



SYMPOSIUM

Perspectives on the Genetic Architecture of Divergence
in Body Shape in SticklebacksDuncan T. Reid^{*,†} and Catherine L. Peichel^{1,*}^{*}Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA; [†]Molecular and Cellular Biology Graduate Program, University of Washington, Seattle, WA 98195, USA

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¹E-mail: cpeichel@fhcrc.org

Synopsis The body shape of fishes encompasses a number of morphological traits that are intrinsically linked to functional systems and affect various measures of performance, including swimming, feeding, and avoiding predators. Changes in shape can allow a species to exploit a new ecological niche and can lead to ecological speciation. Body shape results from the integration of morphological, behavioral and physiological traits. It has been well established that functional interdependency among traits plays a large role in constraining the evolution of shape, affecting both the speed and the repeated evolution of particular body shapes. However, it is less clear what role genetic or developmental constraints might play in biasing the rate or direction of the evolution of body shape. Here, we suggest that the threespine stickleback (*Gasterosteus aculeatus*) is a powerful model system in which to address the extent to which genetic or developmental constraints play a role in the evolution of body shape in fishes. We review the existing data that begins to address these issues in sticklebacks and provide suggestions for future areas of research that will be particularly fruitful for illuminating the mechanisms that contribute to the evolution of body shape in fishes.

Introduction: why do we need to learn about the genetics of body shape?

If we want to understand why and how the shapes of fishes have evolved, it is necessary to consider whether there are genetic or developmental constraints that limit the phenotypes available for selection. In this paper, we will adopt the definition of constraint used by Walker (2007): “constraints are features of systems that bias the rate and direction of phenotypic evolution”. If there are constraints that affect the “rate” of evolution, a trait may evolve more quickly or more slowly, while if there are constraints that affect the “direction” of evolution, some phenotypes may be more likely to evolve than others.

The rate or direction of phenotypic evolution can be biased due to functional constraints resulting from the fact that individual morphological, behavioral and physiological traits can affect multiple functional systems; such functional integration can bias patterns of variation (Arnold 1992;

Endler 1995; Walker 2007). In addition, any given phenotype is subject to many different selective pressures, which can lead to functional trade-offs (Reznick and Travis 1996; Reznick and Ghalambor 2001; Ghalambor et al. 2003). For example, there is a relationship between body shape and swimming performance, but body shape is also influenced by foraging behavior, the risk of predation, and stream velocity (Webb 1984; Walker 1997). Thus, the interaction between many different phenotypic traits and many different selective pressures can create functional constraints, such that only those changes with the greatest positive and fewest negative effects on fitness will be selected.

Although there is good evidence that functional constraints play a role in the evolution of the body shape of fishes, the extent to which genetic or developmental constraints bias the rate or direction of the evolution of body shape is less well understood. The concept of genetic bias was first articulated by

Haldane (1932), who proposed that closely related species might exhibit the same phenotypic traits in similar environments (parallel evolution) because of shared genetic constraints. Similarly, it has been proposed that shared developmental constraints might also produce similar phenotypes in closely related species (Maynard Smith et al. 1985; Wake 1991; Shubin et al. 1995; West-Eberhard 2003). Thus, although natural selection is strongly implicated when parallel evolution is observed (Endler 1986), genetic or developmental bias may also play an important role (Schluter 1996). Because similar body shapes have evolved repeatedly in similar environments across disparate species of fish as well as among closely related species, the body shape of fishes provide an excellent system in which to investigate the contribution of genetic and developmental constraints to evolution.

In particular, investigating the genetic basis of parallel evolution in the body shape of fishes provides an opportunity to tease apart the effects of natural selection and genetic constraint (Schluter et al. 2004). For example, if different genes underlie the same phenotypic changes in closely related species, natural selection, rather than shared genetic constraint, is strongly implicated. However, if the same genes underlie the same phenotypic changes in closely related species, genetic constraint may play a role. In this case, it is necessary to further disentangle whether the same genes are repeatedly involved due to selection on existing standing variation or due to new mutations at the same locus (Barrett and Schluter 2008). Finding evidence for new mutations at the same locus that underlie the same phenotype in independent populations provides strong evidence that genetic constraint might play a role.

In addition to investigating the genetic basis of parallel evolution, more detailed studies of the genetic architecture of divergence in body shape will provide new insights into the role of genetic and developmental constraints in the evolution of shape. For example, the number of genes that contribute to a particular phenotype or the number of phenotypes that a particular gene affects (i.e. pleiotropy) may bias the rate and direction of the evolution of body shape. In addition, the location of genes within the genome may also bias the evolution of body shape. For example, tight linkage between several genes that affect shape phenotypes might allow rapid adaptation to a new environment. Furthermore, if genes are linked to sex chromosomes, differences in shape between the sexes rather than between populations may evolve. Despite the importance of investigating the genetic architecture of

divergence in body shape, very little work has been done in any fish species. Below, we explore how recent developments in sticklebacks have already contributed to our knowledge of the genetic basis of evolution of body shape in fishes and outline future work that will provide additional insight into the role of genetic and developmental constraint in this process.

Why are sticklebacks a good system to study the genetics of body shape?

Threespine sticklebacks (*G. aculeatus*) are small, teleost fish that are found in both marine and coastal freshwater populations throughout the Northern hemisphere. Most freshwater populations were established within the past 12,000 years since the end of the last ice age, when ancestral marine sticklebacks invaded newly created freshwater lakes and streams, with each freshwater population an independent evolutionary event (Bell and Foster 1994). During this time, freshwater sticklebacks have diverged in behavioral, physiological and morphological traits, including body shape as detailed below. In addition, sticklebacks have invaded similar habitats many independent times, providing an opportunity to examine the extent to which similar morphological changes evolve in response to similar environmental conditions. The replicated evolution of similar changes in body shape further provides an opportunity to determine whether the same genetic changes are involved; addressing this question will provide important insights into the potential role of genetic and developmental constraints in the evolution of body shape.

Genetically based adaptive divergence in body shape

Stickleback populations living in different habitats have diverged considerably in shape. The ancestral marine form is pelagic and migratory (Bell and Foster 1994; Walker and Bell 2000) and has a relatively streamlined shape characterized by a large head, deep body, narrow peduncle, anteriorly-placed and widely-spaced dorsal spines, a posteriorly-placed pelvis and longer median fins (Walker and Bell 2000; Spoljaric and Reimchen 2007). This streamlined body form is thought to facilitate both foraging and cruising in open water (Webb 1982, 1984; Walker 1997).

In freshwater habitats, body shape has diverged in predictable patterns based on whether the fish are foraging on zooplankton in open-water (limnetic) habitats or on macroinvertebrates in littoral

(benthic) habitats. For example, sticklebacks found in freshwater habitats with a large littoral area generally have shorter, deeper bodies, as well as more posteriorly-placed dorsal spines and shorter median fins (Walker 1997; Walker and Bell 2000; Spoljaric and Reimchen 2007). Deeper bodies are better for maneuvering and foraging in a more complex littoral area (Webb 1982, 1984; Walker 1997). Divergence in shape is also dependent upon the predation regime. For example, in lakes with predatory fish, sticklebacks tend to be larger, and have longer median fins and more anteriorly-placed dorsal spines (Walker 1997; Walker and Bell 2000; Spoljaric and Reimchen 2007). It has been suggested that the anterior placement of the dorsal spines is important when predatory fish are present because sticklebacks are ingested headfirst by piscivorous fish (Reimchen 1991). In the absence of predatory fish, sticklebacks that occupy limnetic habitats or large lakes actually have more streamlined bodies with larger median fins than do marine sticklebacks; this shape is thought to facilitate acceleration during strikes on fast-moving prey (Walker 1997). These studies highlight the fact that body shape in sticklebacks has evolved in response both to foraging habitat and to predation regime (and likely other factors); thus, the evolution of body shape in sticklebacks should be subject to functional constraints.

The repeatability of the evolution of body shape in sticklebacks is further highlighted when examining pairs of stickleback populations that are found in divergent habitats within the same watershed (McPhail 1994; McKinnon and Rundle 2002). For example, marine sticklebacks have repeatedly invaded freshwater streams, independently establishing resident stickleback populations in streams across the Northern hemisphere (Bell and Foster 1994; McKinnon et al. 2004; Colosimo et al. 2005). Consistent with living in a more littoral habitat, sticklebacks that are resident in streams have a deeper body and a reduction in median fin length relative to parapatric marine sticklebacks (Hagen 1967; Schluter et al. 2004). Similar patterns are seen across multiple parapatric pairs of sticklebacks from streams and lakes; sticklebacks living in lakes and foraging on zooplankton in open water have much shallower bodies than do deep-bodied sticklebacks from adjoining streams where they forage on benthic prey (Moodie 1972; Reimchen et al. 1985; Lavin and McPhail 1993; Hendry et al. 2002; Hendry and Taylor 2004; Berner et al. 2008). Finally, within several lakes in British Columbia, two stickleback species have evolved parallel and predicted changes in shape; the streamlined limnetic

species forages on zooplankton in the open water, while the deeper-bodied benthic species forages on benthic macroinvertebrates in the littoral zone (Schluter and McPhail 1992). These replicated pairs of marine-stream, lake-stream and benthic-limnetic stickleback pairs thus present a remarkable opportunity to examine the genetic basis of parallel evolution in body shape.

There is evidence that phenotypic plasticity and activity patterns do play a role in establishing differences in body shape between stickleback populations (Day et al. 1994; Spoljaric and Reimchen 2007; Sharpe et al. 2008). However, studies on many different stickleback populations suggest that differences in body shape have a strong genetic component (McPhail 1984, 1992, 1994; Lavin and McPhail 1993; Hendry et al. 2002; Schluter et al. 2004; Leinonen et al. 2006; Spoljaric and Reimchen 2007; Sharpe et al. 2008). Thus, we focus here on the prospects for dissecting the genetic components that contribute to the evolution of body shape in sticklebacks.

Availability of genetic tools and genomic resources

Within the past decade, a number of genetic tools and genomic resources have been developed for the threespine stickleback (Peichel et al. 2001; Hosemann et al. 2004; Kingsley et al. 2004; Kingsley and Peichel 2007; Miller et al. 2007a; Baird et al. 2008), making it possible to identify the chromosomal regions, genes and actual mutations that underlie morphological and behavioral traits that differ among stickleback populations (Peichel et al. 2001; Colosimo et al. 2004, 2005; Cresko et al. 2004; Shapiro et al. 2004; Kimmel et al. 2005; Coyle et al. 2007; Miller et al. 2007b; Albert et al. 2008; Chan et al. 2009; Kitano et al. 2009). As of yet, these genetic tools have only begun to be applied to the study of shape in sticklebacks. We next highlight what has been learned so far as well as avenues for future research using the stickleback system.

Do the same genetic changes underlie parallel changes in body shape?

As outlined in the introduction, the question of whether genetic constraints play a role in the evolution of body shape in fishes can be explicitly examined by studying the genetic basis of parallel evolution. In sticklebacks, there is now a growing literature that examines whether the same genetic changes underlie similar phenotypic differences in independent populations. In some cases, the same traits appear to evolve many times in similar

environments because of selection on existing genetic variation in the ancestral population (Colosimo et al. 2005; Miller et al. 2007b). However, a recent study demonstrated that independent mutations at the same locus underlie the repeated evolution of pelvic reduction in threespine stickleback populations (Chan et al. 2009), strongly suggesting a role for genetic constraint. However, in other stickleback species, different genes are responsible for pelvic reduction (Shapiro et al. 2009). Therefore, we do not yet have a comprehensive view of the role of genetic constraint in the evolution of sticklebacks. Body shape is a promising trait to examine because there are likely many genes involved (Albert et al. 2008), as well as many populations with parallel divergence in body shape.

There are relatively few data on the genetic basis of parallel divergence in body shape. One study of differences in shape between marine and stream-resident sticklebacks from Canada and Japan suggests that the genetic architecture of these differences is similar in independent populations (Schluter et al. 2004), but whether the same genes are involved in differences in shape between marine and stream sticklebacks is unknown. However, there are some hints that the same genes might be involved in differences in shape among three independent benthic-limnetic species pairs. For example, markers on linkage group (LG) 12 differentiate benthics and limnetics from Paxton, Priest and Enos lakes (Gow et al. 2006); this same chromosomal region is linked to a quantitative trait locus (QTL) for shape in a cross between a Paxton Lake benthic and a Japanese marine fish (Albert et al. 2008). Furthermore, differences in shape between Enos Lake benthics and limnetics map to LG1, 4 and 7 (T. Malek, J. Boughman and C. Peichel, unpublished data); QTL for shape are found in similar regions on these LGs in the above-mentioned cross involving a Paxton Lake benthic fish (Albert et al. 2008). More direct investigations of the genetic basis of divergence in body shape between the benthic-limnetic, as well as marine-stream and lake-stream pairs, should provide insight into the role of genetic constraint in the evolution of body shape in sticklebacks.

How many genes underlie divergence in body shape?

Another way to examine whether genetic constraints play a role in the evolution of body shape is to investigate the overall genetic architecture of differences in shape. For example, we need to address whether differences in body shape can evolve through a few genetic changes of large effect or

whether it requires many genes of smaller effect. If few genes are involved, it may be easier to change shape rapidly during adaptation to a new environment. By contrast, if many genes are involved, body shape may be less able to evolve rapidly. Thus, the number of genes involved in changes in body shape might particularly constrain the “rate” of evolution.

Although the number of genetic changes required for the evolution of differences in shape among stickleback populations has not been exhaustively studied, two studies suggested that body shape has a complex genetic basis. Analysis of crosses between marine and stream-resident sticklebacks from Japan and Canada suggest that genetic variation for body shape is mostly additive and polygenic (Schluter et al. 2004). To date, only a single study has used a QTL mapping approach to identify the regions of the genome that contribute to variation in shape among stickleback populations (Albert et al. 2008). In this study, an F2 intercross between a Japanese Pacific Ocean marine stickleback and a Paxton Lake benthic stickleback was analyzed. Twenty-seven landmarks, producing 54 x and y coordinates were measured on 372 F2 individuals. Of these 54 coordinates, 47 were mapped to 26 QTL found on 17 different LGs. The x and y coordinates of the same landmark nearly always mapped to different QTL. Therefore, many different genes contribute to the overall differences in body shape between these populations.

Most of these QTL had relatively small effects on phenotype, although a few QTL of larger effect were identified. The distribution of effect sizes followed a gamma distribution, which is similar to that predicted by the geometric model of Orr (1998) when the inability to detect QTL of very small effect is considered (Otto and Jones 2000). There has been only one other QTL analysis of body shape in fishes; in this study, many QTL of relatively small effect and a few QTL of larger effect contribute to differences in body shape among populations of Atlantic salmon (Boulding et al. 2008). Taken together, these data suggest that the rate of evolution in body shape might be constrained by the lack of mutations that have a large effect on body shape. Additional studies of differences in shape among stickleback populations, as well as other fish species, will be required to rigorously address this question.

Does genetic linkage or pleiotropy contribute to the evolution of body shape?

It is also important to address whether the genes that contribute to the evolution of body shape have

pleiotropic effects on other phenotypes, or whether they are tightly linked to genes that affect other adaptive traits. For example, even if a particular allele has beneficial effects on body shape, it may have detrimental effects on other phenotypes; such antagonistic pleiotropy will constrain both the rate and direction of evolution (Lande 1979; Lande and Arnold 1983; Barton 1990; Keightley and Hill 1990; Otto 2004). Even in the absence of pleiotropy, some genes may preferentially contribute to the evolution of body shape because they are tightly linked to genes that underlie other traits required for adaptation to a particular environment (Gratten et al. 2008).

Thus far, the data on the contributions of linkage or pleiotropy to body shape in sticklebacks are limited to the study by Albert et al. (2008). However, some preliminary conclusions can be drawn. First, despite the fact that most QTL only affected a single x or y coordinate, there were several individual QTL that affected multiple coordinates. These data suggest that although body shape has a complex genetic basis, some aspects of differences in shape among populations are controlled by the same genes or by linked genes; this could facilitate rapid divergence in shape between populations. Second, one of the QTL that affected both head and pelvic landmarks in this cross is linked to the *Eda* gene, which is the major locus that controls differences in lateral plate number between marine and freshwater sticklebacks (Colosimo et al. 2005). However, it is unlikely that *Eda* itself is responsible for the differences in shape and more likely that there is linkage between genes for shape and lateral plates (Albert et al. 2008). This study suggests that both linkage and pleiotropy may be important in the evolution of body shape in sticklebacks. Because there are a multitude of phenotypic differences among stickleback populations, comprehensive studies on the genetic architecture of many traits, including shape, will assess the extent to which genetic linkage or pleiotropy plays a role in stickleback adaptation.

Another source of pleiotropy that is important to consider is allometry; i.e., whether divergence in shape is simply the result of differences in body size between individuals of the same age (static allometry) or between individuals of different ages (ontogenetic allometry). There is evidence for both ontogenetic and static allometry in sticklebacks (Walker 1993, 1997; McGuigan et al. this issue). However, the study by McGuigan et al. (this issue) concludes that the evolution of shape in sticklebacks is not simply due to pleiotropic effects resulting from selection for body size. In further support of this

hypothesis, size and shape QTL are found in different chromosomal locations (Albert et al. 2008). Although more work is certainly needed in this area, allometry does not appear to be a prevalent source of genetic or developmental constraints in the evolution of body shape in sticklebacks.

What is the genetic basis of sexual dimorphism in body shape?

In addition to the repeated patterns of divergence in shape that have been identified in different ecological systems, there are also repeated patterns of divergence in shape between male and female sticklebacks. A number of studies have examined sexual dimorphism in many traits in sticklebacks, but until recently, few studies have used geometric morphometrics to explicitly examine sexual dimorphism in body shape (Leinonen et al. 2006; Kitano et al. 2007; Aguirre et al. 2008; Spoljaric and Reimchen 2008). These recent studies reveal similar divergence in body shape between males and females across many stickleback populations (marine, stream and lake populations from Scandinavia, Japan, Alaska, Canada and Washington State). In general, females are larger with longer pelvic girdles, while males have larger and deeper heads, as well as larger median fins; these patterns are seen both in ancestral marine populations as well as derived freshwater populations (Leinonen et al. 2006; Kitano et al. 2007; Aguirre et al. 2008; Spoljaric and Reimchen 2008).

Sexual dimorphism—sexual selection or ecological niche partitioning?

Sexual dimorphism evolves when a trait confers differential fitness effects to males and females. Sexual dimorphism can result from sexual selection (Darwin 1874), but it could alternatively be driven by ecological differences resulting from competition for niche space between the sexes (Slatkin 1984; Shine 1989). Both sexual selection and ecological niche partitioning may play a role in the evolution of sexual dimorphism in shape in sticklebacks. For example, the larger size of females may be the result of sexual selection: larger females have more eggs (Baker 1994), and males prefer larger females in many populations of sticklebacks (Rowland 1994). In addition, several studies demonstrate that males and females differentially utilize benthic and limnetic habitats within lakes, where they exploit different food sources and are exposed to different predators and parasites (Wootton 1976; Reimchen 1980; Bentzen and McPhail 1984; Reimchen and Nelson 1987; Reimchen and Nosil 2001, 2004, 2006; Reimchen

et al. 2008). Consistent with foraging in a more complex benthic habitat, males have deeper bodies, longer median fins and deeper peduncles, which allow for rapid acceleration and maneuverability (Webb 1982, 1984; Walker 1997). By contrast, the more streamlined bodies of females are more suited for cruising in the open water (Webb 1982, 1984; Walker 1997). In addition, the larger pelvic girdles of females may be protective against avian predators in the open water (Reimchen 1994). In addition to these consistent patterns of sexual dimorphism that suggest niche partitioning between the sexes, Spoljaric and Reimchen (2008) found that the sexual dimorphism was greatest in large, clear lakes and reduced in small, shallow, stained lakes. These data support the idea that differences in ecological opportunity can either promote or constrain the evolution of sexual dimorphism in body shape (Nosil and Reimchen 2005). However, it is still difficult to disentangle the effects of ecological niche partitioning and sexual selection on the evolution of sexual dimorphism in sticklebacks (Shine 1989); both have likely played a role.

Antagonism between sexual dimorphism and speciation

As described above, ecologically driven disruptive selection can lead to sexual dimorphism, but disruptive selection can also lead to speciation; these processes may be antagonistic (Bolnick and Doebli 2003). If males and females diverge substantially, then ecological speciation may be inhibited because males and females have already partitioned the available ecological niches, eliminating disruptive selection. Thus, when strong sexual dimorphism exists, disruptive selection on ecological traits, such as body shape, is predicted to be minimal. Only a few empirical studies have explicitly examined whether such a tradeoff between these two phenomena exists. Consistent with the hypothesis of antagonism between ecological sexual dimorphism and speciation, a negative correlation between sexual dimorphism and disruptive selection for gill raker length (a trait associated with trophic ecology) was found within a lake population (Bolnick and Lau 2008). However, there is sexual dimorphism in body shape and other ecologically relevant traits within both benthics and limnetics from the same lake (McPhail 1992; Spoljaric and Reimchen 2008). These same traits are highly divergent between benthics and limnetics, suggesting that there is no trade-off between sexual dimorphism and speciation in this system.

Genetics of sexual dimorphism in body shape

The genetic architecture of sexual dimorphism might also influence whether sexual dimorphism or speciation is the outcome of disruptive selection (Bolnick and Doebli 2003). For example, if the genes that underlie ecologically relevant traits are linked to sex chromosomes, then we might predict sexual dimorphism to evolve more rapidly than would speciation. Theory suggests that sexually dimorphic traits should map to the sex chromosomes, particularly the X chromosome (Rice 1984; Charlesworth et al. 1987). However, the available empirical data across a wide variety of taxa do not always support this prediction (Fairbairn and Roff 2006).

Many of these previous empirical studies have used comparisons between species and populations, whereas studies within populations may be more informative for assessing whether sexually dimorphic traits are preferentially mapped to sex chromosomes (Fairbairn and Roff 2006). The stickleback model is ideal for such a within-population study. There is evidence that sexual dimorphism in body shape does have a strong genetic component (Kitano et al. 2007; Spoljaric and Reimchen 2008). Although no within-population mapping studies have yet been performed in sticklebacks, two studies have used crosses between populations to assess whether sexually dimorphic traits in sticklebacks are preferentially found on sex chromosomes. Body size is sexually dimorphic in many threespine stickleback populations, including marine forms from the Japan Sea and the Pacific Ocean (Kitano et al. 2007); variation in the body size of males between these populations is linked to the X chromosome (Kitano et al. 2009). Interestingly, length of the dorsal spine is only sexually dimorphic in the population from the Japan Sea (Kitano et al. 2007) and variation in the length of the dorsal spine in males maps to a neo-X chromosome found only in the population from the Japan Sea (Kitano et al. 2009).

The only study to explicitly examine the genetic basis of sexual dimorphism in body shape is the previously discussed QTL analysis performed on a cross between a Japanese Pacific Ocean marine female and a Paxton Lake benthic male (Albert et al. 2008). In this study, 30 *x* and *y* coordinates mapped to the sex chromosome, indicating that there were differences in the traits between XX females and XY males. However, in this study, direct linkage of traits to the X chromosome was not assessed by examining variation within each sex. Thus, it is still unknown whether sexual dimorphism in body shape is due to preferential linkage to the X chromosome.



Fig. 1 Representative specimens of a laboratory-reared male (top) and a laboratory-reared female (bottom) blackspotted stickleback (*G. wheatlandi*). When compared to females, males are smaller, have larger heads, longer median fins, and shorter, deeper caudal peduncles. Scale bar = 5 mm. Twenty-one landmarks were chosen based on Albert et al. (2008): (1) anterior extent of maxilla, (2) anterior extent of orbit, (3) ventral extent of orbit, (4) posterior extent of orbit, (5) posterior extent of supraoccipital, (6) first dorsal spine insertion, (7) second dorsal spine insertion, (8) third dorsal spine insertion, (9) posterior insertion of dorsal fin, (10) posterior extent of caudal peduncle, (11) posterior insertion of anal fin, (12) anterior insertion of anal fin, (13) insertion point of pelvic spine into the pelvic girdle, (14) posterior extent of ectocorocoid, (15) dorsal extent of ectocorocoid, (16) dorsal insertion of pectoral fin, (17) posteriodorsal extent of operculum, (18) ventral extent of operculum, (19) dorsal extent of preopercular, (20) posterioventral extent of preopercular, (21) anteroventral extent of preopercular.

Similar patterns of sexual dimorphism have been seen in other stickleback species, such as the blackspotted stickleback (*G. wheatlandi*; Sargent et al. 1984), ninespine stickleback (*Pungitius pungitius*; Ikeda 1933), brook stickleback (*Culaea inconstans*; Moodie 1986), and fourspine stickleback (*Apeltes quadracus*; Blouw and Hagen 1984). Our preliminary work suggests that aspects of sexual dimorphism in body shape do map to the X chromosome in the blackspotted stickleback (Fig. 1). Assessing whether sexual dimorphism in body shape maps to the sex chromosomes across these different species will be particularly revealing, as each stickleback species has a unique sex-chromosome system (Ross et al. 2009). This may reveal whether sex linkage imposes constraints on the evolution of sexual dimorphism in body shape.

Conclusions

Future research aimed at elucidating the genetic architecture of stickleback shape will determine whether genetic or developmental constraints play

an important role in the evolution of shape across stickleback populations. It will be interesting to determine whether the genetic architecture of body shape differs from that of other complex traits in sticklebacks or in other species. This research in sticklebacks will also have implications for the importance of genetic constraints on shape and ecomorphology in other taxa, elucidating the driving forces behind adaptive radiations and the recapitulation of common body forms.

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References

- Aguirre WE, Ellis KE, Kusenda M, Bell MA. 2008. Phenotypic variation and sexual dimorphism in anadromous threespine stickleback: implications for postglacial adaptive radiation. *Biol J Linn Soc* 95:465–78.
- Albert AYK, Sawaya S, Vines TH, Knecht AK, Miller CT, Summers BR, Balabhadra S, Kingsley DM, Schluter D. 2008. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evolution* 62:76–85.
- Arnold SJ. 1992. Constraints on phenotypic evolution. *Am Nat* 140(Suppl):S85–107.
- Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One* 3:e3376.
- Baker J. 1994. Life history variation in female threespine stickleback. In: Bell MA, Foster SA, editors. *The evolutionary biology of the threespine stickleback*. Oxford: Oxford University Press. p. 144–87.
- Barrett RDH, Schluter D. 2008. Adaptation from standing genetic variation. *Trends Ecol Evol* 23:38–44.
- Barton N. 1990. Pleiotropic models of quantitative variation. *Genetics* 124:773–82.
- Bell MA, Foster SA. 1994. *The evolutionary biology of the threespine stickleback*. Oxford: Oxford University Press.
- Bentzen P, McPhail JD. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) – specialization for alternative trophic niches in the Enos Lake species pair. *Can J Zool* 62:2280–6.
- Berner D, Adams DC, Grandchamp A-C, Hendry AP. 2008. Natural selection drives patterns of lake-stream divergence in stickleback foraging morphology. *J Evol Biol* 21:1653–65.
- Blouw DM, Hagen DW. 1984. The adaptive significance of dorsal spine variation in the fourspine stickleback, *Apeltes quadracus*. III. Correlated traits and experimental evidence on predation. *Heredity* 53:371–82.
- Bolnick DI, Doebli M. 2003. Sexual dimorphism and adaptive speciation: two sides of the same ecological coin. *Evolution* 57:2433–49.
- Bolnick DI, Lau OL. 2008. Predictable patterns of disruptive selection in stickleback in postglacial lakes. *Am Nat* 172:1–11.
- Boulding EG, Culling M, Glebe B, Berg PR, Lien S, Moen T. 2008. Conservation genomics of Atlantic salmon: SNPs associated with QTLs for adaptive traits in parr from four trans-Atlantic backcrosses. *Heredity* 101:381–91.
- Chan YF, et al. 2009. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer. *Science* 327:302–5.
- Charlesworth B, Coyne JA, Barton NH. 1987. The relative rates of evolution of sex chromosomes and autosomes. *Am Nat* 130:113–46.
- Colosimo PF, Peichel CL, Nereng K, Blackman BK, Shapiro MD, Schluter D, Kingsley DM. 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. *PLoS Biol* 2:635–41.
- Colosimo PF, Hosemann KE, Balabhadra S, Villareal G, Dickson M, Grimwood J, Schmutz J, Myers RM, Schluter D, Kingsley DM. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of *Ectodysplasin* alleles. *Science* 307:1928–33.
- Coyle SM, Huntingford FA, Peichel CL. 2007. Parallel evolution of *Pitx1* underlies pelvic reduction in threespine stickleback (*Gasterosteus aculeatus*). *J Hered* 98:581–6.
- Cresko WA, Amores A, Wilson C, Murphy J, Currey M, Phillips P, Bell MA, Kimmel CB, Postlethwait JH. 2004. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. *Proc Natl Acad Sci USA* 101:6050–5.
- Darwin C. 1874. *The descent of man; and selection in relation to sex*. New York: Humboldt.
- Day T, Pritchard J, Schluter D. 1994. Ecology and genetics of phenotypic plasticity: a comparison of two sticklebacks. *Evolution* 48:1723–34.
- Endler JA. 1986. *Natural selection in the wild*. Princeton: Princeton University Press.
- Endler JA. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol Evol* 10:22–9.
- Fairbairn DJ, Roff DA. 2006. The quantitative genetics of sexual dimorphism: assessing the importance of sex-linkage. *Heredity* 97:319–28.
- Ghalambor CK, Walker JA, Reznick DN. 2003. Multi-trait selection, adaptation, and constraints on the evolution of burst swimming performance. *Integr Comp Bio* 43:431–8.
- Gow JL, Peichel CL, Taylor EB. 2006. Contrasting hybridization rates between sympatric threespine sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. *Mol Ecol* 15:739–52.
- Gratten J, Wilson AJ, McRae AF, Beraldi D, Visscher PM, Pemberton JM, Slate J. 2008. A localized negative genetic correlation constrains microevolution of coat color in wild sheep. *Science* 319:318–20.
- Hagen DW. 1967. Isolating mechanisms in threespine sticklebacks (*Gasterosteus*). *J Fish Res Bd Can* 24:1637–92.
- Haldane JBS. 1932. *The causes of evolution*. London: Longman.
- Hendry AP, Taylor EB. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. *Evolution* 58:2319–31.
- Hendry AP, Taylor EB, McPhail JD. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. *Evolution* 56:1199–216.
- Hosemann KE, Colosimo PF, Summers BR, Kingsley DM. 2004. A simple and efficient microinjection protocol for making transgenic sticklebacks. *Behaviour* 141:1345–55.
- Ikeda K. 1933. The distribution and morphological variations of the stickleback in Japan. *Zool Mag (Tokyo)* 46:553–72.

- Keightley PD, Hill WG. 1990. Variation maintained in quantitative traits with mutation-selection balance: pleiotropic side-effects on fitness traits. *Proc R Soc Lond B* 242:95–100.
- Kimmel CB, Ullman B, Walker C, Wilson C, Currey M, Phillips PC, Bell MA, Postlethwait JH, Cresko WA. 2005. Evolution and development of facial bone morphology in threespine sticklebacks. *Proc Natl Acad Sci USA* 102:5791–6.
- Kingsley DM, Peichel CL. 2007. The molecular genetics of evolutionary change in sticklebacks. In: Östlund-Nilsson S, Mayer I, Huntingford FA, editors. *Biology of the three-spined stickleback*. Boca Raton, FL: CRC Press. p. 41–81.
- Kingsley DM, et al. 2004. New genomic tools for molecular studies of evolutionary change in sticklebacks. *Behaviour* 141:1331–44.
- Kitano J, Mori S, Peichel CL. 2007. Sexual dimorphism in the external morphology of the threespine stickleback (*Gasterosteus aculeatus*). *Copeia* 2007:336–49.
- Kitano J, et al. 2009. A role for a neo-sex chromosome in stickleback speciation. *Nature* 461:1079–83.
- Lande R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution* 33:402–16.
- Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–26.
- Lavin PA, McPhail JD. 1993. Parapatric lake and stream sticklebacks on northern Vancouver Island – disjunct distribution or parallel evolution? *Can J Zool* 71:11–7.
- Leinonen T, Cano JM, Mäkinen H, Merilä J. 2006. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *J Evol Biol* 19:1803–12.
- Maynard Smith J, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L. 1985. Developmental constraints and evolution. *Q Rev Biol* 60:265–87.
- McGuigan K, Nishimura N, Currey M, Hurwit D, Cresko WA. 2010. Quantitative genetic variation in static allometry in the threespine stickleback. *Integr Comp Biol*. doi:10.1093/icb/icq026.
- McKinnon JS, Rundle HD. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol Evol* 117:480–8.
- McKinnon JS, Mori S, Blackman BK, David L, Kingsley DM, Jamieson L, Chou J, Schluter D. 2004. Evidence for ecology's role in speciation. *Nature* 429:294–8.
- McPhail JD. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Can J Zool* 62:1402–8.
- McPhail JD. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): evidence for a species pair in Paxton Lake, Texada Island, British Columbia. *Can J Zool* 70:361–9.
- McPhail JD. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. In: Bell MA, Foster SA, editors. *The evolutionary biology of the threespine stickleback*. Oxford: Oxford University Press. p. 399–437.
- Miller MR, Dunham JP, Amores A, Cresko WA, Johnson EA. 2007a. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Res* 17:240–8.
- Miller CT, Beleza S, Pollen AA, Schluter D, Kittles RA, Shriver MD, Kingsley DM. 2007b. *cis*-Regulatory changes in *Kit ligand* expression and parallel evolution of pigmentation in sticklebacks and humans. *Cell* 131:1179–89.
- Moodie GEE. 1972. Morphology, life history, and ecology of an unusual stickleback (*Gasterosteus aculeatus*) in the Queen Charlotte Islands. *Can J Zool* 50:721–32.
- Moodie GEE. 1986. The population biology of *Culaea inconstans*, the brook stickleback, in a small prairie lake. *Can J Zool* 64:1709–17.
- Nosil P, Reimchen TE. 2005. Ecological opportunity and levels of morphological variance within freshwater stickleback populations. *Biol J Linn Soc* 86:297–308.
- Orr HA. 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution* 52:935–49.
- Otto SP. 2004. Two steps forward, one step back: the pleiotropic effects of favoured alleles. *Proc R Soc Lond B* 271:705–14.
- Otto SP, Jones CD. 2000. Detecting the undetected: estimating the total number of loci underlying a quantitative trait. *Genetics* 156:2093–107.
- Peichel CL, Nereng KS, Ohgi KA, Cole BLE, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM. 2001. The genetic architecture of divergence between threespine stickleback species. *Nature* 414:901–5.
- Reimchen TE. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? *Can J Zool* 68:1232–44.
- Reimchen TE. 1991. Evolutionary attributes of headfirst prey manipulations and swallowing in piscivores. *Can J Zool* 69:2912–6.
- Reimchen TE. 1994. Predators and morphological evolution in the threespine stickleback. In: Bell MA, Foster SA, editors. *The evolutionary biology of the threespine stickleback*. Oxford: Oxford University Press. p. 240–76.
- Reimchen TE, Nelson JS. 1987. Habitat and morphological correlates to vertebral number in a freshwater teleost, *Gasterosteus aculeatus*. *Copeia* 1987:868–74.
- Reimchen TE, Nosil P. 2001. Ecological causes of sex-biased parasitism in threespine stickleback. *Biol J Linn Soc* 73:51–63.
- Reimchen TE, Nosil P. 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. *Evolution* 58:1274–81.
- Reimchen TE, Nosil P. 2006. Replicated ecological landscapes and the evolution of morphological diversity among *Gasterosteus* populations from an archipelago on the west coast of Canada. *Can J Zool* 84:643–54.

- Reimchen TE, Ingram T, Hansen SC. 2008. Assessing niche differences of sex, armour and asymmetry phenotypes using stable isotope analyses in Haida Gwaii sticklebacks. *Behaviour* 145:561–77.
- Reimchen TE, Stinson EM, Nelson JS. 1985. Multivariate differentiation of parapatric and allpatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. *Can J Zool* 63:2944–51.
- Reznick DN, Ghalambor CK. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112–13:183–98.
- Reznick DN, Travis J. 1996. The empirical study of adaptation in natural populations. In: Rose MR, Lauder GV, editors. *Adaptation*. San Diego: Academic Press. p. 243–89.
- Rice WR. 1984. Sex chromosomes and the evolution of sexual dimorphism. *Evolution* 38:735–42.
- Ross JA, Urton JR, Boland J, Shapiro MD, Peichel CL. 2009. Turnover of sex chromosomes in the stickleback fishes (Gasterosteidae). *PLoS Genet* 5:e1000391.
- Rowland WJ. 1994. Proximate determinants of stickleback behaviour: an evolutionary perspective. In: Bell MA, Foster SA, editors. *The evolutionary biology of the three-spine stickleback*. Oxford: Oxford University Press. p. 297–344.
- Sargent RC, Bell MA, Krueger WH, Baumgartner JV. 1984. A lateral plate cline, sexual dimorphism, and phenotypic variation in the black-spotted stickleback *Gasterosteus wheatlandi*. *Can J Zool* 62:368–76.
- Schluter D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* 50:1766–74.
- Schluter D, McPhail JD. 1992. Ecological character displacement and speciation in sticklebacks. *Am Nat* 140:85–108.
- Schluter D, Clifford EA, Nemethy M, McKinnon JS. 2004. Parallel evolution and inheritance of quantitative traits. *Am Nat* 163:809–22.
- Shapiro MD, Marks ME, Peichel CL, Blackman BK, Nereng KS, Jónsson B, Schluter D, Kingsley DM. 2004. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* 428:717–23.
- Shapiro MD, Summers BR, Balabhadra S, Miller AL, Cunningham CB, Aldenhoven JT, Bell MA, Kingsley DM. 2009. The genetic architecture of skeletal convergence and sex determination in ninespine sticklebacks. *Curr Biol* 19:1140–5.
- Sharpe DMT, Räsänen K, Berner D, Hendry AP. 2008. Genetic and environmental contributions to the morphology of lake and stream stickleback: implications for gene flow and reproductive isolation. *Evol Ecol Res* 10:849–66.
- Shine R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Q Rev Biol* 64:419–61.
- Shubin N, Wake DB, Crawford AJ. 1995. Morphological variation in the limbs of *Taricha granulosa* (Caudata: Salamandridae): evolutionary and phylogenetic implications. *Evolution* 49:874–84.
- Slatkin M. 1984. Ecological causes of sexual dimorphism. *Evolution* 38:622–30.
- Spoljaric MA, Reimchen TE. 2007. 10 000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. *J Fish Biol* 70:1484–503.
- Spoljaric MA, Reimchen TE. 2008. Habitat-dependent reduction of sexual dimorphism in geometric body shapes of Haida Gwaii threespine stickleback. *Biol J Linn Soc* 95:505–16.
- Wake DB. 1991. Homoplasy: the result of natural selection or evidence of design limitations? *Am Nat* 138:543–67.
- Walker JA. 1993. Ontogenetic allometry of threespine stickleback body form using landmark-based morphometrics. In: Marcus LF, Bello E, Garcia-Valdecasas A, editors. *Contributions to morphometrics*. Madrid: Museo Nacional de Ciencias Naturales. p. 193–214.
- Walker JA. 1997. Ecological morphology of lacustrine three-spine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol J Linn Soc* 61:3–50.
- Walker JA. 2007. A general model of functional constraints on phenotypic evolution. *Am Nat* 170:681–9.
- Walker JA, Bell MA. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks. *J Zool Lond* 252:293–302.
- Webb PW. 1982. Locomotor patterns in the evolution of actinopterygian fishes. *Am Zool* 22:329–42.
- Webb PW. 1984. Body form, locomotion and foraging in aquatic vertebrates. *Am Zool* 24:107–20.
- West-Eberhard MJ. 2003. *Developmental plasticity and evolution*. New York: Oxford University Press.
- Wootton RJ. 1976. *The biology of the sticklebacks*. London: Academic Press.