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Natural selection and the genetics of adaptation in threespine stickleback

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Growing knowledge of the molecular basis of adaptation in wild populations is expanding the study of natural selection. We summarize ongoing efforts to infer three aspects of natural selection—mechanism, form and history—from the genetics of adaptive evolution in threespine stickleback that colonized freshwater after the last ice age. We tested a mechanism of selection for reduced bony armour in freshwater by tracking genotype and allele frequency changes at an underlying major locus (*Ectodysplasin*) in transplanted stickleback populations. We inferred disruptive selection on genotypes at the same locus in a population polymorphic for bony armour. Finally, we compared the distribution of phenotypic effect sizes of genes underlying changes in body shape with that predicted by models of adaptive peak shifts following colonization of freshwater. Studies of the effects of selection on genes complement efforts to identify the molecular basis of adaptive differences, and improve our understanding of phenotypic evolution.

Keywords: natural selection; genetics of adaptation; stickleback; ectodysplasin

1. INTRODUCTION

Investigations into the genetics of adaptation in natural populations permit new insights into the process of natural selection. Knowledge of the molecular basis of phenotypic evolution will be useful in uncovering aspects of selection that are otherwise difficult to detect. An obvious example is the genomic 'signature of selection', which has indicated a role for directional selection in the evolution of many species differences in nature (e.g. Rieseberg *et al.* 2002; Nielsen 2005; Storz 2005; Rogers & Bernatchez 2007; Nosil 2009). However, knowledge of underlying genes will also permit direct measurements of the fitnesses of alternative genotypes, and so aid the study of the causes of selection (Bradshaw & Schemske 2003; Lexer *et al.* 2003; Barrett *et al.* 2008; Coberly & Rausher 2008; Gratten *et al.* 2008). Direct measurement of selection will additionally illuminate the form of selection on genetic polymorphisms, whether balancing, disruptive or frequency dependent (Subramaniam & Rausher 2000; Fitzpatrick *et al.* 2007), and contribute to our understanding of how genetic variation is maintained in the wild. As a final example, the distribution of the phenotypic effect sizes of genes may reveal aspects of the recent history of natural selection on traits (Orr 1998, 2005; Collins *et al.* 2007; Kopp & Hermisson 2007; Martin *et al.* 2007).

Here, we summarize three ongoing efforts to infer the mechanism, form and history of natural selection from the genetics of adaptive variation within and among wild populations of threespine stickleback. First, we describe an experiment in which we measured selection on a major locus underlying adaptive differences in a phenotypic trait in order to test a hypothesis about the causes of natural selection. Second, we summarize a new study investigating the form of selection on the same locus in a polymorphic stickleback population. Third, we apply the Fisher–Orr theory for the genetics of adaptation to infer recent adaptive peak shifts in a suite of phenotypic traits from the distribution of genetic effect sizes. Together, these studies illustrate some ways in which the genetics of adaptation is facilitating our studies of natural selection.

Our study system is the assortment of threespine stickleback populations inhabiting lakes and nearby marine waters of coastal British Columbia (BC), Canada. This geographical region was covered by ice until about 12 000 years ago, putting an upper limit on the ages of populations investigated. The lakes formed as coastal lands rebounded from the sea after the weight of ice was removed (Hutchinson *et al.* 2004). Lakes were colonized by marine stickleback shortly thereafter, initiating a process of adaptation to the new environments. The ancestral marine species continues to inhabit marine coastal waters, preserving the ancestral state for comparison.

The process of adaptation to postglacial lakes was repeated many times over. The direction of evolution was largely parallel in each case, though the magnitude of divergence from the marine form now varies from lake to lake according to local conditions (Walker & Bell 2000). In general, lake populations
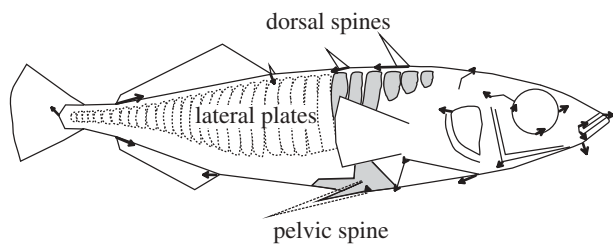
dorsal spines

lateral plates

pelvic spine

Figure 1. Evolution of armour and body shape in freshwater populations of threespine stickleback. Arrows indicate direction and relative magnitude of evolved changes in the position of body landmarks, from ancestral marine to derived freshwater, along the first principal component of among-population shape variation. Calculations are based on measurements of two marine and 15 freshwater populations. Dotted outlines represent lateral plates and pelvic spine present in the marine ancestor but absent or reduced in most freshwater populations.

exhibit reduced external body armour, reduced medial fins, greater overall head size and body depth and a downward rotation of the jaw compared with the ancestral marine form (figure 1). These morphological transitions from ocean to lake are associated with reduced predation by predatory birds and fish in freshwater, greater predation by aquatic insects, an increase in the availability of littoral habitat and benthic invertebrate prey and the loss of a migratory (anadromous) lifestyle. Such parallel evolution, occurring independently in hundreds of lakes, is compelling evidence for natural selection. Certain morphological changes in freshwater have known performance consequences (Taylor & McPhail 1986; Walker 1997). However, most details of the process of selection, such as the specific ecological factors causing fitness differences, remain obscure. As we show below, growing information about the genetic basis of phenotypic differences will aid efforts to fill the gaps.

2. TESTING MECHANISMS OF SELECTION
Repeated, parallel genetic changes between populations in similar environments provide observational evidence for the effects of natural selection at the genetic level (e.g. Balanya *et al.* 2006; Derome *et al.* 2006; Baxter *et al.* 2008; Feldman *et al.* 2009; Gross *et al.* 2009; Chan *et al.* 2010). Molecular signatures of selection at loci underlying ecologically significant phenotypic traits provide additional evidence (e.g. Edelist *et al.* 2006; McBride 2007; Linnen *et al.* 2009). Yet, neither type of pattern is usually enough by itself to infer the adaptive mechanisms—the precise reasons why certain alleles were favoured in those environments in which they spread to fixation. However, it is becoming feasible to carry out field experiments to test hypotheses about the causes of fitness differences between alternative alleles (Bradshaw & Schemske 2003; Lexer *et al.* 2003; Barrett *et al.* 2008; Coberly & Rausher 2008; Gratten *et al.* 2008).

We applied this approach to help us understand mechanisms driving the parallel evolution of reduced lateral plate armour in stickleback populations in postglacial lakes. *Ectodysplasin* (*Eda*) is the major gene underlying this reduction in lateral plates in freshwater

populations (Colosimo *et al.* 2005) (figure 1). Individuals homozygous for the low-armour allele typically have few plates, whereas those homozygous for the high-armour allele have a complete row of lateral plates from head to tail on each side. Heterozygotes are usually fully plated or have an intermediate number of plates. The low-armour *Eda* allele has been repeatedly selected in freshwater from standing genetic variation present in the ancestral marine populations (Colosimo *et al.* 2005). However, the ecological mechanisms of natural selection leading to fixation of the low-armour allele are not well understood.

We conducted an experiment to measure selection on *Eda* in transplanted populations, with the aim of clarifying the mechanisms of natural selection on lateral plates in freshwater (Barrett *et al.* 2008). The high-armour allele is thought to be favoured in the sea because having many lateral plates is advantageous for defence. The plates interfere with ingestion of stickleback by predatory fish and they reduce injury after escape (Reimchen 1995, 2000). The advantages of the low-armour allele in freshwater are less clear. Our experiment investigated a hypothesis arising from a laboratory experiment by Marchinko & Schluter (2007), which showed that juvenile stickleback with reduced lateral plate armour have a growth advantage in freshwater. In a subsequent laboratory experiment, Barrett *et al.* (2008) confirmed that young fish homozygous for the low-armour *Eda* allele grow more rapidly than high-armour homozygotes, whereas heterozygotes grow at an intermediate rate. This suggested that faster growth permitted by a reduction of lateral plates may explain the spread of the low-armour allele in freshwater lakes. In the wild, faster growth is predicted to translate into reduced mortality by predatory insects, which prey on the smallest size classes of stickleback, increased nutritional reserves for overwinter survival and enhanced reproductive success.

To test this, we transplanted adult marine stickleback heterozygous at the *Eda* locus to freshwater ponds and tracked selection on *Eda* genotypes in the offspring over a subsequent year. About 180 adult heterozygotes were used in the experiment, which were located by exhaustive search in a large marine population breeding in a salt water lagoon (Barrett *et al.* 2008). These marine fish were divided equally among four experimental ponds located on the campus of the University of British Columbia. The ponds had been excavated about 20 years previously and contained a natural fauna of plants and invertebrates. Each measured 23 m × 23 m and had a maximum depth of 3 m in the centre (Schluter 1994). Marine stickleback are highly tolerant to freshwater (most populations are anadromous, breeding in freshwater and returning to sea), and the colonists bred successfully in each pond. We took samples of 50 fish from the offspring cohort from each pond every four to six weeks to track growth rates and correlated changes in *Eda* genotype frequencies until the fish reached sexual maturity the following spring.

The number of lateral plates attains its final value in development by the time a stickleback is about 30 mm in length, which happened in the offspring generation
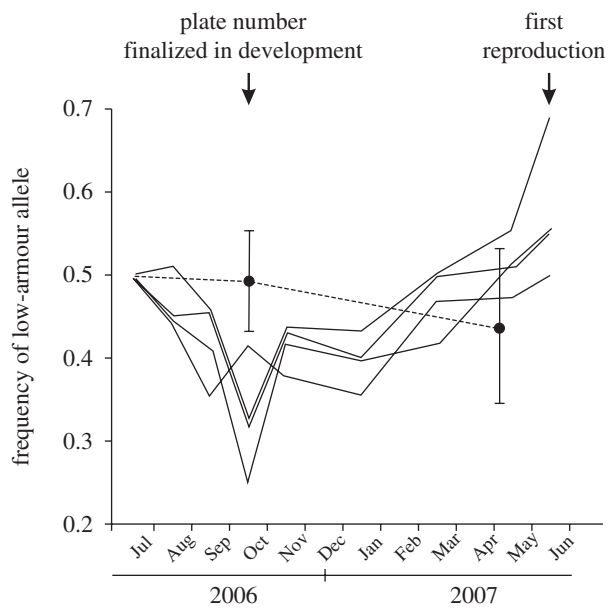
Figure 2. Natural selection on *Eda* in a cohort of marine stickleback transplanted to freshwater. The four solid lines indicate the frequency of the low-amour *Eda* allele in four replicate ponds from birth to adulthood. Indicated are the approximate dates at which the number of lateral plates reached its maximum in developing individual fish, and the date at which the first individuals in the cohort began to reproduce. The dashed line indicates the frequencies of the low-armour *Eda* allele in a laboratory control cohort. Adapted from Barrett & Schluter (2010).

by about October (figure 2). By this time, fish carrying the low-armour *Eda* allele were larger in size than fish homozygous for the high-armour allele, an advantage that persisted to adulthood the following spring (Barrett *et al.* 2008). The frequency of the low-armour *Eda* allele rose steeply over this same period, from 0.33 to 0.51, representing a 1.5-fold survival advantage compared with the high-armour allele. These findings are consistent with the predictions of the hypothesis of a growth advantage for the evolution of reduced lateral plate number in freshwater stickleback.

Unexpectedly, however, we detected nearly equally strong selection in the opposite direction in very young fish, before the number of lateral plates is finalized in development (figure 2). This selection against the low-armour genotypes early in life counteracted the gains by the low-armour allele later in life, yielding weak net selection over the lifespan. No such fluctuations in allele frequency were detected in fish of the same cohort raised in the laboratory (figure 2), implying that the agents responsible for selection both early and later in life were environment-specific and thus have an ecological basis.

Thus, we found some support for the hypothesis that a growth advantage is responsible for the parallel evolution of reduced armour in freshwater stickleback. However, opposing selection prior to completion of plate development suggests that *Eda* or a linked gene also has large effects on additional (unidentified) phenotypic traits under selection. The implication is that evolution of reduced lateral plates in marine colonists to freshwater is partly governed (indeed, opposed) by these pleiotropic or linkage effects.
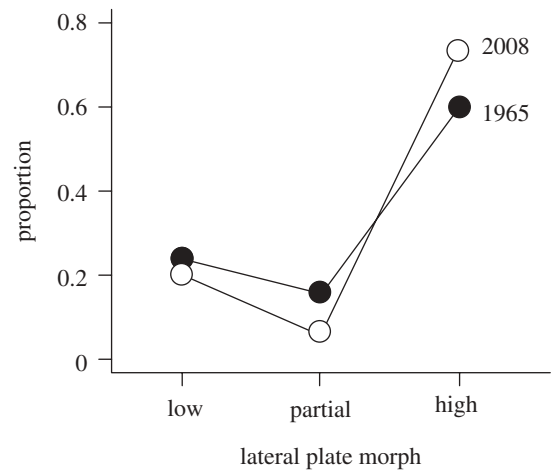
Figure 3. Frequencies of lateral plate morphs in the Kennedy Lake adult stickleback population sampled in 1965 and 2008 (K. B. Marchinko, B. W. Matthews, S. M. Rogers & D. Schluter 2010, unpublished data). Low-plated fish are those having 10 or fewer plates on each side. The high-armour morph has 30 or more plates per side. Partially plated individuals have between 11 and 29 plates on each side (Bell 2001).

It would not have been possible to detect these effects without knowledge of the gene, because selection early in life occurred before it was even possible to distinguish between lateral plate morphs. Our results also underscore the value of experiments for investigating the mechanisms of selection influencing major genes. Following isolation and discovery of a gene underlying an adaptation, it will often pay to place individuals of known genotypes into its environment, in order to evaluate its phenotypic effects and its fitness consequences.

3. THE FORM OF SELECTION ON A POLYMORPHIC TRAIT

Knowledge of the molecular basis of phenotypic variation will permit direct measurement of the form of selection on underlying genetic variation. One benefit will be an enhanced ability to determine whether genetic polymorphism at a given locus affected by selection is maintained by heterozygote advantage, frequency-dependent selection, or by a balance between directional selection and migration. Considerable insight has already been gained into the frequency of directional and nonlinear selection on phenotypic traits in natural populations (Hoekstra *et al.* 2001; Siepielski *et al.* 2009). Direct measurements of selection (or rather, of the effects of selection) on variation at underlying genes are still rare but increasingly within reach (Subramaniam & Rausher 2000; Coberly & Rausher 2008).

K. B. Marchinko, B. W. Matthews, S. M. Rogers & D. Schluter (2010, unpublished data) investigated the form of selection on variation at the *Ectodysplasin* (*Eda*) locus in a stickleback population polymorphic for lateral plates. The focus of the study is the population of Kennedy Lake, BC, which is one of only a few stickleback populations in the region that are polymorphic for lateral plate armour. In contrast, most

lake populations are low plated (Hagen & Gilbertson 1972). The frequency of different lateral plate morphs has been relatively stable over the past 42 years (figure 3). K. B. Marchinko, B. W. Matthews, S. M. Rogers & D. Schluter (2010, unpublished data) have confirmed that the polymorphism is associated with allelic variation at the *Ectodysplasin* (*Eda*) locus. Individuals homozygous for the low-armour allele typically have few plates, whereas those homozygous for the high-armour allele have a complete row of lateral plates. Heterozygotes are either fully plated or have an intermediate number of plates. This population thus presents an outstanding opportunity to investigate the forces maintaining genetic variation at a locus underlying an ecologically important phenotypic trait (Mitchell-Olds *et al.* 2007).

K. B. Marchinko, B. W. Matthews, S. M. Rogers & D. Schluter (2010, unpublished data) discovered that adults sampled from the lake exhibit a significant deficit of *Eda* heterozygotes. The frequency of heterozygotes was only about two-thirds that expected under Hardy–Weinberg equilibrium. Yet putatively neutral microsatellite frequencies found no trace of any genetic population structure within the lake. Moreover, the heterozygote deficit was absent in embryos that were tested. The implication is that disruptive natural selection, rather than non-random mating, is responsible for the deficit of heterozygotes among adult fish.

However, disruptive selection by itself should destabilize rather than maintain a genetic polymorphism. The rarer allele (in this case, the low-armour allele) is expected to be driven to extinction under random mating because a greater fraction of its copies will occur in heterozygotes, which are selected against. Only frequency-dependent selection can stabilize a polymorphism with heterozygote disadvantage, by favouring alleles when they are scarce (Lewontin 1958; Wilson & Turelli 1986). Conceivably, natural or sexual selection in the Kennedy Lake population might favour rare homozygous male genotypes and stabilize the polymorphism, though as yet we have no direct evidence of this. One possibility is that there is ecological niche differentiation between *Eda* genotypes, which can be tested via direct measurement or indirectly using stable isotopes of carbon and nitrogen. Niche differentiation would provide an ecological mechanism for the stability of the polymorphism because it is expected to lead to frequency-dependent selection in favour of rare phenotypes (Wilson & Turelli 1986). Other examples of single-gene polymorphisms persisting in the face of disruptive selection are few, but perhaps more cases will be found as we gain greater information on the genetic basis of ecologically important phenotypic variation in natural populations.

Selection affecting the frequency of armour phenotypes in the Kennedy Lake population would be difficult to study without knowledge of the gene because most heterozygotes at *Eda* have the same number of lateral plates as individuals homozygous for the high-armour allele, yet they have lower fitness (K. B. Marchinko, B. W. Matthews, S. M. Rogers & D. Schluter 2010, unpublished data). This difference in fitness between genotypes having similar numbers

of lateral plates might be caused by effects of *Eda* on other traits (Barrett *et al.* 2008), but in any case, it doubtless affects the equilibrium frequencies of each of the lateral plate morphs (figure 3). Further study of *Eda* in this population should thus shed more light on the factors underlying the observed phenotypic polymorphism.

4. GENETICS OF ADAPTIVE PEAK SHIFTS

Theory predicts that the frequency distribution of effect sizes of genes fixed during adaptation should reflect broad features of the adaptive landscape—the surface describing the relationship between phenotypes and fitness (Orr 1998; Kryazhimskiy *et al.* 2009). If so, then it should be possible to infer some of the features of past selection on natural populations from current genetic data. We are exploring the distribution of effect sizes of genes underlying body shape, which represent a suite of phenotypic traits that evolved in parallel between freshwater stickleback populations after colonization of lakes by the marine species (figure 1). Unlike lateral plate armour, which is strongly affected by the major gene *Eda*, shape is influenced by multiple loci of varying effect size and, as yet, of unknown identity (Albert *et al.* 2008).

The theory we employed makes two assumptions about natural selection on a population adaptive to a new environment (Fisher 1930; Orr 1998). First, selection is presumed to be multivariate, acting on many phenotypic traits at once. Second, a single, roughly stationary adaptive peak is assumed to be present in the new environment. A population beginning at some distance away from the peak adapts by acquiring and fixing a series of advantageous mutations that bring it nearer to the new optimum (figure 4a). Relative to the ancestral population in the old environment, the population in the new environment thus undergoes an 'adaptive peak shift' in the suite of traits. Mutations are assumed to be pleiotropic, such that selection acts on them via their effects on multiple phenotypic traits. The effects of different mutations on the population mean phenotype are also assumed to be additive. Body shape is a useful phenotype with which to evaluate the theory with stickleback because it is multidimensional, yet all traits are in the same units (e.g. millimetres).

Under this model of adaptive peak shift, the predicted distribution of effect sizes of genes that fix during the transition from ancestral state to the new adaptive peak in freshwater is roughly exponential, with most genes having a small effect, a moderate number of genes having medium effects and a small number of genes having a large effect (Orr 1998, 1999; Griswold & Whitlock 2003). When quantitative trait locus (QTL) detection limits are incorporated, the effect sizes are predicted to conform approximately to a gamma distribution with shape parameter greater than one rather than an exponential distribution (Otto & Jones 2000). This was tested in stickleback using QTL effects mapped in an F2 cross between a marine population and a benthic stickleback representing among the most differentiated freshwater populations known. Body shape was quantified using
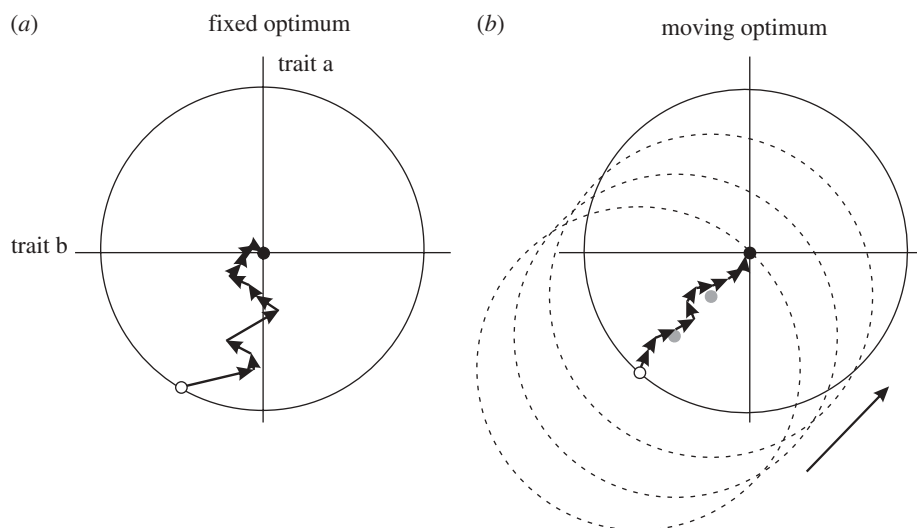
Figure 4. Example sequences of adaptive steps (arrows) as an ancestral population evolves towards a new adaptive peak. Horizontal and vertical dimensions represent two phenotypic traits. Circles represent contours of equal fitness, with fitness higher inside the circle than outside. The ancestral state is indicated by the open circle, and the new optimum by the filled circle. In (*a*), the ancestral population evolves to a distant, fixed optimum. In (*b*), the population evolves towards a near, moving optimum.

the *x*- and *y*-coordinates of landmarks similar to those illustrated in figure 1 (Walker & Bell 2000). Strictly speaking, the theory refers to effect sizes on fitness, but its predictions hold approximately for the absolute value of effect sizes on phenotypic traits (Orr 1999; Griswold & Whitlock 2003). We also assume that a QTL effect is produced by a single mutation, which has some support. In all three cases in which a QTL was eventually traced to the gene, only a very small chromosome region was implicated (Colosimo *et al.* 2005; Miller *et al.* 2007; Chan *et al.* 2010).

The results agreed with theoretical expectation (Albert *et al.* 2008). The distribution of QTL effect sizes was broad and conformed approximately to a gamma distribution (figure 5). The largest effects were between 0.4 and 0.55 mm, which approach in magnitude the average per-trait difference between the parent species (about 0.6 mm). The model of adaptive peak shift is thus supported by these data.

Under a contrasting model of adaptation, the adaptive peak is assumed to be near the ancestral state when a population colonizes a new environment and gradually moves to its current position, with the population tracking the optimum (figure 4*b*). This alternative model of selection is possible to imagine for a new lake formed at the end of the last ice age. For example, it may have taken a long time for the littoral zones of lakes to develop to their current level of resource production. However, this alternative model is not supported by the genetic data. Under this alternative model, mutations of mainly small effect are predicted (Collins *et al.* 2007; Kopp & Hermisson 2007, 2009), which is not what we observed (figure 5).

The expectation of a roughly gamma distribution of effect sizes may not be unique to the adaptive peak shift model; other scenarios of selection might predict a similar distribution (Kopp & Hermisson 2009; Kryazhimskiy *et al.* 2009). However, a second prediction of the model of adaptive peak shift (figure 4*a*) is probably more distinctive: the largest effects should
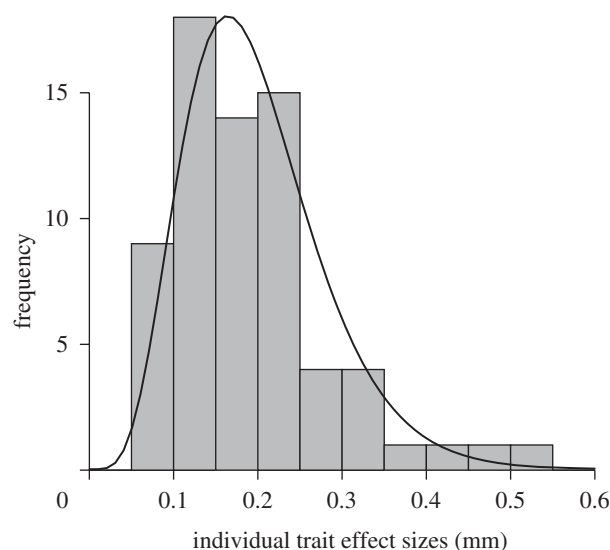

Figure 5. The distribution of effect sizes of QTL underlying shape differences between an ancestral marine and a derived freshwater stickleback population. The curve indicates the fitted gamma distribution. Differences in the landmark position between the populations were measured in millimetres. For comparison, the distance between the means of the parent species was about 0.6 mm, averaged over the 54 landmark traits. QTL effects are absolute values and do not include the sex chromosome. Adapted from Albert *et al.* (2008).

fix while the population is still far from the optimum in the new environment (Orr 1998). Because of pleiotropy, the closer the population is to the optimum, the greater is the chance that a large-effect mutation will take the population away from (rather than towards) the optimum. We are in the process of testing this prediction by comparing the distribution of QTL effect sizes for shape between freshwater populations residing in two contrasting lake types that differ in whether another fish species, the prickly sculpin (*Cottus asper*), is present (Vamosi 2003). Sculpin is

an intraguild predator, and stickleback inhabiting lakes with sculpin repeatedly have a body shape intermediate between that of the marine form and that of stickleback in non-sculpin lakes (S. M. Rogers, P. Tamkee, B. Summers, S. Balabahadra, M. Marks, R. Svanbäck, D. M. Kingsley, & D. Schluter 2010, unpublished data). Because the stickleback populations in the two lake types are of similar age, and the pattern is consistent among multiple lakes, it is reasonable to conclude that the shape differences between sculpin and non-sculpin lakes represent two distinct phenotypic optima in freshwater, with lakes containing sculpin having a phenotypic optimum closer to the ancestral state than the optimum in non-sculpin lakes. In this case theory predicts that the frequency of large-effect QTL will be significantly higher in lakes representing the far optimum (sculpin absent) than in lakes representing the near optimum (sculpin present). Under a contrasting model, the adaptive peak is assumed to be near the ancestral state when a population colonizes a new environment and gradually moves to its current position (figure 4b). This model clearly predicts no difference in the frequency of large-effect mutations between sculpin and non-sculpin lakes. To test the idea, we have generated F2 crosses between four lake populations, two from sculpin lakes and two from non-sculpin lakes, to the same marine population representing the ancestral type. Analysis of these crosses will make it possible to compare the frequency of large-effect QTL between populations that have evolved to adaptive peaks at varying distance from the ancestral state.

5. DISCUSSION

Modern efforts to understand the molecular basis of adaptation are leading to a better understanding not only of the genes underlying phenotypic traits, but also of natural selection itself, the process driving divergence. The studies summarized here illustrate how increased knowledge of the genetic basis of phenotypic evolution in stickleback is aiding our studies of natural selection on traits. In our first example, we showed how measuring the effects of selection on a specific locus in an experimental population can inform us about the mechanisms of selection on phenotypes. Our experiment found some support for a causal hypothesis to explain the evolution of reduced lateral plate armour in freshwater populations, but we were surprised to discover additional large effects on fitness of the underlying major gene, or linked genes, via other traits as yet undiscovered. Selection on these other traits therefore affects the evolution of armour, a discovery that would not have been possible without knowing the major underlying gene. Similar effects of pleiotropy and linkage have been made in other studies of selection on known genes (Coberly & Rausher 2008; Gratten et al. 2008), and the finding might turn out to be common.

Our second example, on a polymorphic stickleback population, adds to a growing number of studies investigating the role of selection in the maintenance of ecologically important genetic polymorphisms (Subramaniam & Rausher 2000; Fitzpatrick et al.

2007; Mitchell-Olds et al. 2007). It would be difficult if not impossible to make progress on the mechanisms maintaining the polymorphism without knowing the major gene underlying it. For example, the armour phenotype of most heterozygotes resembles that of one of the homozygotes, yet the heterozygote has a lower fitness (K. B. Marchinko, B. W. Matthews, S. M. Rogers & D. Schluter 2010, unpublished data), perhaps because the gene also affects other phenotypic traits (Barrett et al. 2008). This case, which found evidence for selection against heterozygotes at *Eda*, might be unusual in nature but there are not enough similar studies yet to allow generalization. Genetic polymorphism with disruptive selection is a precursor to sympatric speciation (Maynard Smith 1966; Wilson & Turelli 1986) and the evolution of dominance (Charlesworth & Charlesworth 1975; Durinx & Van Dooren 2009), and these represent conceivable long-term outcomes for the stickleback population in Kennedy Lake. However, most freshwater populations in the region are low plated and presumably are fixed for the low-armour *Eda* allele (Colosimo et al. 2005), and this is a possible endpoint in a future Kennedy Lake population as well.

Our third example was an investigation of the distribution of genetic effect sizes on a suite of morphological traits in stickleback adapting to postglacial lakes. This distribution matches the theoretical expectation of a model in which an ancestral population adapts to a new relatively distant optimum phenotype in a suite of traits. Ongoing studies will evaluate the model further with a test of the prediction that large-effect mutations are more likely to contribute to adaptation when the population is far from the optimum than when it is nearer (S. M. Rogers, P. Tamkee, B. Summers, S. Balabahadra, M. Marks, R. Svanbäck, D. M. Kingsley, & D. Schluter 2010, unpublished data). Alternative scenarios, in which mutations affect only single traits, or populations adapt to a moving optimum phenotype, do not make the same prediction (Kopp & Hermisson 2007, 2009).

The results of this work may help to explain more generally why large-effect mutations appear to be involved in adaptive evolution in some instances but not others. Numerous studies have reported the presence of a few genes of large effect underlying adaptive divergence in specific traits (e.g., Bradshaw et al. 1998; Albertson et al. 2003; Rogers & Bernatchez 2007; Steiner et al. 2007; Gratten et al. 2008), whereas others have found evidence only for many genes of small effect (e.g. Jones 1998; Sawamura et al. 2000; Fishman et al. 2002). Another striking feature of these studies is the variation between them in the number of genes detected that influences species differences (Orr 2001). Both aspects, gene number and frequency of large-effect genes, are likely to depend on the kinds of traits analysed and the time that separates populations and species analysed. However, the nature of selection should also affect the number and effect sizes of genes that fix. In turn, it may be possible to infer some of these features of selection by measuring the properties of genes.

Although we are enthusiastic about our growing ability to measure selection directly on genes (or

more specifically, the effects of phenotypic selection on the fitnesses of alternative alleles), and about the improvements to our understanding of the process of selection that will result, we are also realistic about the number of genes in the genome that can be studied in this way. For example, it is difficult to imagine that an experimental approach to measuring selection will be feasible for every selected gene in the genome. As well, there are lower limits to the strength of selection that can be detected in this way. Nevertheless, it is likely that studies of a few key genes will teach us general lessons about the process of natural selection at the genomic level. As well, direct measurements on individual genes will complement efforts to generate a macroscopic view of selection by mapping the rate of adaptive evolution across the genome using patterns of nucleotide variation (Eyre-Walker 2006; Sella *et al.* 2009). Combining information from both approaches will lead to yet further insights, such as estimates of the timing of origin and spread of adaptive mutations (Linnen *et al.* 2009). Hence, we see direct measurements of selection on genes as a vital component of research into the genetics of adaptation.

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