The Population Genomics of Parallel Adaptation: Lessons from Threespine Stickleback

Paul A. Hohenlohe and Isabel Santos Magalhaes

Abstract Threespine stickleback fish (*Gasterosteus aculeatus*) have long been an ecological and evolutionary model system. Stickleback exhibit remarkable patterns of parallel adaptation among populations across their range, most notably repeated colonization and adaptation in freshwater habitats from ancestral marine or anadromous forms and repeated diversification into different freshwater ecotypes such as lake/stream and benthic/limnetic. The phenotypic traits involved in this adaptive evolution include physiology, behavior, life history, pigmentation, and numerous aspects of body size, shape, and morphology, the genetic basis of which has been elucidated through laboratory-based genetic mapping. With the advent of next-generation sequencing and the availability of a well-assembled reference genome for the species, numerous studies have identified genomic regions exhibiting signatures of selection in natural populations. The combination of these approaches has established numerous linkages among genotype, phenotype, environment, and adaptation. Here we review these results and assess alternative modes for the genetic basis of parallel phenotypic adaptation in terms of the genetic architecture of the traits and the source of adaptive variation across populations. We highlight examples ranging from single genes of major effect to polygenic traits and from reuse of allelic variation shared among populations to independent mutations across loci. Demographic scenarios such as serial colonization and adaptation, along with genomic features such as inversion polymorphism, provide insights into how widespread parallel adaptation in multiple phenotypes can occur. The diversity of genetic mechanisms for parallel evolution in stickleback leads to the "Everyone Wins" principle of biology—nearly any alternative mechanism plays a role in at least some cases, and often multiple mechanisms act concurrently. Because of the wealth of natural evolutionary experiments and the ever-expanding set of genomics and

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other tools available in this species, threespine stickleback will likely remain a key model system for population genomics studies of adaptation.

Keywords Adaptive radiation · *Gasterosteus aculeatus* · Genome scan · GWAS · Parallel evolution · QTL mapping

1 Adaptive Evolution in Threespine Stickleback

1.1 Ecological Diversity and Parallel Evolution

With a nearly circumpolar marine distribution in the Northern Hemisphere and ongoing repeated colonization of freshwater habitats, threespine stickleback fish (*Gasterosteus aculeatus*) exhibits remarkable diversity in a wide range of phenotypes (Fig. 1). This diversity, combined with their local abundance and ease of collection, has made threespine stickleback an evolutionary model system for the study of rapid and dramatic evolution of morphological, physiological, and behavioral adaptations to new environments and, in particular, replicated colonization and adaptation to a set of habitats distributed across the species range. Early studies attempting to characterize the species were presented with a bewildering amount of phenotypic diversity that challenged taxonomic groupings (Bell and Foster 1994). Further research in the twentieth century began correlating phenotypic diversity with habitat, suggesting that natural selection could probably account for much of the observed variation (Hagen and McPhail 1970; Bell 1976). Stickleback became a model system for adaptive radiation and sympatric diversification with the discovery of evidence for character displacement in lakes where morphologically distinct types coexisted, with links between morphology and resource use, and reduced gene flow between morphotypes even in sympatry (Schluter and McPhail 1992; Schluter 1993, 1996).

A remarkable feature of stickleback diversity is that similar phenotypes can be found repeatedly in similar environments in geographically isolated locations across the species range, suggesting independent but phenotypically parallel adaptation. For instance, Lavin and McPhail (1993) found repeated phenotypic differences between lake and stream populations in British Columbia, arguing that phenotypic similarities could be the result of parallel evolution. Since then the number of studies of parallel evolution and adaptive radiation in stickleback has continued to grow, along multiple phenotypic and environmental axes. With the advent of genomics and other experimental tools that are readily applied in this species, many researchers have combined various types of data to address the connections among genotype, phenotype, environment, and adaptation. Table 1 presents a representative set of publications focused on parallel evolution in threespine stickleback, based on a simple and far-from-exhaustive web search. Not only has the frequency of such publications steadily increased, but they are well-cited in the broader literature, showing the influence of stickleback research on the field of evolutionary biology (Fig. 2).

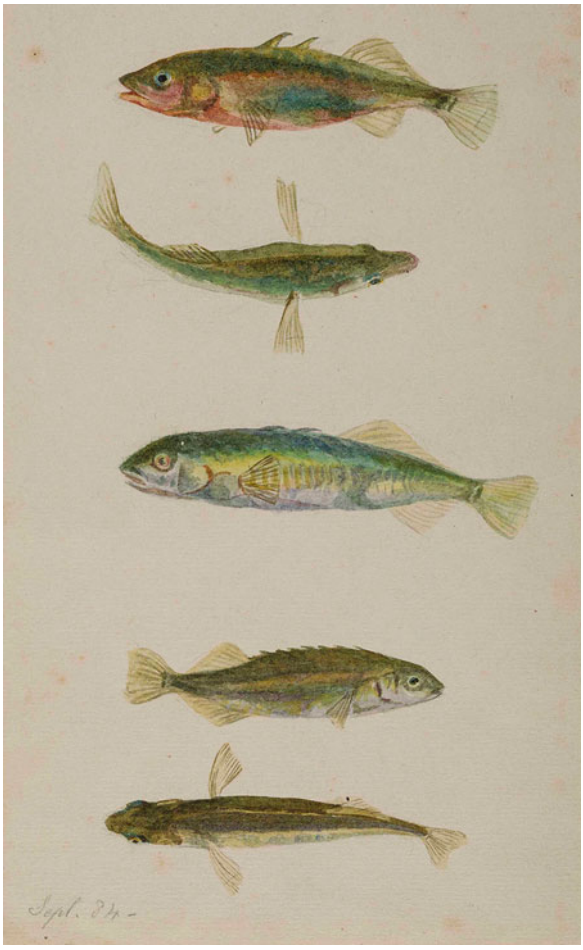

Fig. 1 Phenotypic variation and popular appreciation of threespine stickleback (*Gasterosteus aculeatus*; top three fish), as well as ninespine stickleback (*Pungitius pungitius*; bottom two individuals). Illustration by children's book author and naturalist Beatrix Potter (1866–1943), reproduced courtesy of the Armitt Trust

Three main pairwise comparisons have been the focus of studies of stickleback evolution and divergence: (1) marine versus freshwater, (2) stream versus lake, and (3) benthic versus limnetic forms. It is generally accepted that extant marine populations represent the ancestral threespine stickleback form (Bell 1977; Schluter and McPhail 1992; Bell and Foster 1994; but see Morris et al. 2018), and the diverse freshwater ecotypes are derived. There is also differentiation between truly marine and anadromous populations, although this distinction is poorly known (Ahnelt 2018). Many studies have focused on external morphology, particularly the reduction in armor traits, such as lateral plates and spines, which often occurs with colonization of freshwater from marine habitats (Bell et al. 2004). Marine stickleback nearly always have three dorsal spines, two pelvic spines (one on each side of the body), an armored pelvis, and a full set of lateral armor plates running from behind the head to the tail. Freshwater populations, however, exhibit a wide variation in the reduction in the number of dorsal spines, pelvic armor and spines, and

Table 1 A non-exhaustive survey of studies of parallel evolution in threespine stickleback, showing the region in which populations were sampled, the primary axis of comparison, and phenotypic and genetic data collected

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Rudman et al. (2019)	British Columbia, Canada	Marine, freshwater	Lateral plate morph (high, low), ionome, calcium uptake, excretions	–
Verta and Jones (2019)	Scotland; British Columbia, Canada	Marine, freshwater	Gene expression	RNAseq
Miller et al. (2019)	British Columbia, Canada	Sculpin presence, absence	Body shape, armor traits	mtDNA, WGS
Haenel et al. (2019)	Scotland	Marine, acid lakes, alkaline lakes	Armor traits	Pooled RADseq
Xie et al. (2019)	California, Alaska, USA; British Columbia, Canada	Marine, freshwater	Pelvic armor	Mutational mechanism
Kitano et al. (2019)	Japan; British Columbia, Canada	Marine, stream	Gene expression	eQTL from targeted SNP genotyping
Liu et al. (2018)	Denmark; Greenland	Marine, freshwater	Lateral plates, keel plates	RADseq
Bassham et al. (2018)	Alaska, USA	Marine, freshwater	–	RADseq
Nelson and Cresko (2018)	Alaska, USA	Marine, freshwater	–	RADseq
Hanson et al. (2017)	British Columbia, Canada	Lake, stream	Gene expression	Transcriptome sequencing
Pujolar et al. (2017)	Denmark	Marine, freshwater	Lateral plates	Targeted SNP genotyping
Mobley et al. (2016)	British Columbia, Canada	Benthic, limnetic	Mating preference	–
Erickson et al. (2016)	British Columbia, Canada	Marine, benthic freshwater	Skeletal morphology, armor traits	GBS
Hanson et al. (2016)	British Columbia, Canada	Lake, stream	Sexual maturity (body color for males, gravidity for females)	–
Oke et al. (2016)	British Columbia, Canada	Lake, stream	Morphological measurements, gill rakers	–

(continued)

Table 1 (continued)

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Conte et al. (2015)	British Columbia	Benthic, limnetic	Body shape, armor traits, gill rakers	Targeted SNP genotyping
Mazzarella et al. (2015)	Norway	Salinity	Body shape	–
Hirase et al. (2014)	California, Washington, Alaska, USA; British Columbia, Nova Scotia, Canada; Japan; Germany; Norway; Scotland; Iceland	Marine, freshwater	Gene copy number	WGS
Glazer et al. (2014)	British Columbia, Canada; Washington, Alaska, USA	Marine, freshwater	Gill rakers	Microsatellites, InDel markers
Lucek et al. (2013)	Switzerland	Lake, stream	Body shape, armor traits, gill rakers	Microsatellites
Ravinet et al. (2013)	Northern Ireland	Lake, stream	Body shape, armor traits, gill rakers, diet	Microsatellites
Moser et al. (2012)	Germany; Austria; Switzerland	Lake, stream	Otoliths, body shape, fecundity, stomach content, lateral plates	Microsatellites, mtDNA
Natsopoulou et al. (2012)	Iceland	Rocky, lava, mud substrates	Parasite load	MHC diversity by SSCP
Deagle et al. (2012)	British Columbia, Canada	Lake, stream	Morphological measurements, lateral plates	Targeted SNP genotyping
Dalziel et al. (2012)	British Columbia, Canada	Marine, stream	Gill rakers, ventricular and pectoral muscle, hemoglobin concentration, hematocrit	–
Hohenlohe et al. (2012)	Alaska, USA	Marine, freshwater	–	RADseq
Kaeuffer et al. (2012)	British Columbia, Canada	Lake, stream	Body shape, armor traits, gill rakers, diet, trophic position	Microsatellites
Kimmel et al. (2012)	Alaska, Oregon, USA; British Columbia, Canada; Iceland	Marine, freshwater	Opercle morphology	–

(continued)

Table 1 (continued)

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Jones et al. (2012)	California, Washington, Alaska, USA; British Columbia, Nova Scotia, Canada; Japan; Germany; Norway; Scotland; Iceland	Marine, freshwater, benthic, limnetic	Body shape	WGS
Hohenlohe et al. (2010)	Alaska, USA	Marine, freshwater	–	RADseq
Ólafsdóttir and Snorrason (2009)	Iceland	Rocky, lava, mud substrates	Microhabitat, body shape, armor traits	Microsatellites
Chan et al. (2009)	British Columbia, Canada; Alaska, USA; Japan	Marine, freshwater	Pelvic armor	Microsatellites, targeted sequencing of *PitxI*, transgenics
Marchinko (2009)	British Columbia, Canada	Marine, freshwater	Armor traits	Targeted genotyping of *Eda*
Miller et al. (2007)	British Columbia, Canada; Washington, California, USA; Japan	Marine, freshwater	Gill and skin pigmentation	Microsatellites
Coyle et al. (2007)	Scotland	Lake, stream	Pelvic girdle and pelvic spine	Microsatellites
Marchinko and Schluter (2007)	British Columbia, Canada	Marine, freshwater	Lateral plates, growth rate	–
Ólafsdóttir et al. (2007)	Iceland	Marine, freshwater	Spine length and lateral plate morphology, microsatellite loci	–
Colosimo et al. (2005)	British Columbia, Canada	Marine, freshwater	Lateral plates	Microsatellites, targeted sequencing of *Eda*
Boughman et al. (2005)	British Columbia, Canada	Benthic, limnetic	Behavior (courtship), gill raker, armor plate numbers	–
Colosimo et al. (2004)	California, USA; British Columbia, Canada	Marine, freshwater	Lateral plates	Microsatellites
Cresko et al. (2004)	Alaska, USA	Marine, freshwater	Armor traits	Microsatellites
Rundle et al. (2000)	British Columbia, Canada	Benthic, limnetic	Spawning probability	–
Thompson et al. (1997)	British Columbia, Canada	Lake, stream	–	mtDNA

(continued)

Table 1 (continued)

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Lavin and McPhail (1993)	British Columbia, Canada	Lake, stream	Gill rakers, body shape	–

We searched Web of Science for *TITLE*: (stickleback∗) *AND TITLE*: (parallel∗ or repeat∗) *NOT TITLE*: (nine∗) as of June 2019. Ten publications were removed as the word "repeated" in the title did not refer to parallel evolution, leaving a total of 44 publications. This excludes relevant studies without the specific keywords in the title (e.g., Raeymaekers et al. 2017; Stuart et al. 2017)
mtDNA mitochondrial DNA sequence, *RADseq* restriction site-associated DNA sequencing, *eQTL* expression quantitative trait locus, *SNP* single-nucleotide polymorphism, *WGS* whole-genome sequencing, *MHC* major histocompatibility complex, *SSCP* single-stranded conformation polymorphism

lateral plates, with some populations having lost nearly all of these. Early comparisons of lake and stream populations focused on the ecology of adaptive radiations (Schluter and McPhail 1992; Schluter 1993, 1996) and parallel evolution (Lavin and McPhail 1993), particularly in body shape phenotypes.

Both marine/freshwater and lake/stream stickleback population pairs are widespread across the species range, including both sides of the North Atlantic and North Pacific. In contrast, the coexistence of distinct bottom-dwelling (benthic) and open-water (limnetic) forms within freshwater habitats is much less common, with examples primarily from a few lakes in British Columbia. Their rarity may be explained by the fact that the pairs most likely resulted from double invasions facilitated by fluctuations in sea level in this region (Schluter 1996): the first oceanic colonizers of these lakes evolved into a freshwater form, and then a second invasion by oceanic sticklebacks displaced the first population to the benthic niche while adapting to the alternative open-water limnetic niche (Taylor and McPhail 1999). These pairs have provided examples of divergence in size, shape, feeding morphology, body armor, mate preference, and behavior, which confer fitness advantages when tested in the corresponding benthic and limnetic environments (Schluter and McPhail 1992; Erickson et al. 2016).

1.2 Threespine Stickleback as a Model System

The threespine stickleback has become a model system for adaptive evolution from multiple perspectives (Hendry et al. 2013). While the taxonomic implications of diversification have been a continuing source of debate, the mechanisms of ecotype formation and evolution of partial or complete reproductive isolation between stickleback forms provide a model for understanding the processes of adaptive radiation and speciation (Foster et al. 1998; McKinnon et al. 2004). Because of the adaptation to different habitats and ecological niches in stickleback diversification, the species has played a key role in the concept of ecological speciation—speciation

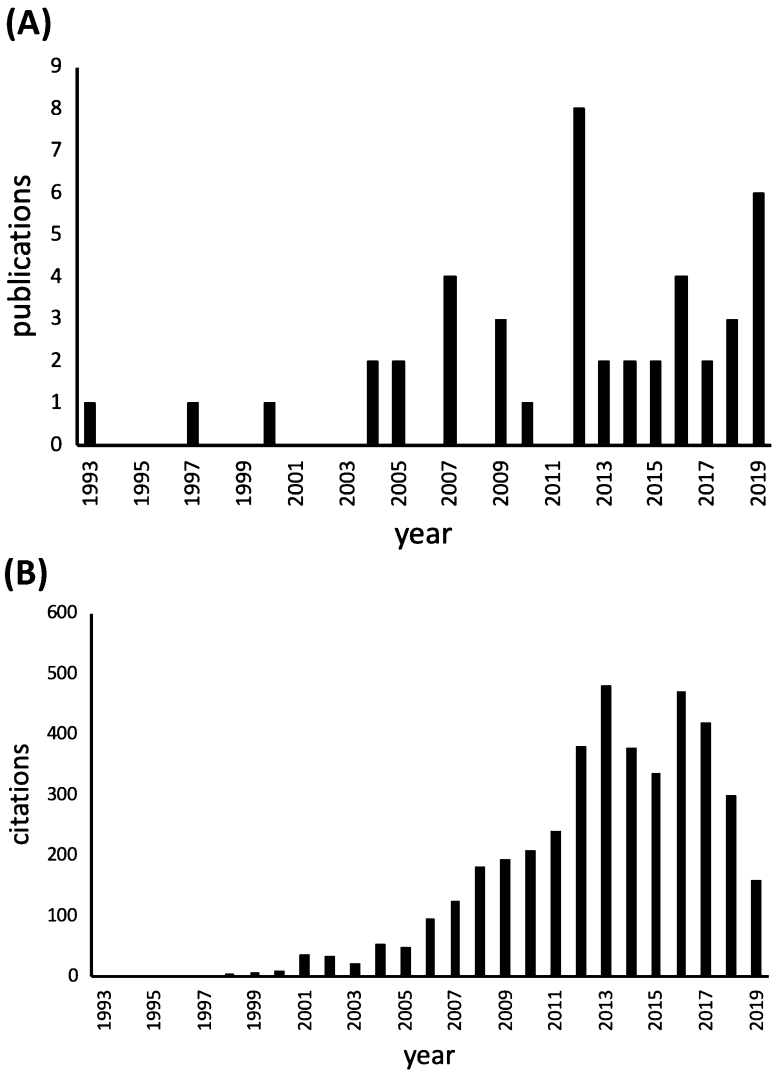

Fig. 2 (**a**) Annual counts of published studies of parallel evolution in threespine stickleback as of June 2019 that are shown in Table 1. (**b**) Total number of times that all of these publications have been cited per year

in which reproductive isolation occurs as a by-product of phenotypic divergence resulting from adaptation to different ecological roles (Schluter 2001, 2009; Nosil 2012). Ecological communities feel the effects of these processes. For example, divergence into benthic and limnetic ecotypes has been shown to have cascading ecological effects on prey community structure, primary productivity, and dissolved organic material (Harmon et al. 2009). Conversely, the collapse of ecotypes into

a single interbreeding population (termed "reverse speciation" or "introgressive extinction") can also have ecological consequences. For instance, Rudman and Schluter (2016) found that when benthic and limnetic forms combined into a single intermediate form, effects on relative abundances of prey included changes in the pupating aquatic insects that emerged into the surrounding terrestrial environment.

A number of genomics and laboratory tools and resources have facilitated stickleback research. The genome of the threespine stickleback is of a tractable size (~460 Mb, in 21 chromosomes), and a high-quality reference genome assembly has been available for some time (Kingsley et al. 2004; Jones et al. 2012). With the advent of next-generation sequencing, threespine stickleback have been the focus of early empirical studies in the field of population genomics (Hohenlohe et al. 2010; Jones et al. 2012). They are also easily raised in the lab and subject to experimental manipulation for developmental or physiological studies. To the extent that research can uncover the developmental genetic basis of traits that play important roles in ecology and parallel adaptation, stickleback can be a model for connecting evolutionary patterns to developmental processes ("evo-devo"; Cresko et al. 2007; Miller et al. 2014).

Despite the strong evidence that phenotypic changes in stickleback have evolved in response to environmental conditions, particularly along the ecological axes described above, there are comparatively few studies of the specific environmental drivers of divergence. Most of these have focused on predation, parasites, and salinity and/or pH. A few studies have found positive associations between spine length and predation intensity (Moodie et al. 1973; Gross 1978; Reimchen 1995). Large variation in pH and calcium among lakes has been linked to the evolution of body size or armor in stickleback in these lakes (Giles 1983; Spence et al. 2013; MacColl and Aucott 2014). Studies of environmental drivers of stickleback adaptation have traditionally focused on the relationship between a single environmental factor and the evolution of one or a small number of traits (Vamosi and Schluter 2002; Marchinko 2009), although this has begun to change (Bourgeois et al. 1994; Raeymaekers et al. 2017; Stuart et al. 2017). The understanding of stickleback diversity can benefit from viewing both the environment and phenotype as highly multivariate and with complex relationships to fitness.

1.3 Genetics of Parallel Evolution

Parallel phenotypic evolution has been observed in a number of taxa, such as cichlid and salmonid fishes (Elmer and Meyer 2011) and *Anolis* lizards (Mahler et al. 2013). Several authors (Arendt and Reznick 2008; Elmer and Meyer 2011; Rosenblum et al. 2014; Bolnick et al. 2018) have addressed the distinction between "parallel" and "convergent" evolution, which depends on how two populations or lineages arrived at a similar phenotypic state; parallel implies a similar starting point (i.e., more recent common ancestor or similar genetic basis), while convergent implies different

starting points (i.e., distant and phenotypically distinct common ancestor or different genetic mechanisms). The wealth of natural experiments and genomics tools in stickleback allow direct investigation of the genetic basis of adaptive phenotypes. However, the genetic basis of phenotypes shared among populations can be similar or different in a multitude of ways (Arendt and Reznick 2008). First, it is important to consider the level of biological organization at which the relevant genetic variation occurs (e.g., nucleotide, gene, network) (Rosenblum et al. 2014). For example, mutations that affect different nucleotide positions within the same gene and thus result in similar phenotypes could be considered convergent at the gene level but not at the nucleotide level (Fig. 3). Variation may be inherited from a common ancestor,

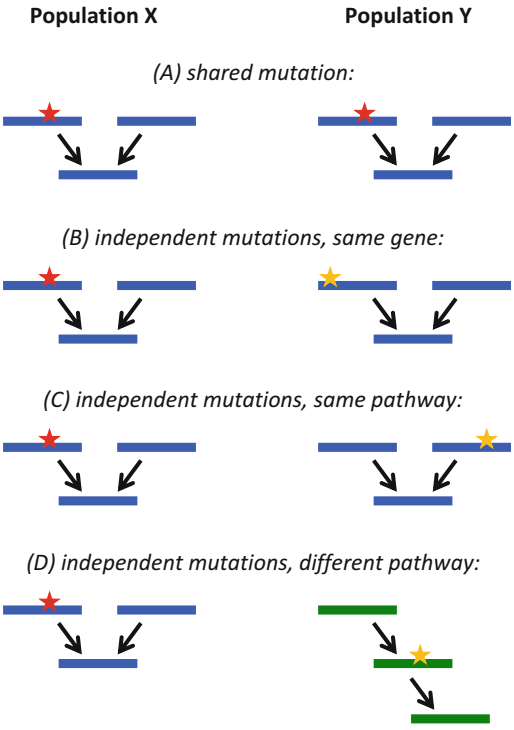

Fig. 3 Alternative genetic scenarios for parallel phenotypic evolution (Elmer and Meyer 2011; Rosenblum et al. 2014). Similar phenotypes evolve in similar habitats in two independent populations X and Y (e.g., stickleback in two freshwater bodies) from a divergent common ancestor (e.g., marine). Bars represent genes interacting with each other in a pathway that affects the phenotype, and stars represent any type of mutation (nucleotide substitution, insertion/deletion, etc.) that affects either regulatory or coding regions of the gene. (**a**) A single mutation in one gene creates an allele that is present in the ancestral population, and selection acts on this allele in both the descendant populations. (**b**) Two different mutations in the same gene lead to similar phenotypes in each population. (**c**) Two different mutations in different genes produce similar phenotypes by affecting the same genetic pathway. (**d**) Independent mutations affect genes in different pathways, but nonetheless have similar phenotypic effects. In the case of polygenic phenotypes, some combination of any or all of these scenarios may play a role together

thus providing the shared genetic mechanism of parallel evolution, in the case of adaptation from standing variation (Barrett and Schluter 2008). Alternatively, it may reflect independent genetic changes between populations or lineages. Phenotypic variation may also be polygenic, so that parallel phenotypic change may depend on genetic changes in overlapping suites of loci or pathways. To address these cases, and to make use of quantitative trait locus mapping studies, Conte et al. (2012) developed a metric of proportional similarity to reflect the proportional contributions of genes to parallel phenotypes.

Whether parallel phenotypic evolution relies on one or many genes, or independent variants versus shared ancestral variation, depends on a large number of factors that are specific to the genetic basis of the phenotype and the demographic history of the populations (Rosenblum et al. 2014). It is possible that different phenotypes show different patterns within the same set of populations or that parallel evolution of a polygenic phenotype reflects a mixture of shared ancestral variation and independent mutations (Fig. 3). Indeed, examples of all of these scenarios can be found in threespine stickleback. Below we describe the primary population genomics approaches that have been taken to understand the genetics of adaptation in stickleback, highlight examples of the various genetic modes of parallel phenotypic evolution, and discuss how demographic and genomic conditions can facilitate repeated, rapid adaptation in this species. With the power of population genomics, threespine stickleback continue to reveal insights into the genetics of adaptation.

2 Identifying Functional Loci in Stickleback

The advent of molecular population genetics has enabled direct investigations of important factors in the evolution of threespine stickleback and the relationships among genotype, phenotype, fitness, and the environment (Hendry et al. 2013). Two broad areas of focus have been most widely applied to understand the genetic basis of adaptation: first, genetic mapping of traits—identifying loci in the genome that explain some proportion of variation in a particular phenotype, directly linking genotype to phenotype. Second, genome scans for selection or genotype–environment association (GEA)—identifying loci that show either evidence of a response to selection or correlation with environmental variables in natural populations, linking genotype to fitness or the environment. Mapping studies can be grouped as traditional genetic mapping approaches, which use a laboratory cross of individuals with divergent phenotypes and identify marker loci that segregate with phenotypic variation, termed quantitative trait loci (QTL), and genome-wide association studies (GWAS), which identify associations between marker loci and phenotypic variation in an outbred population (Wellenreuther and Hansson 2016). Genetic markers, such as microsatellites, can be used for traditional mapping because the relatively large linkage blocks present in a laboratory cross can be genotyped with fewer markers. However, GWAS, genome scans for selection, and GEA require larger numbers of

markers to survey the entire genome, because they use information from outbred populations in which linkage blocks are much smaller and a higher density of markers is required to detect functional loci.

2.1 Mapping

Accordingly, the first major insights into the genetic basis of parallel adaptation in stickleback grew out of traditional mapping studies using laboratory crosses of phenotypically divergent individuals. For instance, Peichel et al. (2001) created a linkage map based on 227 informative microsatellite markers and used it to map traits involved in benthic–limnetic differentiation in freshwater stickleback in British Columbia. Shapiro et al. (2004) used an overlapping set of markers to link the pelvic armor phenotype to the gene *Pitx1*, which also affects hind limb development in mice. Other genes identified in QTL studies of stickleback are also known to have similar functions in widely divergent model organisms (Miller et al. 2007). The well-studied lateral plate phenotype, which typically diverges rapidly between marine and freshwater habitats, was mapped to a single Mendelian locus of major effect and traced to the gene *Ectodysplasin* (*Eda*) in a series of microsatellite-based mapping studies (Colosimo et al. 2004, 2005; Cresko et al. 2004). Other studies have used microsatellite or single-nucleotide polymorphism (SNP) markers to identify QTL for multivariate phenotypes such as body shape (Albert et al. 2008; Liu et al. 2014), skeletal morphology (Kimmel et al. 2005; Miller et al. 2014), and pigmentation (Greenwood et al. 2011). The advent of genomics tools has greatly increased the number of genetic markers that can be efficiently genotyped in non-model organisms (Ellegren 2014; Kratochwil and Meyer 2015). This means that the density of markers possible across the stickleback genome with tools such as restriction site-associated DNA sequencing (RADseq) (Hohenlohe et al. 2010) is sufficient to identify the relatively small linkage blocks present in outbred populations (Hohenlohe et al. 2018). For instance, while much of the repeated evolution of lateral plate number in freshwater stickleback populations involves substantial reduction in number of lateral plates, it is quite rare for lateral plates to be lost altogether (Magalhaes et al. 2016). Mazzarella et al. (2016) used RADseq in a GWAS framework to identify the genetic basis of the plateless phenotype in Norwegian stickleback populations, finding this trait to be polygenic.

A few general conclusions can be reached about the genetic basis of phenotypic variation in stickleback from the results of QTL mapping studies. Not surprisingly, there are relatively few loci with a large effect on phenotypic variation and many more loci with small effect (Peichel and Marques 2016). QTL also appear to be clustered across the genome; for instance, chromosomes IV and XXI have a higher-than-expected number of QTL across phenotypic traits after accounting for chromosome size and gene number (Peichel and Marques 2016). Of course it should be noted that the phenotypes that have been the subject of QTL mapping studies in stickleback are not a random sample of variable phenotypes but instead are focused

on ecologically important traits and those that exhibit parallel evolution across stickleback ecotypes.

To compare across replicate natural populations, phenotypic variance can be attributed to multiple loci and their proportional effect quantified. The growing number of comparable mapping studies allows for meta-analysis of the degree of overlap in loci that contribute to parallel phenotypic evolution (Conte et al. 2012; Peichel and Marques 2016). For instance, in a pair of comprehensive QTL mapping studies of parallel evolution in benthic freshwater sticklebacks in British Columbia, Conte et al. (2015) and Erickson et al. (2016) mapped multiple phenotypic traits and compared overlap of QTL across lakes. Traits included body shape morphometrics and skeletal meristic traits, including lateral plates, and the authors genotyped a large number of SNP markers in each case. Again the results were mixed (Fig. 4); Conte et al. (2015) found that at nearly half of the QTL, alleles showed the same association with the same trait in the same direction. At other loci, genotype was associated with the trait in one lake but not the other. At a smaller number of QTL, the genotype was associated with the same phenotype in both lakes but in opposite directions, suggesting different patterns of linkage disequilibrium between the marker and the causative mutation. Erickson et al. (2016) found that just over half of the QTL were unique to one of the three lakes tested, although some loci were significant for all three.

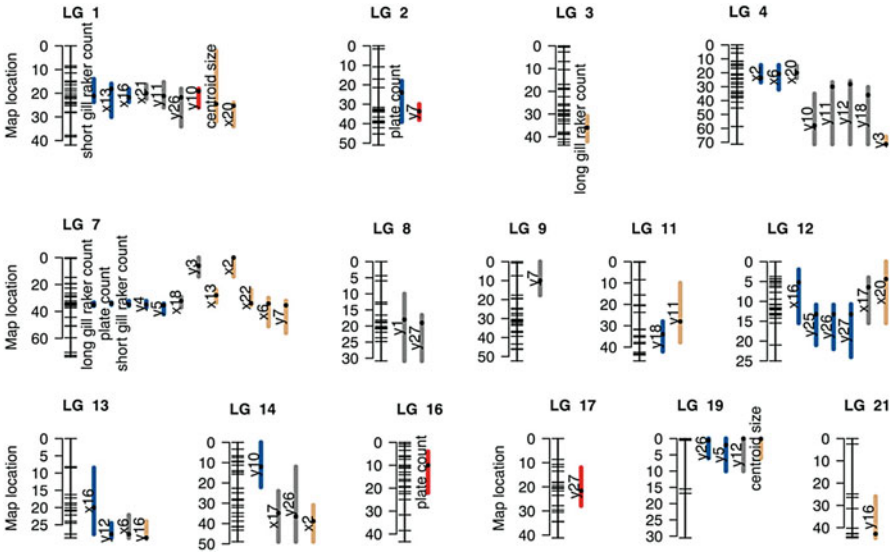

Fig. 4 Overlapping QTL associated with phenotypic differentiation between benthic and limnetic stickleback forms in two lakes in British Columbia. Only linkage groups (chromosomes) on which QTL were found are shown, and the trait is given next to each QTL. Blue indicates parallel effects in both the lakes; gray indicates effects in one lake but not the other; red indicates effects in opposite directions in each lake; tan indicates QTL for which two or more models cannot be distinguished. Reproduced with permission from Conte et al. (2015)

To extend from QTL mapping to selection and parallel evolution in nature, QTL identified in a laboratory cross can be tested for association with the same phenotype or signatures of selection across multiple natural populations, using targeted marker genotyping or sequencing. Examples can be found between the ecotypes of estuarine and freshwater (Raeymaekers et al. 2007), lake and stream (Berner et al. 2014), or benthic and limnetic comparisons (Erickson et al. 2016). The results are mixed. Many QTL in these studies do not show consistent patterns across populations, suggesting that parallel phenotypic evolution is not attributable to divergence at these loci. However, many other QTL, particularly those with large phenotypic effect, do show consistent association with particular phenotypes or signatures of selection across populations. Perhaps the most striking and well-studied example is *Eda*, discussed in more detail below.

2.2 Genome Scans for Selection

Genome scans for selection assess the patterns of allelic variation, haplotype structure, and other features across populations to identify signatures of natural selection acting on the genome (Fig. 5). Genome scans in threespine stickleback have made use of both reduced representation genomic sequencing techniques like RADseq (e.g., Hohenlohe et al. 2010; Liu et al. 2018) and whole-genome sequencing (e.g., Jones et al. 2012). These genomics techniques provide a dense set of markers across the genome. When placed on the periodically improving stickleback genome assembly (Glazer et al. 2015), a genomic set of markers can identify significant genomic regions either by finding clusters of significant markers such as SNPs or by using sliding window analyses (e.g., Fig. 5d shows both individual SNPs and a smoothed sliding window average), and then candidate genes can often be identified in the chromosomal neighborhood of such significant regions. This illustrates the value of a physical map of the genome in population genomics research (Luikart et al. 2018). Because of the role of adaptive divergence between habitats in stickleback evolution, genome scans for selection have most commonly searched for outlier loci—loci with differentiation (often quantified by F_{ST}) between populations significantly greater than the genome-wide background. Most commonly, these studies have tested for outliers between replicate habitat pairs such as marine–freshwater (Fig. 5b, e), lake–stream (Fig. 5c, d, f), or benthic–limnetic, while a few have focused on biotic factors such as the presence of prickly sculpin (*Cottus asper*), which is both a predator and a competitor (Miller et al. 2019).

Outlier-based genome scans do not directly indicate which phenotype or environmental variable is associated with the genetic signature of selection. In contrast, genotype–environment association (GEA) analyses specifically test for relationships between loci and specific environmental variables, such as temperature or salinity (Hoban et al. 2016). For example, Guo et al. (2015) surveyed 10 stickleback populations across temperature and salinity gradients in the Baltic Sea and used RADseq to genotype a large number of SNP markers. They identified several loci

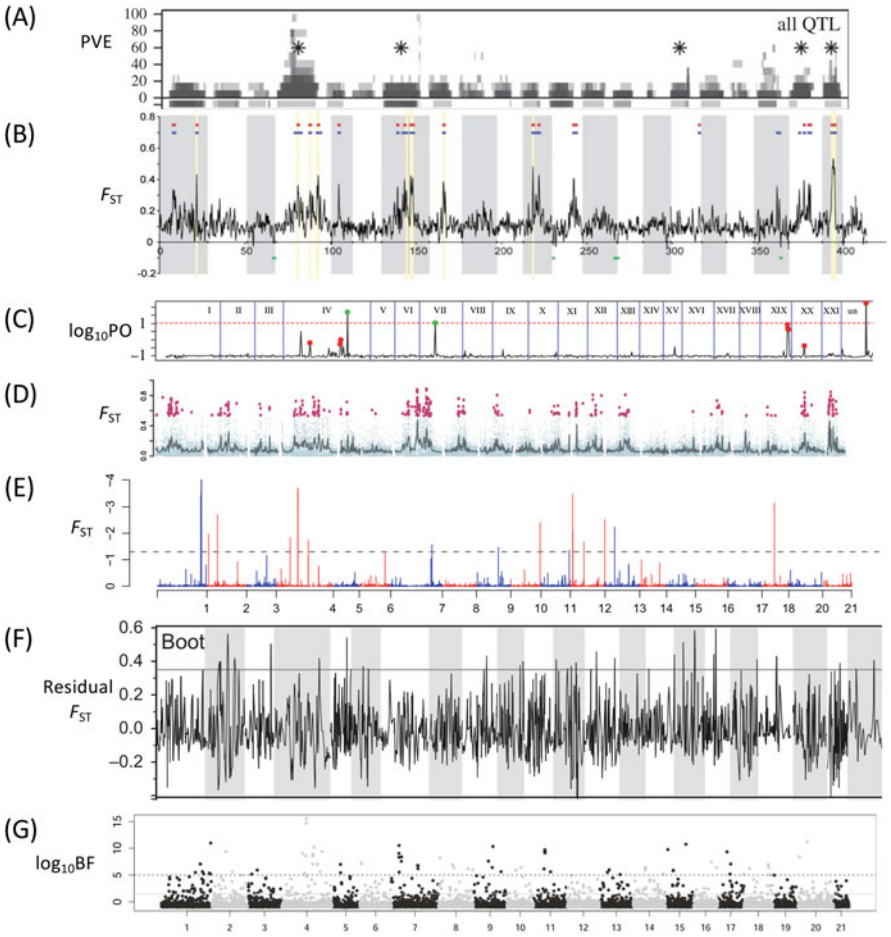

Fig. 5 Examples of genetic mapping and genome scan studies in threespine stickleback. In all cases, chromosomes I through XXI are aligned along the horizontal axis, and plots are rescaled to correspond with each other; (**b**, **c**, and **f**) also show unassembled scaffolds, and (**d** and **e**) have removed the sex chromosome XIX. (**a**) Percent variance explained (PVE) by QTL across multiple studies (Peichel and Marques 2016). (**b**) Differentiation (F_{ST}) between three independent freshwater and two marine populations in Alaska (Hohenlohe et al. 2010). (**c**) Significance of selection signatures in a Bayesian analysis (log of posterior odds, $\log_{10}$PO) in lake–stream comparisons in British Columbia (Deagle et al. 2012). (**d**) Differentiation (F_{ST}) between lake and stream populations in Alaska (Feulner et al. 2015). (**e**) Differentiation (F_{ST}) between marine and freshwater populations in Denmark (Liu et al. 2018). (**f**) Differentiation (residual F_{ST}) between lake and stream populations in British Columbia (Roesti et al. 2012). (**g**) Genotype–environment association with salinity (log of Bayes factors) in populations across the Baltic Sea (Guo et al. 2015)

across the genome associated with each of these environmental variables (Fig. 5g), including several that matched outlier loci found in previously published genome scans of marine–freshwater comparisons. Rennison et al. (2019) combined a genome scan for outlier genomic windows of differentiation between lake and stream populations with GEA methods to detect associations between genotype and both environmental variables and morphology. Multiple genomic regions were associated with lake–stream differentiation, abiotic environmental factors, diet, and morphology, and these regions exhibited some clustering on particular chromosomes, such as IV and VII (as evident in Fig. 5). However, while there was some overlap among categories, it was not significant at a genome-wide scale. Less overlap in genomic regions associated with adaptation to salinity was observed between threespine and ninespine stickleback (*Pungitius pungitius*) (Raeymaekers et al. 2017).

The large and growing number of mapping and genome scan studies in stickleback allows for comparative meta-analyses. Multiple studies have identified concentrations of functional loci in the stickleback genome (Peichel and Marques 2016). For instance, note the prevalence across the studies in Fig. 5 of significant loci on chromosome IV, which is the chromosome containing *Eda*. Many of the same tools have been shared by the stickleback research community and applied across QTL mapping, genome scan, and GEA studies, allowing direct comparison of the same loci across a large number of populations and studies. For instance, a largely overlapping set of microsatellite loci has been used following Peichel et al. (2001) (Table 1). Most RADseq-based studies, starting with Hohenlohe et al. (2010), have applied a similar protocol even down to the restriction enzyme used (SbfI), which again means that a common set of loci are interrogated. As whole-genome sequencing becomes more prevalent (Jones et al. 2012), this pattern of comparability among studies continues.

3 Population Genomics of Parallel Adaptation

3.1 Shared Variation at a Gene of Major Effect

The best-known example of a gene of major effect in threespine stickleback is *Ectodysplasin* (*Eda*), associated with the repeated reduction in lateral plates commonly seen in adaptation to freshwater habitats. A region of linkage group IV was first linked to the lateral plate phenotype by genetic mapping (Colosimo et al. 2004; Cresko et al. 2004). Laboratory complementation studies established that the same gene was involved in parallel adaptation to freshwater habitats (Cresko et al. 2004), and fine-scale mapping and sequencing determined that repeated evolution of the low-plated phenotype resulted from a shared allele at *Eda* estimated to be 2 million years old (Fig. 6; Colosimo et al. 2005). Further work established that selection on the *Eda* region, particularly evidenced by elevated differentiation at this locus between marine and freshwater populations, was widespread across the Atlantic (Mäkinen et al. 2008) and Pacific Ocean basins (DeFaveri et al. 2011). Subsequent

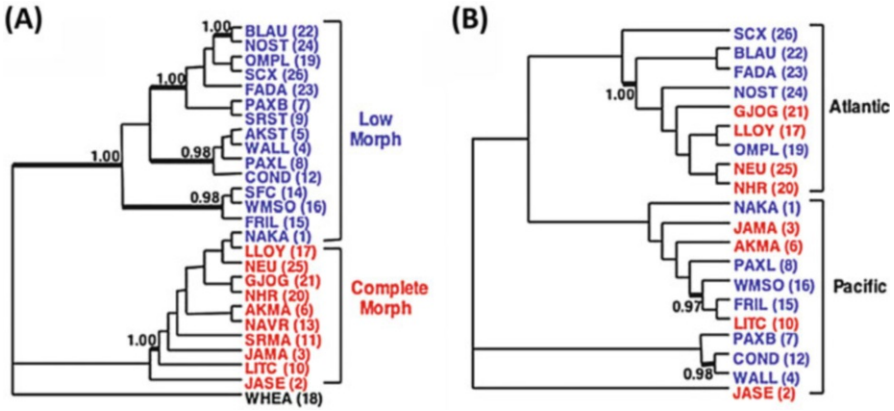

Fig. 6 Widespread reuse of a shared haplotype at the *Eda* gene across the threespine stickleback range in adaptation to freshwater habitats. (**a**) Relationships among sequences at *Eda* show two distinct clades corresponding to lateral plate morph, and the "low morph" allele is shared across ocean basins. (**b**) Relationships among sequences at an unrelated locus reflect geographic region rather than plate morph. Reproduced with permission from Colosimo et al. (2005)

genome scans for selection between marine and freshwater populations have found elevated genetic differentiation around *Eda* across the range of the species (Hohenlohe et al. 2010; Jones et al. 2012; Terekhanova et al. 2014; Ferchaud and Hansen 2016). Roesti et al. (2015) also found elevated differentiation in the chromosomal region around *Eda* in lake–stream comparisons, also corresponding to differences in lateral plate phenotypes.

The repeated use of a shared, ancient allele at *Eda* in lateral plate reduction demonstrates that this parallel evolution relies on standing genetic variation present in the marine population (Fig. 3a). Evolution from standing genetic variation can be remarkably rapid, occurring over just a few decades (Lescak et al. 2015; Marques et al. 2018), demonstrating the strength of selection in newly colonized freshwater habitats. However, the situation is more complex than it may seem. Selection for reduced lateral plates has been linked to predation by insect larvae in freshwater (Marchinko 2009), but *Eda* haplotypes also appear linked to growth rate (Barrett et al. 2008, 2009a), behavior including propensity to migrate between saltwater and freshwater habitats (Barrett et al. 2009b), and immune system function (Robertson et al. 2017). In some cases, for example in northern Europe, it appears that standing variation at *Eda* is not available, and a similar phenotype is achieved by reduction in lateral plate size (Leinonen et al. 2012) or by genetic changes at other loci (Pujolar et al. 2017) (Fig. 3c, d).

3.2 Independent Mutations at a Large-Effect Gene

In contrast to the ancient *Eda* haplotype clade shared in stickleback populations across the species range, a different major-effect locus shows evidence of repeated independent mutations with similar phenotypic effects (Fig. 3b). The pelvic girdle and spines found in marine stickleback led to the genus name *Gasterosteus* ("bony stomach"), but like the lateral plates, pelvic armor is often reduced in the adaptation of stickleback to newly colonized freshwater habitats from ancestral marine populations (Bell and Foster 1994). Also like lateral plates, loss of pelvic armor may be linked to changes in vertebrate and invertebrate predators as well as calcium ion availability in the different habitats (Shapiro et al. 2004). Laboratory crosses found pelvic armor to be a Mendelian trait, and genetic mapping traced the variation to the *pituitary homeobox transcription factor I* (*PitxI*), which is remarkable because of this gene's role in hind limb development in mice (Cresko et al. 2004; Shapiro et al. 2004). Stickleback with reduced pelvic structures exhibited reduced expression of *PitxI* in pelvic precursor tissue during development (Shapiro et al. 2004), and *PitxI* expression was implicated in pelvic reduction in both Atlantic and Pacific stickleback populations (Cresko et al. 2004; Shapiro et al. 2004; Coyle et al. 2007).

Chan et al. (2010) tested allele-specific expression patterns in F1 crosses and used fine-mapping and transgenic techniques to determine that multiple independent deletion mutations in a tissue-specific enhancer of *PitxI* had resulted in the parallel phenotype of loss of pelvic structures. These mutations were positively selected during adaptation to freshwater habitats, meaning that parallel evolution in this case was driven by independent mutations that nonetheless had very similar genetic mechanisms leading to similar phenotypes. This genomic region appears to be particularly prone to a high rate of double-stranded DNA breaks and deletion mutation (Chan et al. 2010; Xie et al. 2019), so this is an example of mutational bias facilitating parallel evolution (Rosenblum et al. 2014).

3.3 Polygenic Adaptation

Single genes of major effect on an important phenotype, such as *Eda* and *PitxI*, may be more the exception than the rule in adaptive evolution of stickleback. A large number of QTL mapping studies and genome scans have generally identified a larger number of loci contributing to phenotypic variation and adaptation. In their review of QTL studies, Peichel and Marques (2016) found a similar pattern of few genes of large effect and many genes of smaller effect, and this pattern held across traits related to feeding, body shape, defense, and other categories. Similarly, genome scans for local adaptation typically identify multiple outlier loci or loci associated with environmental variables (Fig. 5). While there may be some clustering of genes that contribute to ecologically relevant phenotypes, multiple genes across multiple chromosomes still contribute to phenotypic variation that is under selection during

colonization of novel habitats. This is particularly true for complex, multivariate traits, for example, the body shape differences that play an important role in benthic–limnetic divergence (Schluter 1993; Erickson et al. 2016).

4 Mechanisms of Rapid, Parallel Evolution

4.1 Recurrent Colonization and Standing Genetic Variation

Several factors may play a role in the remarkable parallel evolution observed in stickleback. As described above, parallel phenotypic evolution in stickleback appears to rely on a mix of independent mutations that produce similar phenotypes and shared ancestral variation that is subject to repeated selection in independent populations. Evolution from standing genetic variation can occur quickly because there is no waiting time for mutations to appear, and so the remarkably rapid evolution observed in some stickleback populations (e.g., Lescak et al. 2015; Marques et al. 2018) depends on selection acting on existing alleles.

Schluter and Conte (2009) proposed the "transporter hypothesis" to explain this phenomenon, named for the transporter in the television series *Star Trek*, in which humans or objects could be disintegrated in one place and transported to another location, where they are then rapidly reassembled. Under this model, adaptation to freshwater habitats involves alleles at multiple loci affecting traits such as morphology, life history, and behavior, so that a freshwater-adapted genotype is a multi-locus combination. Because most marine stickleback are either anadromous or breed in estuarine or coastal habitats, despite some reproductive isolation, there is still opportunity for gene flow between freshwater and marine populations. This will carry freshwater alleles into the marine population, where the multi-locus, freshwater-adapted genotypes will be broken up by recombination in subsequent generations and exist at low frequency, potentially subject to negative selection. Nonetheless, colonization into new freshwater habitats will carry some of these alleles, where they will again be favored by selection. The actions of selection and recombination will reassemble the multi-locus freshwater genotype, and the transporter process is then complete. The rapid evolution observed in stickleback from marine to freshwater, and subsequently into multiple different freshwater ecotypes, relies on an ancestral marine population that is relatively old, large, and able to maintain high levels of genetic diversity, with repeated colonizations into relatively new freshwater habitats (Liu et al. 2016).

This demographic model of standing variation is consistent with several observations (Terekhanova et al. 2014; Marques et al. 2018; Haenel et al. 2019). For instance, the low-plated *Eda* haplotype that has contributed to parallel evolution is known to occur in marine stickleback, although at very low frequencies (Colosimo et al. 2005; Barrett et al. 2008). Many of the freshwater-adapted alleles, including *Eda*, are known to be very old and much older than many of the freshwater habitats in which stickleback are currently found, such as those that appeared only after the

Pleistocene glaciation (Nelson and Cresko 2018). In fact, variants that characterize marine–freshwater divergence average several million years old, suggesting that they have persisted through multiple recurrent cycles of selection in freshwater habitats and gene flow back into the marine population (Nelson and Cresko 2018). Roesti et al. (2014) described a characteristic pattern of genomic variation around these recurrently selected loci, a peak-valley-peak pattern of F_{ST}, which is predicted based on population genomic models and observed in marine–freshwater stickleback comparisons. Barrett et al. (2009b) even found the intriguing result that the *Eda* haplotype is associated with migration behavior, facilitating the movement of this freshwater-adapted allele back into the marine population where it can contribute to subsequent freshwater colonization. Finally, the results described above in which multi-trait parallel evolution tends to involve a mix of shared and non-shared variation among derived populations are also consistent with the transporter model. Because freshwater-adapted alleles are at low frequency in the marine ancestor, each new colonization of freshwater habitat may by chance include some and not others in the founding individuals, and this will drive the degree of parallelism (Leinonen et al. 2012; Pujolar et al. 2017).

4.2 Genomic Mechanisms

Several genomic features may also facilitate parallel evolution in stickleback by making it easier for a population to respond to selection in a newly colonized habitat. Multi-locus genotypes can be maintained in several ways, so that selection does not need to act independently on each locus; instead, if multiple favored alleles co-occur in individuals, selection on multiple phenotypic traits can act synergistically. First, for a few generations after gene flow from a derived (e.g., freshwater) population back into the ancestral (e.g., marine) one, freshwater-adapted alleles will continue to be in linkage disequilibrium (LD) with each other, meaning that they are statistically more likely to co-occur than the expectation based on their frequencies in the population. Even when they are on different chromosomes with free recombination between them, LD between these alleles decays in an exponential process, and there is evidence that freshwater-adapted alleles exist to some extent in LD with each other in the marine stickleback population even across chromosomes (Hohenlohe et al. 2012). Within chromosomes, many freshwater alleles appear clustered as described above, which will reduce the recombination rate between them and allow LD to persist for longer periods. In fact, especially with the recurrent colonization of the transporter model, there is a theoretical reason to expect that these loci will become clustered over evolutionary time (Yeaman 2013). Rates of recombination also vary across the genome, so that freshwater alleles that are co-localized in regions of low recombination will be maintained longer in LD, and indeed several authors (Roesti et al. 2013; Marques et al. 2016; Samuk et al. 2017) have found that regions of low recombination contribute to rapid adaptation. Finally, chromosomal inversions greatly reduce the rate of recombination, and there is ample evidence that inversion

polymorphisms are common in stickleback and that they contain clusters of functionally important loci contributing to parallel adaptation (Jones et al. 2012; Feulner et al. 2013; Bassham et al. 2018). All of these genomic features suggest that the transporter model of rapidly reassembling multi-locus genotypes adapted to a newly colonized habitat is not as unlikely as it may seem.

5 Conclusions: The "Everyone Wins" Principle of Biology

Threespine stickleback have become a model system in evolutionary biology and population genomics for a number of reasons. Aside from being amenable to genetic and laboratory studies, they exhibit remarkable patterns of parallel evolution across a number of phenotypic and environmental axes at multiple spatial and temporal scales, giving biologists a wealth of replicate natural experiments to investigate. Stickleback have been the focus of multiple genome scans for selection, using reduced representation sequencing approaches like RADseq, helping to refine these techniques and improve their applicability to non-model taxa (Jensen et al. 2015). Stickleback also provide an example for how genome scans can be extended with specific data on environmental variables, ecology, or specific phenotypes (Haasl and Payseur 2015). Although the taxonomy of stickleback ecomorphs may continue to be a source of debate, the divergence of stickleback forms has informed our understanding of speciation processes (Schluter 2009; Hendry et al. 2013; Lackey and Boughman 2017).

A large number of studies have identified the genetic mechanisms of parallel evolution in threespine stickleback, ranging from single genes of major effect to highly polygenic phenotypes, from shared variation to novel mutations, and from single-nucleotide changes to structural variations such as inversion polymorphisms. This leads to what we might call the "Everyone Wins" principle: When multiple plausible mechanisms are proposed to explain some biological pattern, it is likely that all of them play a role in at least some instances, and further they are likely sometimes to co-occur with interesting and important interactions. Threespine stickleback exemplify this view, as they show examples of nearly all the mechanisms of parallel evolution proposed (Rosenblum et al. 2014; Bolnick et al. 2018). This is certainly in part because of the research effort that has been directed toward this taxon, but we suggest that the Everyone Wins principle is more generally an inherent outcome of the complexities of biological systems.

6 Future Directions

Threespine stickleback are likely to continue to be a valuable evolutionary model system. As the costs of DNA sequencing continue to drop, we anticipate that more studies will use whole-genome sequencing rather than the reduced representation

approaches that have been applied. To date, whole-genome sequencing has been used on a relatively small number of representative individuals (e.g., Jones et al. 2012; Liu et al. 2016), but it is now becoming feasible for studies that require genetic data on larger numbers of individuals across many populations. This will allow fine-mapping of causal variants in a single experiment, identification of genomic structural variation, and more. Because stickleback are relatively easy to work with in the laboratory, they are amenable to the ever-expanding toolkit of genetic manipulation and developmental and physiological studies. This will allow continued understanding of the mechanistic basis of stickleback phenotypes and direct linkages between mechanism and adaptation in natural populations.

A few avenues are promising for future work in threespine stickleback. Studies that explicitly combine data on genotype, phenotype, fitness, and the environment (e.g., Rennison et al. 2019) are best-suited to illuminate all the interactions among these factors and gain a comprehensive understanding. Expanding from DNA sequencing to transcriptomics to provide direct estimates of gene expression, as well as directly assessing the role of phenotypic plasticity, will further reveal important aspects of genetic variation (Morris et al. 2014). The role of epigenetics, particularly in rapid parallel evolution, is still relatively unknown but may be critical (Heckwolf et al. 2019). Behavior is a notoriously difficult phenotype to unravel, in part because of the potential roles of plasticity and epigenetics in addition to genetics, but stickleback are a tractable system for behavioral genomics, particularly for behaviors related to mate choice and parental care (e.g., Mobley et al. 2016; Stein and Bell 2019). Finally, the microbiome is a fairly unexplored area that may have a substantial impact on stickleback phenotypes and adaptation (Small et al. 2017; Steury et al. 2019).

These research directions will keep threespine stickleback relevant into the future for continuing progress in understanding the processes of evolution. Specific knowledge about the genetic modes of adaptation, such as the saltwater–freshwater transition, can be extended to related species, such as other fish taxa facing similar environmental challenges. More generally, because stickleback exhibit such a diversity of modes of adaptation across replicate populations, they will continue as a model for understanding the interactions among multiple genetic processes during adaptation to novel or recurrent environments.

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