased to 52.2% (± 3.7% SEM), which reflected the similar genotypic ratios of breeding and nonbreeding adults later in the breeding season.

These patterns linking the low *Eda* allele with higher growth, improved survival, and earlier breeding are consistent with the hypothesis that positive selection stemmed from a reduced burden of producing armor plates in freshwater. This effect, combined with the possibility of reduced vertebrate predation pressure in freshwater compared with the sea (*25*, *40*), may account for the evolution of low genotype populations with reduced plates in freshwater. At the same time, selection against plate production does not fully

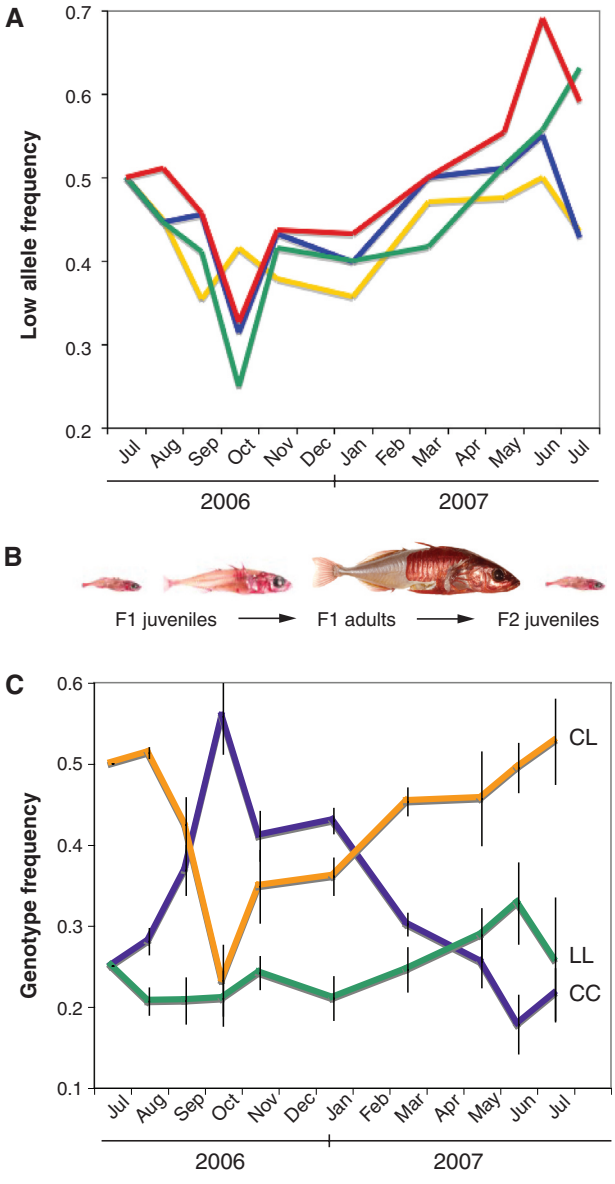

Fig. 2. (**A**) Frequency of the low allele in four replicate ponds (different colored lines). All samples are from the first (F_1) cohort of offspring, except the June and July 2007 samples, which are from the second (F_2) pond generation. (**B**) Approximate life history stages through the course of the experiment. Fish stained as in Fig. 1. (**C**) Genotype frequencies averaged across all four ponds. All samples are as in (A). Purple, homozygous complete genotype (CC); orange, heterozygote genotype (CL); green, homozygous low genotype (LL). Vertical bars show standard errors on the basis of n = 4 ponds.

explain the observed changes in *Eda* allele frequencies. We noted selection favoring the complete allele in all four ponds (Fig. 2A) very early in life, before the fish attain the size at which number of lateral plates is finalized (about 30 mm). The calculated selection coefficient (S) against the low allele between July and October 2006 was 0.50 (± 0.16 SEM) (Fig. 2C), which offset the gains occurring later in life. We also observed oscillations in the relative fitness of heterozygotes at *Eda*, which are difficult to explain solely in terms of the burden of lateral plates, because the size and number of plates in heterozygotes are intermediate between low and complete homozygotes (*22*). The decline in low *Eda* allele frequencies early in life was associated with a drop in the frequency of heterozygous fish and a rise in the frequency of the homozygous complete genotype, which suggested that there is heterozygote underdominance for fitness at this stage [h = –1.38 (± 0.23 SEM)]. Underdominance was especially apparent by October 2006, when heterozygous fish made up less than 25% of the total in our samples, instead of the 50% observed at the start of the F_1 cohort. This episode was followed by a period between November 2006 and May 2007 during which the heterozygotes at *Eda* had the highest fitness of all three genotypes [h = 2.57 (± 0.98 SEM)]. Although positive selection favored the low allele during this period, heterozygotes increased in frequency much faster than the homozygous low genotype (Fig. 2C). These findings suggest that either variation at the *Eda* gene has direct or epistatic effects on other phenotypic traits contributing to fitness, or it is linked to another, unidentified locus affecting fitness.

Our results highlight the utility of direct measurements of natural selection on genes for understanding the genetic basis of adaptation by enabling us to test a mechanism favoring reduction of lateral plates in freshwater environments. Many of our results are consistent with selection against high plate number, although they do not rule out the possibility that selection is also occurring on genes tightly linked to *Eda* (*1*). Our results also expose opposing selection on *Eda* early in life similar in magnitude to the measured advantage of the low allele later in life. This demonstrates not only that countervailing selection pressures diminish the advantage of the low allele over the whole life span but also that the overall fitness effects of *Eda* do not seem to be determined solely by differences in lateral plate number. Along with the fluctuating dominance in fitness at the *Eda* locus, these results indicate that there may be additional pleiotropic effects of this gene. This work underscores the need for a synthesis of population biology and genomics, to determine the genetic basis of fitness differences in natural populations (*41*).

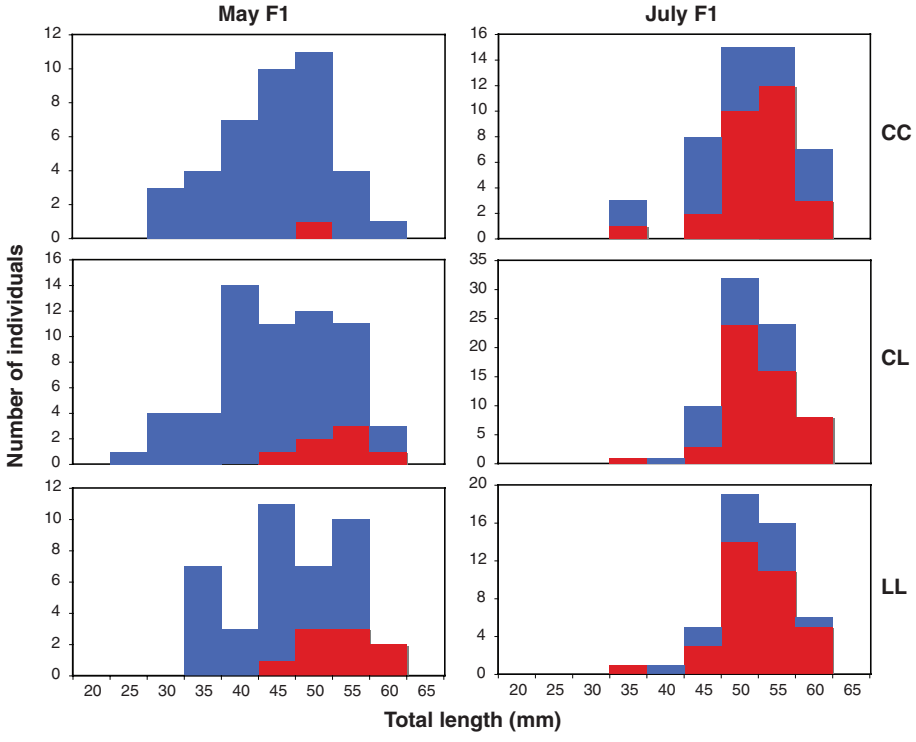

Fig. 3. Body length of individuals in the first (F_1) pond cohort during the breeding season, in May and July 2007, summed across all ponds. Red, individuals in reproductive condition; blue, individuals not in reproductive condition. *Eda* genotypes are labeled on the right axis: homozygous complete (CC), heterozygous (CL), and homozygous low (LL).

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