

Fishing for the Secrets of Vertebrate Evolution in Threespine Sticklebacks

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The threespine stickleback (*Gasterosteus aculeatus*) is rapidly emerging as a new model genetic system to study questions at the interface of evolution and development. The relatively rapid and recent diversification of this small teleost fish, combined with the development of genetic and genomic tools for this fish, provides an unprecedented opportunity to identify the genetic and molecular basis of morphological variation in natural populations of vertebrates. Recently, the genes underlying two different adaptive morphological traits in stickleback have been identified. This work has provided answers to four longstanding questions in the field of evolution and development: (1) How many genes underlie morphological variation in natural populations? (2) What are the genes that underlie morphological variation in natural populations? (3) Do coding or regulatory mutations underlie morphological evolution? (4) What is the molecular and genetic basis of parallel morphological evolution? Because stickleback populations also display natural variation in morphology, life history, physiology, and behavior, extending the approaches used to identify the genetic basis of morphological variation in sticklebacks to other phenotypes is sure to yield further important insights into the genetic and developmental basis of diversity in natural populations. *Developmental Dynamics* 234:815–823, 2005. © 2005 Wiley-Liss, Inc.

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INTRODUCTION

What are the mechanisms that underlie the variation of forms found in nature? Are the differences between species due to the effects of many genes, each with a small phenotypic effect, or can differences between species occur as a result of mutations in genes with large phenotypic effects? Are there particular classes of genes that are more likely to be modified to produce novel phenotypes? Do mutations in these genes occur in protein coding regions or regulatory regions? When similar traits evolve in independent populations, are the same genetic and

molecular pathways used to achieve the same phenotype?

Many of these questions have challenged evolutionary biologists for over a century. More recently, a number of researchers working at the interface of evolution and development have also begun to study these questions. In order to take a genetic approach to answering these questions, it is necessary to identify natural populations that have significant phenotypic variation, but can still be intercrossed in the laboratory in order to identify the genetic and molecular mechanisms that underlie morphological variation.

THE THREESPINNE STICKLEBACK AS A GENETIC MODEL SYSTEM IN EVOLUTION AND DEVELOPMENT

Threespine sticklebacks (*Gasterosteus aculeatus*) are small teleost fish that have been widely and intensively studied by ethologists, ecologists, and evolutionary biologists, resulting in thousands of research papers and several books on their morphology, physiology, life history, ecology, evolution, and behavior. Sticklebacks are widely distributed in both marine and fresh-

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water populations throughout the northern hemisphere. The ancestral sticklebacks are found in marine habitats and are relatively uniform in life history, morphology, and behavior. In contrast, the sticklebacks found in post-glacial freshwater lakes and streams have evolved a diversity of morphologies, life histories, and behaviors from the ancestral marine form in response to local ecological conditions in a relatively short time scale of 12,000 years (Bell and Foster, 1994). Despite this diversity, sticklebacks from virtually any two populations from around the world can be crossed in the laboratory to generate a large number of viable and fertile progeny, thereby facilitating the forward genetic approach outlined in Figure 1 to identify the molecular basis of natural phenotypic variation.

In order to identify the genes and molecular changes that underlie natural variation, a variety of genetic and genomic tools are required. Despite the long history of study in sticklebacks, there were virtually no molecular or genetic tools available for this fish until a few years ago. There are now hundreds of microsatellite markers available, which are distributed across the genome and have been ordered into a genetic linkage map for use in genetic linkage analysis in virtually any stickleback population (Peichel et al., 2001). Several bacterial artificial chromosome (BAC) libraries have been constructed to facilitate positional cloning of genetic loci identified by genetic linkage analysis (Kingsley et al., 2004). Nearly 100,000 ESTs from a variety of tissues and developmental stages have now been deposited in GenBank, and provide a resource for the cloning of interesting candidate genes (Kingsley et al., 2004). Expression patterns of these candidate genes can be assayed using *in situ* hybridization (Ahn and Gibson, 1999a, 1999b; Cresko et al., 2003; Shapiro et al., 2004; Tanaka et al., 2005). Transgenic technologies have also been developed for sticklebacks (Hosemann et al., 2004), which make it possible to perform genetic rescue experiments to demonstrate that a candidate gene is actually responsible for a phenotype (Colosimo et al., 2005). Additionally, transgenic approaches can be used to identify the

cis-regulatory elements that drive expression of genes in particular anatomical locations (DiLeone et al., 2000; Mortlock et al., 2003). With the development of many of these tools that were previously only available in more traditional model organisms, threespine sticklebacks are rapidly emerging as a "supermodel" (Gibson, 2005).

Variation in skeletal armor, highlighted in Figure 2, is the most striking phenotype found in natural populations of sticklebacks. Marine sticklebacks are covered in bony armor, with three dorsal spines, two pelvic spines, and a continuous row of bony lateral plates (Fig. 2A). The dorsal and pelvic spines (Fig. 2A) are thought to be protective against gape-limited predators, such as birds and piscivorous fish (Hoogland et al., 1957; Reimchen, 1983). However, several freshwater populations from around the world have evolved a complete or partial loss of the pelvic skeleton (Fig. 2B), including sticklebacks from Paxton Lake, British Columbia (Bell, 1974), the Queen Charlotte Islands, British Columbia (Moodie and Reimchen, 1976; Reimchen, 1984), Quebec (Edge and Coad, 1983), the Cook Inlet region of Alaska (Bell et al., 1993), southern California (Bell, 1987), the Outer Hebrides in Scotland (Campbell, 1979, 1985), and Iceland (Shapiro et al., 2004). It has been hypothesized that loss of pelvic structures in sticklebacks could be the result of the absence of predatory fish, reduced levels of calcium availability, or predation by macroinvertebrates (Reimchen, 1980, 1983; Reist, 1980a,b; Giles, 1983; Bell et al., 1993; Ziuganov and Zotin, 1995), although none of these hypotheses can completely account for all cases of pelvic reduction.

The most commonly observed morphological variation in freshwater sticklebacks is a reduction in the number of lateral plates (Fig. 2B). Most marine sticklebacks, although not all (Klepaker, 1996), have a complete set of lateral plates along their side (Fig. 2A, complete morph), and most freshwater populations have either retained only the anterior plates (low morph) or anterior plates and a posterior keel (partial morph). Low calcium levels (Giles, 1983), salinity tolerance (Heuts, 1947), stream gradients

(Baumgartner and Bell, 1984), parasite susceptibility (MacLean, 1980), increased body flexibility and changes in swimming performance (Taylor and McPhail, 1986; Bergstrom, 2002), as well as changes in predation regime (Hagen and Gilbertson, 1973; Moodie et al., 1973; Reimchen, 1992, 1995, 2000) and climate (Hagen and Moodie, 1982) have all been suggested as factors contributing to the evolution of lateral plate reduction. However, none of these factors have been directly proven to account for the repeated evolution of lateral plate morphs in thousands of freshwater populations across the Northern hemisphere (Bell and Foster, 1994).

Using a forward genetics approach (Fig. 1), the genetic and molecular basis of pelvic reduction and plate morph variation has now been identified (Colosimo et al., 2004; 2005; Cresko et al., 2004; Shapiro et al., 2004). This work in sticklebacks has provided answers to some long-standing and fundamental questions in evolutionary biology. In this review, I will frame each question in a historical context, briefly summarize some relevant work in other taxa, and then describe the recent work in sticklebacks and its relevance to these questions. I will end by discussing some of the future research directions that can be taken in this newly emerging model for integrative research in evolution and development.

HOW MANY GENES UNDERLIE MORPHOLOGICAL VARIATION IN NATURAL POPULATIONS?

Does evolution proceed via the accumulation of small changes with minor phenotypic effects or can mutations of large phenotypic effect underlie evolutionary change? This question has been debated since the time of Darwin, and Darwin himself believed that natural selection could only occur via small steps with minor effects on fitness (Darwin, 1859). The micromutationist view of evolution was championed by R.A. Fisher, who created a theoretical framework that reconciled gradualism and Mendelian genetics (Fisher, 1930). Although challenged

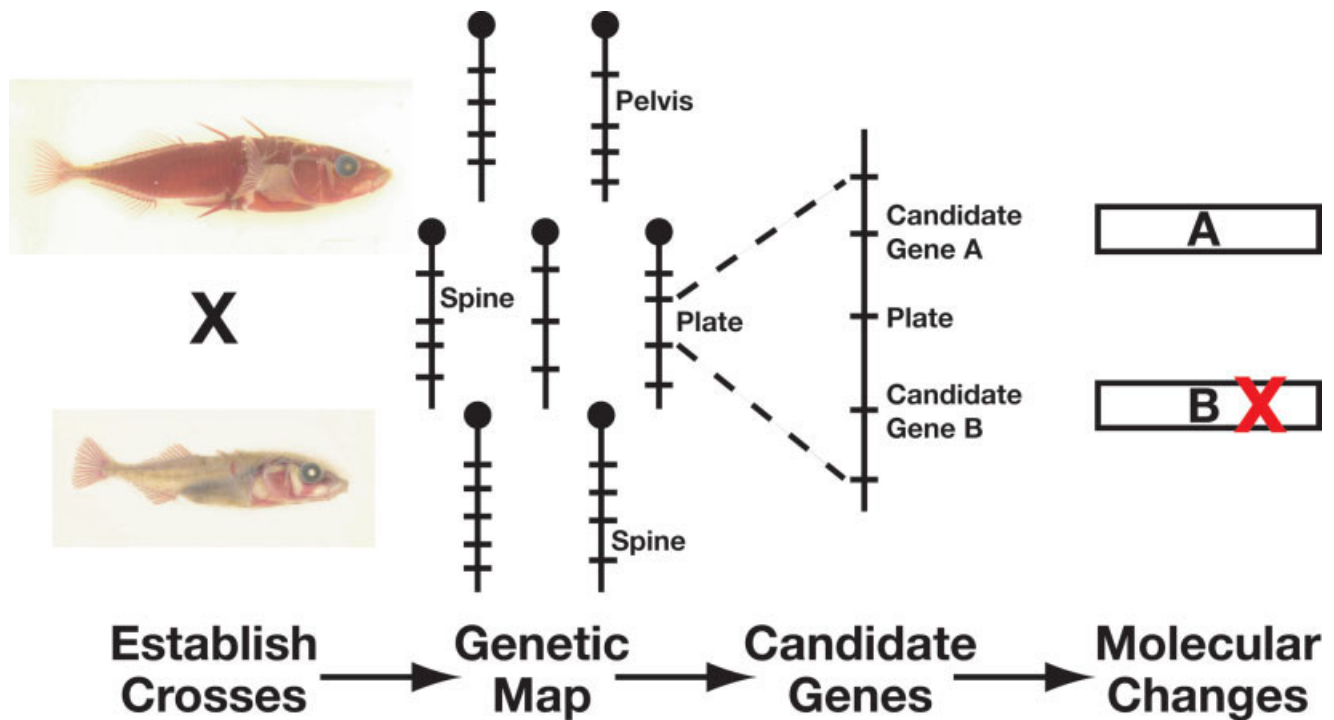


Fig. 1. Forward genetic approach to identify the genes and molecular changes that underlie natural variation in sticklebacks. Fish with divergent phenotypes (morphologies, physiologies, behaviors) are crossed in the laboratory to generate an F1 generation, which are then intercrossed to generate hundreds of F2 progeny. The progeny are then phenotyped for traits of interest and genotyped with a panel of polymorphic molecular markers. Region(s) of the genome in which genotypes are correlated with phenotypes are defined. Candidate genes in these regions are identified, either through positional cloning using BACs and sequencing of the genetic interval, or by genetic mapping of interesting candidate genes to these regions. These candidate genes must then be analyzed for the presence of sequence or expression differences between the starting populations, which correlate with the phenotypic differences between the starting populations. Ultimately, rescue and complementation experiments are required to prove that a sequence variant is responsible for the phenotype observed.

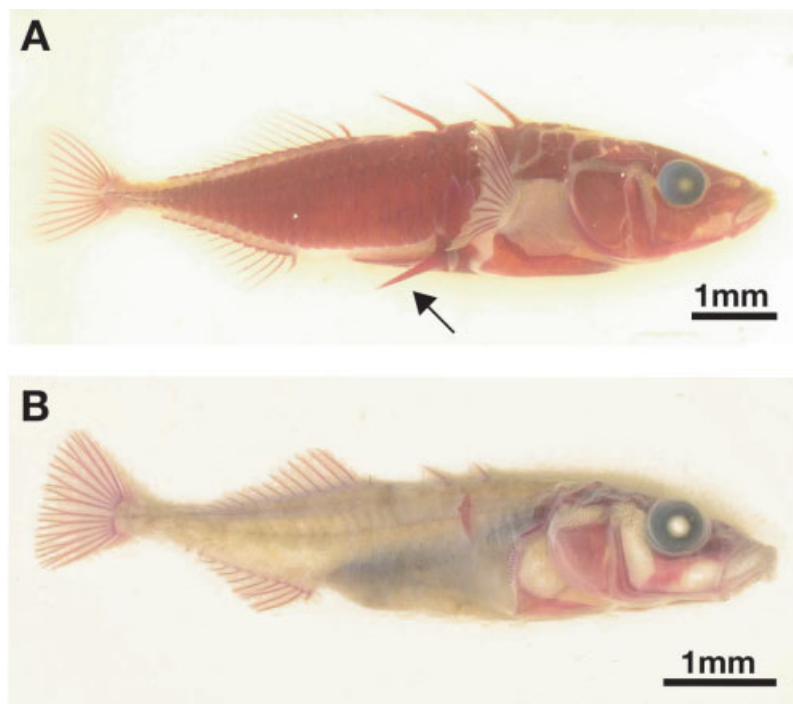


Fig. 2

by scientists such as Bateson and Goldschmidt (Bateson, 1894; Goldschmidt 1940), micromutationism remained the prevailing theory until nearly the end of the 20th century (reviewed in Orr, 2005). However, the advent of quantitative trait locus (QTL) analysis in the 1980s provided an experimental approach to determine both the number of loci and their relative contributions to phenotypic differences between natural popula-

Fig. 2. Threespine stickleback populations have striking differences in overall morphology and skeletal structures. **A:** Alizarin red-stained stickleback from a Japanese Pacific marine population. The fish has robust dorsal and pelvic spines (arrow), and a set of fully developed lateral plates along its side. The morphology of this fish is typical for a marine population. **B:** Alizarin red-stained stickleback from the Paxton Benthic freshwater population. The fish has smaller dorsal spines, completely absent pelvic structures, and only a single lateral plate. In addition, fish from this population are smaller than the marine fish and have differences in body and head shape.

tions. A growing body of experimental evidence, mostly from work in insects and plants, suggests that relatively few genetic loci with major effects on phenotype can contribute to morphological differences between natural populations (reviewed in Orr, 2001, 2005).

To determine the genetic architecture that underlies skeletal changes in natural populations of sticklebacks, a Japanese marine fish with a complete set of lateral plate and pelvic structures (Fig. 2A) was crossed with a Paxton benthic fish with only a few plates and a missing pelvis (Fig. 2B). Intercrossing F1 progeny resulted in a large number of F2 progeny with segregating variation in plate number and pelvic size, as well as in a number of other traits that differed between the starting populations. When the progeny were genotyped with a large number of microsatellite markers spanning the threespine stickleback genome (Peichel et al., 2001), one major locus and four minor loci were identified that together explained a large proportion of the variance in pelvic phenotypes (Shapiro et al., 2004). The major locus accounts for close to 70% of the variance in pelvic reduction in sticklebacks from Paxton Lake, British Columbia, and maps to the end of linkage group (LG) 7. A similar analysis for the plate morph phenotype identified a near Mendelian locus on LG 4 that controlled nearly 80% of the phenotypic variation in plate counts. Four additional loci were identified that contributed to significant variation in plate number and plate size. These loci interact in a semi-additive manner to determine overall plate counts (Colosimo et al., 2004). These data suggest that evolution of significant morphological differences between populations can indeed occur by changes in a relatively small number of loci with major phenotypic effects.

For both pelvic girdle and plate morph variation, it was interesting that a single major locus with a large phenotypic effect and multiple loci with smaller phenotypic effects were found. This experimental evidence supports the theoretical prediction that the distribution of loci fixed during adaptation will be exponential, with a single locus of large effect and

many more loci of smaller effect (Orr, 1998). Genetic mapping of other skeletal traits in sticklebacks, such as facial bone morphology (Kimmel et al., 2005), gill raker number, and dorsal spine length (Peichel et al., 2001) also support the hypothesis that relatively few genes of major effect underlie morphological adaptations in sticklebacks. However, these crosses were too small to detect loci of smaller effect. Future studies in both sticklebacks and other taxa are required to assess the generality of the theoretical prediction about the distribution of loci fixed during adaptation.

WHAT ARE THE GENES THAT UNDERLIE MORPHOLOGICAL VARIATION IN NATURAL POPULATIONS?

Although we still know very little about the genes that underlie morphological variation in natural populations, work during the past 20 years in developmental genetics has yielded insights into the genes that underlie the development of many different organs and structures within model organisms such as fruit flies (*Drosophila melanogaster*), nematodes (*Caenorhabditis elegans*), and mice (*Mus musculus*). Surprisingly, most developmental regulatory genes are conserved across metazoans (Carroll, 2000). Early work in evolutionary developmental biology correlated differences in the expression patterns of these conserved genes, such as *Hox* genes, with morphological differences across widely divergent taxa (Averof and Akam, 1995; Burke et al., 1995; Carroll, 1995; Sordino et al., 1995; Holland and Garcia-Fernandez, 1996; Averof and Patel, 1997; Cohn and Tickle, 1999; Hughes and Kaufman, 2002). However, this approach cannot identify the actual genetic changes that contribute to the evolution of morphologies. Furthermore, it is limited to only looking for variation in known genes. A more direct forward genetic approach provides an unbiased search for the genes that underlie evolutionary changes in natural populations. Genetic approaches in *Drosophila* have provided some evidence that known developmental

genes can underlie morphological differences between species (Stern, 1998; Sucena and Stern, 2000; Wittkopp et al., 2003); however, very few studies have been conducted.

The genes that underlie pelvic and plate morph variation in sticklebacks have also been identified, providing the first insights into the types of genes that control morphological variation in vertebrates. The major pelvic locus on LG7 does not recombine with the *Pitx1* gene, a homeodomain containing protein originally identified in a screen for hindlimb-specific genes in mouse (Shang et al., 1997). The phenotype of mice with a targeted mutation in the *Pitx1* gene is remarkably similar to the phenotype of sticklebacks with pelvic reduction in two aspects. First, *Pitx1* null mice have reduced hindlimbs and normal forelimbs (Lancôt et al., 1999; Szeto et al., 1999), and Paxton Lake sticklebacks have reduced pelvic spines and girdles (fish hindlimbs) and normal pectoral fins (fish forelimbs). Second, hindlimb reduction is more severe on the right side of the body in the *Pitx1* mutant, due to compensation by the closely related *Pitx2* gene, which is expressed at higher levels on the left side of the body in the mouse (Marcil et al., 2003). The reduction of pelvic structures in the Paxton benthic population, as well as several other pelvic reduced stickleback populations (Bell et al., 1985; Cole et al., 2003), is also more severe on the right side than the left. This directional asymmetry also maps to the *Pitx1* locus in the Paxton benthic cross (Shapiro et al., 2004).

To identify the gene that underlies the major plate morph locus, a completely unbiased positional cloning approach was taken, which makes no assumption about the identity of the genes involved (Colosimo et al., 2005). The plate morph locus was genetically mapped to a small (0.68 cM) genetic interval on LG4. Bacterial artificial chromosome (BAC) clones covering the genetic interval were then identified and two BACs covering most of the interval were sequenced. A number of genes were present in this 400-kb interval, but linkage disequilibrium mapping narrowed the region to a small 16-kb interval that contained only a handful of genes (Colosimo et al., 2005). One of these genes,

Ectodysplasin (*Eda*), a member of the tumor necrosis family, was particularly interesting. Mutations in the *Eda* gene, as well as in the *Ectodysplasin* receptor (*Edar*) and downstream signaling molecules in the pathway, result in alterations in skin, hair, and tooth formation in humans and mice (Mikkola and Thesleff, 2003). In addition, a mutation in *Edar* causes the loss of scales in medaka fish (Kondo et al., 2001). Sticklebacks do not have scales, but the lateral plates are dermal bone, which shares a common developmental origin with scales (Sire and Huysseune, 2003). Although *Eda* proved to be a compelling candidate once it was discovered to lie within the plate morph interval, the *Eda* gene would probably not have topped the candidate gene list for the plate morph phenotype. Therefore, this story highlights the power of an unbiased forward genetics approach, rather than relying on a candidate gene approach, to identify genes that underlie morphological evolution. Regardless of the fact that *Eda* may not have been the gene expected to underlie plate morph evolution, transgenic rescue experiments prove that the *Eda* gene is in fact responsible for the loss of lateral plates in freshwater fish (Colosimo et al., 2005).

DO CODING OR REGULATORY MUTATIONS UNDERLIE MORPHOLOGICAL EVOLUTION?

Mutations of *cis*-regulatory elements in genes have been proposed to provide the fodder for the evolution of morphological diversity as it provides a mechanism to alter expression in a specific structure while preserving expression at other sites required for viability of the organism (King and Wilson, 1975; Stern, 2000; Davidson, 2001; Carroll et al., 2004). Genetic studies have also provided evidence for *cis*-regulatory evolution underlying morphological variation in natural populations of fruit flies (Stern, 1998; Sucena and Stern, 2000; Wittkopp et al., 2002; Sucena et al., 2003; Gompel et al., 2005), worms (Wang and Chamberlin, 2002), mammals (Belting et al., 1998; Van Laere et al., 2003), and plants (Wang et al.,

1999). However, with few exceptions (Belting et al., 1998; Wang and Chamberlin, 2002; Gompel et al., 2005), the actual molecular changes that underlie evolution at *cis*-regulatory elements have yet to be identified.

Although many cases of morphological evolution are the result of regulatory evolution, there are examples of dramatic morphological changes in natural populations that result from amino acid changes within the protein. One of the most striking is the occurrence of activating mutations in the *melanocortin1 receptor* (*Mc1r*) in melanistic populations of birds (Theron et al., 2001; Mundy et al., 2004), jaguars (Eizirik et al., 2003), and pocket mice (Nachman et al., 2003). The occurrence of natural variation in the coding region of this gene may result from the fact that the role of *Mc1r* is largely confined to pigment cells (Robbins et al., 1993); therefore, there may be no negative pleiotropic effects on fitness when the coding region of this gene is mutated in the wild. Given the limited nature of these studies, further comparative analysis is required to determine what types of genes might tolerate coding or regulatory variation in natural populations.

In the case of pelvic reduction in sticklebacks, no changes in the amino acid sequence of *Pitx1* were found between the Japanese marine (complete pelvis) and Paxton benthic (reduced pelvis) populations (Fig. 2). Therefore, the expression pattern of *Pitx1* was examined by *in situ* hybridization at time points prior to the appearance of pelvic structures. *Pitx1* is expressed in a variety of larval structures, including the prospective pelvic region in marine larvae with full pelvic development. In Paxton benthic larvae, *Pitx1* is expressed normally in most structures but is not expressed in the presumptive pelvic region. These data suggest that a *cis*-regulatory element, which specifically drives expression of *Pitx1* in the developing pelvis, is altered in the Paxton benthic pelvic reduced sticklebacks. Although this putative *cis*-regulatory element has not yet been identified in sticklebacks, these data provide strong support for the hypothesis that *cis*-regulatory evolution of developmental control genes can provide a simple mechanism for natural selection to tinker with one structure without affecting the

overall viability of the organism in the wild (Stern, 2000; Davidson, 2001; Carroll et al., 2004).

Support for the *cis*-regulatory hypothesis is less clear in the case of the *Eda* gene and lateral plate morph evolution. Although there are four predicted amino acid differences between the completely plated marine and low plated Paxton benthic in the *Eda* protein sequence, none of the changes are in particularly well-conserved residues (Colosimo et al., 2005). In addition, there are no coding differences in *Eda* between the marine population and a second low-plated population, in which the low phenotype fails to complement the low phenotype of a population known to carry the low-plated *Eda* allele (Schluter et al., 2004; Colosimo et al., 2005). Taken together, these data suggest that the difference in the plate phenotypes is due to a regulatory rather than a protein coding change in *Eda*. However, attempts to compare the expression of the *Eda* gene in complete and low morph fish have not yet been successful. This future work will contribute to a greater understanding of both the development of the lateral plates and the mechanisms by which they are lost in evolution.

In the future, it will be particularly exciting to identify the *cis*-acting elements that drive expression of both *Pitx1* and *Eda* in particular anatomical areas and to then compare the sequence of these elements in different stickleback populations. The ongoing sequencing of the stickleback genome, as well as the ability to make transgenic sticklebacks (Hosemann et al., 2004) makes this a worthwhile and feasible, though challenging, goal.

WHAT IS THE MOLECULAR AND GENETIC BASIS OF PARALLEL MORPHOLOGICAL EVOLUTION?

The evolution of similar phenotypes in independent populations in association with similar environmental factors implies that a trait evolved in response to natural selection, rather than by genetic drift (Simpson, 1953; Schluter and Nagel, 1995; Rundle et al., 2001). The evolution of similar phenotypes can result from the same

underlying mechanisms, termed “parallel evolution,” or as a result of different mechanisms, termed “convergent evolution” (Hodin, 2000). However, the mechanisms underlying the evolution of similar phenotypes in independent populations have not been well understood. It has been suggested that genetic and developmental constraints (Haldane, 1932; Maynard Smith et al., 1985; Wake, 1991; Shubin et al., 1995; West-Eberhard, 2003) shared between closely related species may bias the direction of phenotypic evolution and lead to parallel evolution. These hypotheses predict that the same genes will underlie parallel evolution because there are only a limited number of loci that will not have negative pleiotropic effects on fitness when mutated. An alternative explanation for the existence of parallel evolution in closely related lineages is that cryptic genetic variation that is present in the ancestral population is uncovered when the organism is exposed to a novel environment (Bell, 1974, 1988; Gibson and Dworkin, 2004).

Parallel phenotypic evolution has been recently demonstrated in drosophilid flies, where variation in pigmentation and hair patterns is widespread, and can result from genetic changes at the same locus (Gompel and Carroll, 2003; Sucena et al., 2003). However, the molecular nature of these changes is unknown; therefore, it is unclear from these studies whether parallel evolution is the result of new mutations or pre-existing genetic variation in an ancestral population. Melanism in multiple vertebrate species results from mutations in the *Mc1r* gene (Theron et al., 2001; Eizirik et al., 2003; Nachman et al., 2003; Mundy et al., 2004). In these cases, the changes in *Mc1r* are clearly the result of independent mutation at the same locus, providing evidence to support the idea that negative pleiotropic effects of some loci may limit the number of targets of selection.

In sticklebacks, the genetic basis of pelvic reduction has been investigated in multiple populations with pelvic reduction. Genetic mapping and complementation studies were performed using three different pelvic-reduced populations from Alaska, and these studies all revealed a near-Mendelian

locus for pelvic reduction at the end of LG7 (Cresko et al., 2004). Crosses between pelvic reduced sticklebacks from Paxton Lake and pelvic reduced sticklebacks from Iceland resulted in progeny lacking pelvic structures (Shapiro et al., 2004). These data suggest that pelvic reduction in several independent populations of threespine sticklebacks is controlled by the same major genetic locus. However, the expression of the *Pitx1* gene has not yet been analyzed in Alaskan or Icelandic pelvic reduced populations. The expression of *Pitx1* has been examined and was found to be lost in a Scottish pelvic reduced population, as was seen in the Paxton benthic population (Cole et al., 2003; Shapiro et al., 2004). However, neither genetic mapping studies nor complementation analysis have been performed with this population, so it is currently unknown whether pelvic reduction in this Scottish population is due to a mutation at the *Pitx1* gene or in another gene upstream of *Pitx1*. Ultimately, it will be interesting to determine whether the same or different molecular alterations at the *Pitx1* locus underlie pelvic reduction in independent stickleback populations from across the world to determine whether genetic and developmental constraints or pre-existing variation plays a role in the parallel evolution of pelvic reduction.

The positional cloning of the plate morph locus has revealed that pre-existing variation in the ancestral marine population can explain the rapid (Klepaker, 1993; Bell, 2001; Kristjánsson et al., 2002; Bell et al., 2004) and repeated evolution of lateral plate morphs in freshwater populations from around the world (Colosimo et al., 2005). Genetic mapping studies as well as complementation crosses suggested that the repeated evolution of the low morph phenotype is the result of the same major genetic locus (Avisé, 1976; Colosimo et al., 2004; Cresko et al., 2004; Schluter et al., 2004). Sequencing around the plate morph locus revealed a common DNA haplotype that is shared between 15 low-plated populations from around the world, while a different DNA haplotype is shared between 10 completely plated populations from around the world (Colosimo et al., 2005). How-

ever, the low-plated populations do not share a common origin when analyzed with 25 random nuclear markers. The most parsimonious explanation for this data is that there is an ancient low-plated allele present at a low frequency in the ancestral marine population that is selected for when the fish move into fresh water. In fact, this low-plated allele can be found at low frequencies in anadromous populations from both Canada and California (Colosimo et al., 2005). However, there is evidence that at least one low-plated population in Japan does not share the low-plated *Eda* haplotype; despite the fact that complementation crosses indicate the low-plated phenotype is allelic to *Eda* (Schluter et al., 2004; Colosimo et al., 2005). Therefore, this may represent a situation in which new mutation at the same locus, rather than standing genetic variation, contributes to the evolution of a trait.

There are also cases of convergent evolution in which the same genes do not underlie traits in independent populations. Consistent with this, there are stickleback populations in which the developmental mode of pelvic reduction does not look similar to the populations that have been studied genetically (Bell, 1987). These may represent populations in which other genes are responsible for pelvic reduction. In addition, the minor loci that contribute to pelvic spine and plate variation do differ between stickleback populations (Peichel et al., 2001; Colosimo et al., 2004; Cresko et al., 2004). Convergence of pigment patterning in *Drosophila* is not always due to the same genetic loci (Wittkopp et al., 2003) and *Mc1r* does not always underlie melanism in vertebrates (Hoekstra and Nachman, 2003). Future comparative work in multiple taxa will be required to assess the relative contributions of new mutations, pre-existing genetic variation, and developmental and genetic constraints to the evolution of similar phenotypes in independent populations.

FUTURE DIRECTIONS

Perhaps the most exciting aspect of research in sticklebacks is not what we have learned so far, but the things we have yet to learn using this sys-

tem! This review has focused on the genetic basis of morphological variation in skeletal traits, but stickleback populations have extensive variation in other morphological traits, such as feeding morphologies, body shape and size, and breeding coloration; behavioral traits, such as courtship behaviors, social aggregation, levels of aggression, and predator avoidance; and physiological traits, such as lifespan, salinity tolerance, and temperature preference (Bell and Foster, 1994).

Studying multiple traits in sticklebacks will allow us to discern if there are more general rules concerning the genetic basis of phenotypic variation in natural populations. Are there particular classes of traits that are more likely to have a simple genetic basis? Are there differences in the genetic architecture of traits that are lost during evolution vs. traits that are gained during evolution? Will we identify novel genes and gene functions through this approach? What are the relative contributions of *cis*-regulatory and protein coding variation to evolution? Will parallel evolution always involve mutations at the same loci?

In order to address these questions and gain a fuller understanding of the processes driving evolution in natural populations, it will also be necessary to analyze multiple traits from taxonomically diverse species. In this post-genomic age, genetic and molecular tools can be brought to virtually any organism with interesting biology, and we need not limit our studies to a small handful of traditional genetic model systems. The threespine stickleback system provides a compelling example of what can be learned by using a system with biology that is uniquely suited to the questions at hand.

One of the really exciting aspects of studying the genetic basis of natural variation in sticklebacks is the possibility of vertical integration across traditionally disparate fields. For example, genetic studies of skeletal variation in sticklebacks have brought together developmental geneticists, evolutionary biologists, and field ecologists. Although evolutionary biologists have long been concerned with identifying the causes of phenotypic evolution, they have traditionally

used different model systems to identify different levels of causation. For example, the genetic basis of adaptation has been studied in laboratory organisms such as *Drosophila*, while population level processes, such as the selective forces that lead to adaptation have been studied in a different set of organisms in the field. In sticklebacks, we have the opportunity to understand evolutionary causation at many levels, from the selective forces that lead to phenotypic evolution down to the single nucleotide changes in the genome upon which selection acts. It is only through these integrated, multidisciplinary approaches that we will achieve a greater understanding of the basic principles that have resulted in the biological diversity we see around us.

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