ANNUAL REVIEWS

Annual Review of Genomics and Human Genetics

Threespine Stickleback: A Model System For Evolutionary Genomics

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Annu. Rev. Genom. Hum. Genet. 2021. 22:357–83

First published as a Review in Advance on April 28, 2021

The *Annual Review of Genomics and Human Genetics* is online at genom.annualreviews.org

https://doi.org/10.1146/annurev-genom-111720-081402

Keywords

behavior, rapid parallel adaptation, genetic architecture, host–microbiome interactions, parasites, standing genetic variation, vertebrate evolution, *Gasterosteus aculeatus*

Abstract

The repeated adaptation of oceanic threespine sticklebacks to fresh water has made it a premier organism to study parallel evolution. These small fish have multiple distinct ecotypes that display a wide range of diverse phenotypic traits. Ecotypes are easily crossed in the laboratory, and families are large and develop quickly enough for quantitative trait locus analyses, positioning the threespine stickleback as a versatile model organism to address a wide range of biological questions. Extensive genomic resources, including linkage maps, a high-quality reference genome, and developmental genetics tools have led to insights into the genomic basis of adaptation and the identification of genomic changes controlling traits in vertebrates. Recently, threespine sticklebacks have been used as a model system to identify the genomic basis of highly complex traits, such as behavior and host–microbiome and host–parasite interactions. We review the latest findings and new avenues of research that have led the threespine stickleback to be considered a supermodel of evolutionary genomics.

1. INTRODUCTION

Our understanding of the molecular basis of evolution is being revolutionized by increasing access to an ever-expanding number of genomic tools and resources (84, 100, 162). Vast strides are being made in addressing some of the most fundamental and long-standing questions in evolutionary biology. How does speciation occur, and how do new adaptations drive this process? What kinds of genetic changes (e.g., coding or regulatory changes, de novo or preexisting mutations, point mutations or structural variants) underlie morphological, physiological, and behavioral traits? Can we theoretically integrate observations of genetic and molecular mechanisms in the laboratory and in nature into generalized models of evolution? For several decades, *Gasterosteus aculeatus*, commonly known as the threespine stickleback, has been a valuable tool for behavioral and evolutionary research, but the emergence of versatile molecular methods in the past 20 years has facilitated its use to address previously intractable problems in adaptation, developmental evolutionary biology, and speciation.

The threespine stickleback is ancestrally a marine species of bony fish, but marine and anadromous (collectively referred to here as oceanic) populations have been colonizing fresh water for at least 10 million years (22). This species has most recently experienced a burst of rapid diversification by colonizing freshwater habitats that became available at the end of the Last Glacial Maximum (LGM) (~10–20 kya) (21). Through these numerous independent colonizations of freshwater habitats from marine waters, particularly in western North America (46), it has become evident that a shared pool of standing genetic variation has formed the basis for the reproducibility of phenotypes over such a short evolutionary timescale (84). Early studies extensively characterized the ecology, behavior, and diversity of traits of the many stickleback populations, building a foundation for subsequent investigation by an array of genetic and genomic tools that facilitated key discoveries across many biological fields (**Figure 1**).

Over the past 50 years, the threespine stickleback has provided insights to the fields of ecology, behavior, toxicology, vertebrate evolution, speciation, and developmental biology, contributions that have been extensively reviewed previously (6–10, 21, 23, 39, 68, 69, 75, 77, 104). However, in the past decade it has emerged as a particularly powerful model system in the field of evolutionary genomics (86, 87) (**Figure 1**). In this review, we describe the numerous attributes that

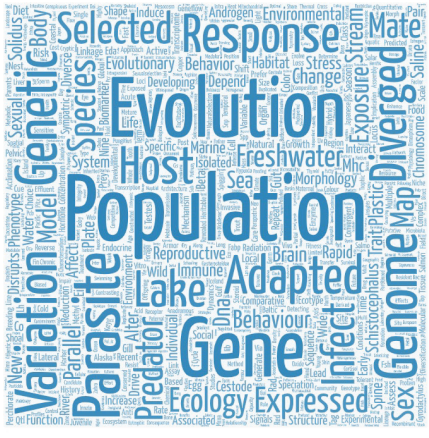

Figure 1

Word cloud constructed from a PubMed search of all titles containing "threespine stickleback" from 2000 to 2020. Figure created at **https://wordart.com**.

make the threespine stickleback an exceptional model system for studying vertebrate biology. We then present a detailed synthesis of recent research that has provided important insights into the genomics of repeated and rapid adaptation, and how the genomic architecture underlies the evolution of both simple and complex phenotypic traits. Finally, we describe how researchers are now using the threespine stickleback as a model system to understand the genomic basis of behavior and assess the interactions among hosts, their microbiomes, and their parasites.

2. THREESPINE STICKLEBACKS ARE A MODEL SYSTEM FOR VERTEBRATE BIOLOGY

For several decades, the threespine stickleback has been considered one of the foremost model systems for studying adaptation. This species has several attributes that make it a superb model organism: small body size, great abundance, wide geographic distribution, high fecundity, and a modest generation time (16, 21). Repeated derivation of freshwater populations from oceanic ones followed by local adaptation has created a large set of replicate natural experiments for adaptation to fresh water, which enables inference of adaptive versus neutral divergence, and numerous biological species have arisen within the nominal species *Gasterosteus aculeatus*. These repeated divergences have led to phenotypically distinct ecotypes associated with specific habitats. Sympatric and parapatric species pairs that repeatedly form include oceanic–freshwater, lake–stream, benthic–limnetic, and lava–mud, among others. This iterative evolution perplexed systematists (17) but makes the threespine stickleback desirable for analysis of evolutionary processes.

Although suitable for laboratory experiments, the threespine stickleback can also be used to study processes of vertebrate adaptation in a natural setting (62, 105). In particular, it has revealed the importance of standing genetic variation for rapid adaptation in vertebrate species compared with the prevalence of de novo mutations (10). However, when different ecotypes come into contact, they can produce viable offspring, which, for example, can occur naturally when oceanic sticklebacks (which are often anadromous) return to spawn in fresh water simultaneously with breeding freshwater sticklebacks. Prezygotic barriers to reproduction, such as habitat preference and mate choice, have been detected (103, 104), while hybrids between ecotypes are present at low frequencies, with studies showing likely selection against hybrids as a postzygotic barrier (57). However, even with continued gene flow, threespine sticklebacks do not often merge into hybrid swarms [although exceptions occur (157)], and the integrity of ecotypes is maintained and can arise again within decades when an ecotype is placed in a habitat better suited to another ecotype (1, 89, 90, 96). This feature of the threespine stickleback is particularly intriguing because it links phenotypic and heritable genetic variation to ecological variation, pointing to natural selection repeatedly driving adaptation to specific environments, thus providing evidence for the role of determinism in evolution (24, 103, 136). Ecotypes associate strongly with their respective habitat type, even when living sympatrically in the same lakes, as is seen, for example, in the case of benthic and limnetic ecotypes (37).

2.1. Practical Features of Threespine Sticklebacks for Experimentation

Threespine sticklebacks range in size from approximately 3 to 10 cm, with adult oceanic sticklebacks tending to be larger than freshwater sticklebacks (54). The conspicuous behavior of this species led to the development of husbandry methods and experimental protocols for behavioral traits in the mid-twentieth century, and interest in the inheritance of its adaptive variation led to the development of methods to cross and rear it in the laboratory (16, 154). Crosses can be performed in the laboratory via either natural matings or artificial fertilization (87), and sticklebacks

have short generation times, typically one to three years in the wild [although exceptions have been documented (54)] and approximately six months in the laboratory. Due to their large mean clutch sizes [ranging from 40 to 450 eggs laid after courtship (167), depending on individual female size and ecotype], they are practical for large genetic mapping studies, where two morphotypes are crossed to produce F1 and F2 progeny, among which segregating traits can then be followed. In addition, stickleback hatchlings are transparent, making them practical for monitoring and screening fluorescently tagged regions during development and for screening the development of organs and other traits after genome or microbiome manipulation (91). This species has also been established as a gnotobiotic organism, allowing for sterile rearing for host–microbiome studies (110). In addition, it is amenable to a range of experiments in laboratories, including parasite introductions (7), common garden experiments to evaluate phenotypic plasticity (118), and various behavioral assays (23, 164).

2.2. Linkage Maps and Quantitative Trait Loci

The recent productivity of the threespine stickleback as a model system is the result of considerable foresight by the stickleback research community in developing an array of genetic and genomic resources to take advantage of future technological advances that would emerge at the turn of the century. A pedigree-based linkage map based on approximately 200 microsatellite loci was developed in 2001 (125), and further maps have since been developed, the most recent of which was constructed using genotyping-by-sequencing methods containing tens of thousands of single-nucleotide polymorphisms (SNPs) (56, 128). These high-resolution maps have greatly facilitated the mapping of several quantitative trait loci (QTLs) from crossing divergent ecotypes, such as oceanic–freshwater, benthic–limnetic, lake–stream, and stream–limnetic, and even other stickleback species (e.g., *G. aculeatus* × *G. nipponicus*) (170), with more than 1,000 QTLs of varying effect sizes identified to date (123).

2.3. High-Quality Reference Genome

A proposal for sequencing the threespine stickleback genome was first formally described in a National Human Genome Research Institute white paper in 2003 (86). In it, David Kingsley identified many of the salient features that would motivate such a project, particularly to develop a better understanding of vertebrate adaptation. Favorable features to effectively sequence the genome included a fairly compact total genome size (~650 Mb) made up of 21 cytologically visible chromosomes, a relatively reduced repeat content, and an X–Y sex system. The first draft of the threespine stickleback genome, *gasAcu-1*, was first available from the National Center for Biotechnology Information in 2006 and was published in 2012 (84). The genome is based on paired-end Sanger sequencing of an inbred female from Bear Paw Lake, Alaska (and thus is a freshwater ecotype genome). The assembled genome consisted of approximately 450 Mb of sequence, and combining it with the previously determined linkage map (125) allowed the construction of a high-quality sequence build with 9× coverage and a scaffold N50 of >10 Mb, a substantially larger value than those of comparable teleost genomes (especially compared with other genomes available at the time). The genome assembly was further improved by utilizing a Hi-C-based proximity-guided assembly (126) and long-read Pacific Biosciences sequencing (112) from a benthic individual from Paxton Lake, British Columbia, leading to a fivefold improvement in continuity (76% of gaps filled) over the previous version (version 4) (126). This long-read Pacific Biosciences sequencing of a male stickleback from Paxton Lake combined with Hi-C proximity-guided assembly has allowed for the construction of an approximately 16-Mb

Y chromosome along with a likely candidate for a sex-determining gene, *Amhy* (124), providing a model system for studying the evolution of sex chromosomes.

3. THE GENOMIC BASIS OF PARALLEL ADAPTATION

3.1. The Chronology and Distribution of Oceanic and Freshwater Ecotypes

Oceanic (marine or anadromous) threespine sticklebacks are widespread across arctic, boreal, and temperate regions of the Northern Hemisphere, and freshwater populations occur in adjacent (plus slightly southward) low-elevation habitats (15, 21). Allopatric and parapatric stream and lake ecotypes are widespread within fresh water, while the well-studied sympatric benthic and limnetic species pairs in lakes are rare and confined to the Strait of Georgia near Vancouver, British Columbia (106) (**Figure 2*a***). Fossil threespine sticklebacks stretch back to at least 13 Mya (19, 22) and possibly 16 Mya (19, 22), with the earliest fossils appearing to originate in the Pacific, before appearing much later in the Atlantic (85). Fossil forms are within the phenotypic range of extant populations (but see 113), with fossils from marine deposits resembling extant oceanic sticklebacks, while those from freshwater deposits fall within the range of diverse freshwater populations (18, 155) (**Figure 2*b***). Given that most related species, such as the blackspotted,

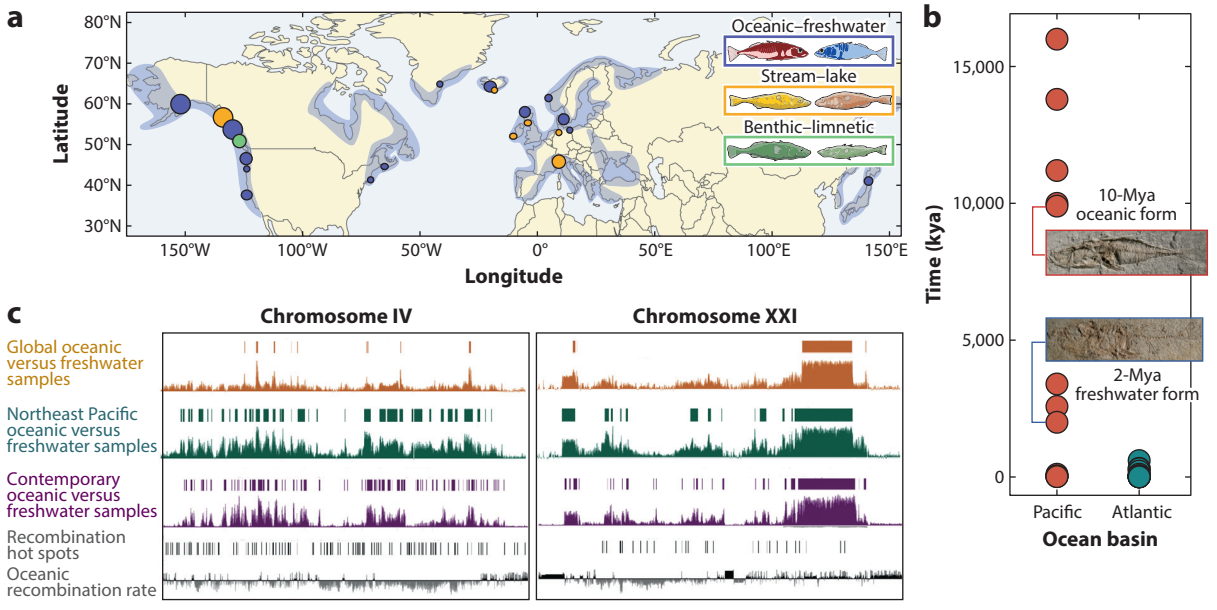

Figure 2

Threespine stickleback distribution, fossil record, and oceanic–freshwater diverging alleles. (*a*) Approximate circumpolar distribution of threespine sticklebacks (*light blue shading*). Circle sizes represent the number of studies done on parallel adaptation in a region, and box colors indicate the ecotypic pair compared. (Differing colors of fish in the boxes indicate ecotype.) There is a clear focus of studies in the northeast Pacific, with the major focus being oceanic–freshwater comparisons. (*b*) Time line of fossils identified in the stickleback species complex in the Pacific and Atlantic, showing that the earliest fossils are found in the Pacific; these fossil dates align well with phylogenetic evidence. Images of fossils show that even 2 Mya there were freshwater phenotypes circulating in the Pacific. Images provided by M.A. Bell. (*c*) Chromosomes IV and XXI from the stickleback genome sequence in the UCSC Genome Browser, showing genome-wide single-nucleotide polymorphism divergence among global oceanic and freshwater samples, northeast Pacific oceanic and freshwater samples, and three contemporary lake populations (10–30 years old). Tracks are from Roberts Kingman et al. (132), and vertical bars above each plot show the significant peaks. There are also tracks available for the recombination rate ($\log_{10}$ scale) and recombination hot spots (>20 times the background rate).

ninespine, and fourspine sticklebacks plus the closely related aulorhynchids (85), are predominantly or strictly marine fish, and that freshwater threespine sticklebacks occur in regions that they must have colonized from the ocean since deglaciation (15), the oceanic ecotype is considered the ancestral state of the threespine stickleback, persisting largely in the same phenotypic form since at least the Middle Miocene (21).

It is apparent that the center for stickleback diversity is the northeast Pacific and that sticklebacks colonized the Atlantic basin more recently. Despite an evolutionary history that spans millions of years, the time to the most recent common ancestor is estimated to range from 29.5 to 226.6 kya for the eastern and western Pacific anadromous sticklebacks and from 11.3 to 95.2 kya for the transatlantic population (48). These recent periods emphasize the importance of events following the LGM for understanding the extant stickleback distribution, with a natural history that indicates a major radiation following the continental deglaciation that resulted in thousands of independent freshwater colonization events by oceanic sticklebacks along coastal waters, rather than any single origin of freshwater ecotypes throughout the Northern Hemisphere (21).

Indeed, the observation of repeated adaptation across multiple timescales is the main reason why threespine sticklebacks have garnered such interest from evolutionary biologists, providing a unique opportunity to investigate the mechanisms underlying parallel evolution in a vertebrate species in the wild. Much attention has been given to the evolution of specific traits that consistently change once oceanic sticklebacks become isolated in fresh water, such as the reduction of defensive traits (bony lateral armor plates and spines) and shifts in feeding morphology traits (gill raker length and number) (63, 70). This has naturally led to an interest in understanding the genetic basis for the repeated convergent evolution of these diverse freshwater phenotypes, forming the core of genomic research into threespine sticklebacks over the past decade.

3.2. Repeated and Rapid Genomic Freshwater Adaptation from Standing Genetic Variation

The first hint of the genetic basis for repeated freshwater adaptation was the observation that parallel evolution of reduced lateral armor plate phenotypes in freshwater populations worldwide is driven by reuse of a single ancient (>2 million years old) clade of haplotypes at the *Ectodysplasin* (*Eda*) gene (35, 36, 38). This now classic result provided the initial evidence that the repeated evolution of freshwater phenotypic traits was the result of standing genetic variation that exists at low frequency in oceanic populations, rather than numerous de novo mutations occurring independently in each new freshwater population (35). Shortly following the *Eda* discovery, a similar pattern of allele reuse near the *Kitlg* gene was found to be responsible for a lighter skin pigmentation in some freshwater populations (108).

The phylogenetic pattern observed at *Eda* and *Kitlg*, where freshwater populations show significant genetic differentiation from oceanic ecotypes regardless of geographic location (i.e., parallel sequence divergence), became a general framework for future genome-wide studies. In particular, building on observations from a localized low-resolution restriction site–associated DNA sequencing (RAD-seq) study (74), Jones et al. (84) provided the first comprehensive look at the genomic landscape of freshwater adaptation using low-coverage (~2×) whole-genome sequencing of 10 geographic pairs of oceanic and well-established adjacent freshwater stickleback populations distributed worldwide. The authors found more than 150 distinct genomic regions (hereafter termed divergent loci) encompassing approximately 1.2 Mb that showed a clear pattern of allele reuse among freshwater stickleback across the species range, thus implicating adaptation from multiple standing genetic variants as the primary mechanism for repeated freshwater adaptation since at least the LGM (84).

More recently, expanded sample sizes (46, 132) have shown that while substantial freshwater-adaptive standing genetic variation is shared globally, the northern Pacific contains five times as many divergent standing genetic variant loci as other populations elsewhere in the Northern Hemisphere, comprising seven times as much of the genome (**Figure 2*c***). Simulations by Fang et al. (46) suggest that a sizable proportion of freshwater-adaptive alleles may have been stochastically lost as sticklebacks expanded out of the Pacific following the LGM. Thus, northern Pacific sticklebacks—the populations on which most studies have focused (**Figure 2*a***)—likely have a greater potential for repeated adaptation to fresh water than those in the Atlantic basin (88).

The reliance on standing genetic variation also explains why the adaptation of threespine sticklebacks to fresh water is not only repeated but also rapid (see the sidebar titled Adaptation from Standing Genetic Variation). Though Jones et al. (84) focused on genetic variation in freshwater populations established by colonization up to thousands of years ago, freshwater populations founded recently by anadromous sticklebacks show a remarkable ability to evolve the same freshwater ecotype within decades (particularly in the northern Pacific), exhibiting what is termed contemporary evolution (1, 11, 20, 90). These newly evolved freshwater populations exhibit significant genomic changes relative to known or putative anadromous ancestors at genomic locations that overlap almost exclusively with the divergent loci used repeatedly by established freshwater populations (11, 90, 158) and often include QTLs for phenotypes that are divergent between oceanic and freshwater stickleback (see below).

ADAPTATION FROM STANDING GENETIC VARIATION

Population genetic theory has traditionally focused on how adaptation proceeds after the occurrence of new (de novo) beneficial mutations. However, it is becoming increasingly clear that mutations may be carried for millions of generations as rare, neutral, or even deleterious variants, known as standing genetic variation, and can also become important for adaptation to different conditions (10). Standing genetic variation seems to be important to allow species to respond quickly to selection, with examples ranging from the adaptive radiation of cichlid fishes in the African Great Lakes (100) to the formation of modern dog breeds (121). There is particular interest in how standing genetic variation may contribute to human evolution (127), for which there is little evidence for classic selective sweeps associated with positive selection on de novo mutation (72).

There are three primary explanations for why standing genetic variation may outperform de novo mutations in these circumstances. First, standing genetic variation already exists and therefore can begin acting immediately; the waiting time for a de novo mutation is eliminated, which can be particularly important in species with the effective population sizes that characterize vertebrate species. Second, while de novo mutations appear as a single copy in the population and are likely to be lost by genetic drift, standing genetic variation can be present at increased frequencies at the onset of selection, providing a major boost in fixation probability, especially for alleles with smaller fitness effects (71). And third, standing genetic variation can be old and may have been tested previously by natural selection under similar conditions. Thus, advantageous standing genetic variation alleles with large effects would be more likely than de novo mutations under Fisher's geometric model to reach a fitness optimum without overshooting the fitness peak. In addition, older standing genetic variation has the potential to be refined over time, continuously accumulating new mutations of small effect to create haplotypes of large effect overall (102). All of these factors appear to be part of the standing genetic variation package that drives parallel evolution in threespine sticklebacks, thus providing essential real data for comparison with an increasing body of theoretical work on standing genetic variation (99, 160).

Increasing the temporal resolution even further, Roberts Kingman et al. (132) recently performed a high-coverage, large-sample-size genomic analysis of pooled DNA samples (Pool-seq) from three young (<35 years old) freshwater populations in Alaska that had been founded by anadromous sticklebacks (one naturally, two experimentally) and sampled annually since founding, providing a unique time series of phenotypic and genomic contemporary evolution. As in previous studies (11, 61, 90, 96, 97, 115), this study found that almost all loci demonstrating significant allele frequency change across this time series overlapped with divergent loci identified in established freshwater populations, particularly from the northern Pacific (**Figure 2*c***). Perhaps even more strikingly, it found that most freshwater-adaptive alleles (>300, depending on the criteria used) increased from a frequency of less than 1% in the founding anadromous population to more than 50% within just eight years after freshwater colonization, resulting in a mean selection coefficient estimate of 0.3 across loci (5th–95th percentile 0.08–0.53), similar to the estimate for *Eda* (143). This is an incredibly high range for a vertebrate species, though it should be noted that individual alleles among the stickleback standing genetic variation likely do not affect fitness independently, but rather reinforce each other via complex interactions, including additive or epistatic fitness effects and haplotype linkage (132).

3.3. Other Molecular Mechanisms Driving Contemporary Evolution

Because of such repeated and rapid adaptation, the threespine stickleback has emerged as the premier model system for studying the importance of standing genetic variation in evolution. Indeed, as genomic tools have become increasingly universal, standing genetic variation is emerging as a critical mechanism for rapid change in a host of other species, including other fishes (like cichlids), plants, and possibly humans (100 129, 162).

However, while adaptation from standing genetic variation is clearly the dominant mechanism in threespine sticklebacks, this does not entirely preclude the contribution of de novo mutations, especially within the context of structural variation. For example, the repeated pelvic reduction in freshwater ecotypes is often due to independent deletions of the pelvic enhancer of *Pitx1* (32, 147, 169). These deletions are located within a fragile genomic region with long TG-dinucleotide repeats in the telomeric region of chromosome VII (169). In this example, the occurrence of a deletion at this fragile site was estimated to be approximately 10^4 times more likely than a point mutation and thus may point to a type of alternative mechanism to standing genetic variation for repeated and rapid freshwater adaptation, though a genomic survey of the general importance of this type of mutation has been hampered by difficulties in sequencing through repetitive regions (and thus is an area where future long-read sequencing may prove particularly useful). Similarly, dynamic copy number variation and movement of transposable elements may provide another pathway for repeated freshwater adaptation (31), and Lowe et al. (94) found consistent differences in copy number variation between oceanic and freshwater ecotypes using the same stickleback genomes sequenced by Jones et al. (84). However, the extent to which such variation actually contributes to repeated stickleback adaptation as opposed to neutrally hitchhiking on adaptive haplotypes is still uncertain.

3.4. The Transporter Model for Multiallele Reassembly in Freshwater

Given how fundamental standing genetic variation is for stickleback evolution, an obvious question arises: How has such a large pool of freshwater-adaptive standing genetic variation been conserved within oceanic populations, such that the same alleles that were fixed during colonization of fresh water immediately following the LGM can also be taken from oceanic populations to

found new freshwater populations today? The observation that the *Eda* freshwater allele appeared to be largely recessive for the low-armor-plate phenotype and was found at very low frequencies in multiple samples from oceanic populations (35) suggested a general model in which freshwater-adaptive alleles could be acquired by oceanic populations and persist as low-frequency heterozygotes without major deleterious fitness consequences. Subsequent F1 crosses between lake and anadromous sticklebacks suggested that many freshwater phenotypes are recessive to their typical homologs in oceanic stickleback (20).

Schluter & Conte (142) provided a more complete demographic explanation for the maintenance of freshwater-adaptive standing genetic variation, which they termed the transporter hypothesis, and which has since become the dominant model for contextualizing patterns of genomic divergence. Under this model, a key consideration is not only the establishment of freshwater populations from oceanic populations but also constant gene flow from freshwater populations back into the oceanic populations. Starting from the position that all the underlying genetic variants responsible for freshwater adaptation exist (meaning that the model does not deal with the generation of new freshwater adaptive alleles), a network of established freshwater populations are continuously recycling freshwater-adaptive alleles (many of which will be linked) via gene flow into oceanic populations. Once in the marine environment, such alleles are no longer advantageous (and are perhaps even deleterious), creating a gene flow–selection equilibrium frequency and resulting in recombination that breaks apart multiallelic haplotypes and disperses these smaller freshwater-adaptive alleles among individuals as low-frequency standing genetic variation. Single oceanic sticklebacks will be unlikely to possess many freshwater-adaptive alleles, but many will have a few. Upon colonization of new freshwater habitats by oceanic sticklebacks, directional selection favoring freshwater-adaptive phenotypes will cause the underlying alleles from standing genetic variation to increase in frequency, resulting in rapid reassembly of the original multiallelic haplotype, with linkage between alleles reinforcing any effects on fitness. Indeed, many loci with freshwater-adaptive alleles occur on a few chromosomes (83, 132) (e.g., 20% of all divergent loci are found on chromosome IV), facilitating rapid adaptation after oceanic sticklebacks colonize fresh water (20).

The most thorough exploration of the transport model to date was by Galloway et al. (53), who used forward simulations at genome-wide scales to demonstrate that realistic parameters can largely recapitulate the rapid and repeated freshwater adaptation observed in the threespine stickleback system. One of their findings was that even in newly established freshwater populations with continuous gene flow from oceanic populations, it was the initial oceanic colonizers that contributed most of the alleles relevant for the subsequent freshwater adaptation to lakes. The prevalence and identification of freshwater-adaptive alleles in founding oceanic individuals are clearly the key to the extent and rate of adaptation in new freshwater environments. RAD-seq analysis of ~50-year-old Alaskan freshwater populations and their likely oceanic ancestor suggests that the presence of so-called jackpot carriers among founders (i.e., recent descendants of freshwater–oceanic hybrids possessing multiple freshwater-adaptive alleles) may be an important element for rapid adaptation (11), rather than many oceanic founders needing to possess a few freshwater alleles, which may take longer to reassemble into genome-wide freshwater haplotypes.

3.5. Genomic Features of Standing Genetic Variation in the Transporter Model

While Schluter & Conte's (142) model provided a general framework for the demographics of stickleback evolution (20), it was proposed before the evolutionary, genomic, and sequence features of such standing genetic variation were known. One notable element is that even though most of the freshwater populations present today are younger than the LGM, the haplotypes

found at divergent loci are millions of years old and at least approximately twice as old as the rest of the genome (115, 132). In addition, recent work has shown, somewhat surprisingly, that the oldest divergent regions tend to be larger than the younger adaptive regions and that the center of each region is the most ancient part (132). This suggests that, over millions of years of repeated freshwater colonization and gene flow from freshwater to oceanic populations, the original oldest adaptive alleles have accumulated additional advantageous mutations, creating a set of finely tuned mini-haplotypes (rather than individual SNPs) that provide the raw material for freshwater adaptation in extant populations today (47, 132).

These mini-haplotypes generally span only kilobase scales and are distinct from larger haplotypes relevant to reassembly under the transporter model, which can involve loci spread across tens of megabases and appear to be closely associated with the underlying recombination landscape (**Figure 2*c***). Genome-wide recombination rate maps based on both pedigrees (56, 135) and patterns of linkage disequilibrium (132, 146) show significantly decreased recombination rates where loci with alternative marine and recycled freshwater alleles are clustered. The effect of this clustering is particularly marked for the chromosomes with the most divergent loci, such as chromosome IV, where adaptive regions span 60% of the chromosome when using physical distance but only 20% when considering genetic distance (132). While this lower recombination rate may generally act to keep individual freshwater divergent loci together (particularly mutations on the individual mini-haplotypes) (114), recombination hot spots are frequently found immediately between these mini-haplotypes, such that the larger chromosome-wide freshwater-adaptive haplotypes can disassemble in marine environments but reassemble and avoid Hill–Robertson effects when entering new freshwater environments.

3.6. The Heterogeneity of Global Patterns of Genomic Evolution of Other Ecotypic Pairs

In contrast to the generally consistent signature of allele reuse during the oceanic–freshwater transition (specifically in the eastern Pacific), a more complex picture has emerged for divergence among other ecotypic pairs within freshwater environments (40, 156). Despite also being geographically widespread, SNP array (40), RAD-seq (97, 134), and whole-genome sequencing (49) data from distinct parapatric (and occasionally sympatric) lake and stream ecotype pairs show fewer globally shared divergent loci. Instead, geographically restricted (i.e., local) standing genetic variation and phenotypic plasticity (118) seem to dominate. Both adaptive and nonadaptive (i.e., the result of population demography) effects have been shown to be important in the formation of lake–stream pairs (156). Phenotypic divergence between these ecotypes will be influenced by the range of global standing genetic variation that is available at founding. In addition, the need to adapt to specific local ecological conditions within lake–stream systems, such as specific parasite populations (49) (see Section 5.3), and environmental heterogeneity within habitats (156) will also play a role. Many of these traits may also have a polygenic basis, based on QTL analyses (see below), leading to even greater variation in which loci ultimately are selected for following independent episodes of lake–stream divergence. Oceanic sticklebacks have comparatively larger effective population sizes (57, 146) than other ecotypes and thus greater potential to maintain and circulate substantial standing genetic variation over large distances than more fragmented freshwater lake–river systems (97). However, it should be noted that loci diverging among these lake–river systems still tends to occur near a few important genomic regions that are associated with oceanic–freshwater divergence (83), including around *Eda* and other parts of chromosome IV, chromosome VII, opsin genes, and the inversion on chromosome I (96–98). These selected regions are generally found in low-recombination regions and overlap QTLs (96), thus suggesting some common molecular basis for oceanic–freshwater, lake–stream, and benthic–limnetic adaptation.

4. THE GENOMIC ARCHITECTURE OF THREESPINE STICKLEBACK PHENOTYPES

4.1. Large-Effect Versus Small-Effect Alleles

The relative contributions of mutations with small and large effects on fitness have been a subject of intense debate for nearly a century. Building on Fisher's geometric model of adaptive phenotypic evolution (50), Orr (119) predicted that adaptation is likely to take place through a few mutations of large effect and many mutations of small effect via an adaptive walk. On the surface, quantitative mapping studies in threespine sticklebacks support this prediction (123), with a largely exponential distribution of presumptive fitness effects across QTLs, inferred from the proportion of phenotypic variance explained (PVE) (**Figure 3*b***). Threespine stickleback QTLs (n = 1,104, based on 28 QTL studies up until 2017) are associated with several prominent phenotypic categories, such as body shape (39%), feeding (36%), and defense traits (15%), and are distributed across multiple chromosomes (123) (**Figure 3*a***). These quantitative mapping studies have commonly identified the same QTLs in different threespine stickleback populations. However, there is considerable enrichment of QTLs in regions of the genome that display ecotypic divergence (123), and thus it is reasonable to conclude that most QTLs found to date mark standing genetic variation. Therefore, caution should be applied in relating the apparent exponential pattern of QTL PVEs observed in threespine sticklebacks directly to Orr's prediction, which was based on the theory that adaptation was occurring via unlinked de novo mutations (120) and not standing genetic variation. The expected distribution of fitness effects for adaptation based on standing genetic variation is less certain (41) and is likely dependent on the specific scenario [e.g., how and to what degree the optimum trait is changing (99) and how polygenic the trait is (79)]. Also, as described above (Section 3.5), strongly selected standing genetic variation mini-haplotypes associated with large-effect QTLs in extant sticklebacks today are possibly the result of multiple mutations, with much smaller effects accumulating over millions of years. Though a tremendous challenge, being able to parse the fitness effects of specific mutations will be key to understanding the extent to which large- and small-effect alleles have acted in threespine stickleback evolution (143).

A handful (n = 6) of stickleback QTLs, all associated with pelvic and plate morphology, have particularly large effects (PVE > 80%) (**Figure 3*b***). Two of these are major skeletal changes—reduction in the number of lateral armor plates and pelvic reduction—which are phenotypically equivalent to modifications in higher taxa of vertebrates, including homologs for human disease (36, 38, 147, 159) (**Table 1**). These two traits show surprisingly simple, almost Mendelian, inheritance (36, 38), with single large-effect QTLs on chromosome IV (associated with *Eda*) and chromosome VII (associated with *Pitx1*). Interestingly, the *Eda* gene is highly pleiotropic (**Figure 3*d***) and influences diverse traits, such as lateral line patterning, body shape, and schooling behavior (2, 4, 36, 58, 60, 111), while variation in bony lateral plate number is also affected by potential modifier genes with a much smaller effect on other chromosomes (35, 36).

However, despite the high scientific profile of *Eda* and *Pitx1*, only a few QTLs actually have such a high PVE for stickleback traits (as shown in **Figure 3*b***), with smaller effects being much more frequent (7.6% median PVE across all QTLs). For example, while two moderate-effect QTLs for gill raker number and spacing occur on chromosome XX (25% PVE) and chromosome IV (23% PVE), other QTLs with much smaller effects have been identified for this trait on 15 other chromosomes (~5.4% mean PVE) (109). Adding to this complexity for certain traits, different smaller-effect QTLs have been shown to influence the same traits in different populations, indicating likely independent loci leading to these convergent phenotypic traits (55, 56, 109). Though it is difficult to elucidate genes underlying these smaller-effect QTLs, high-resolution

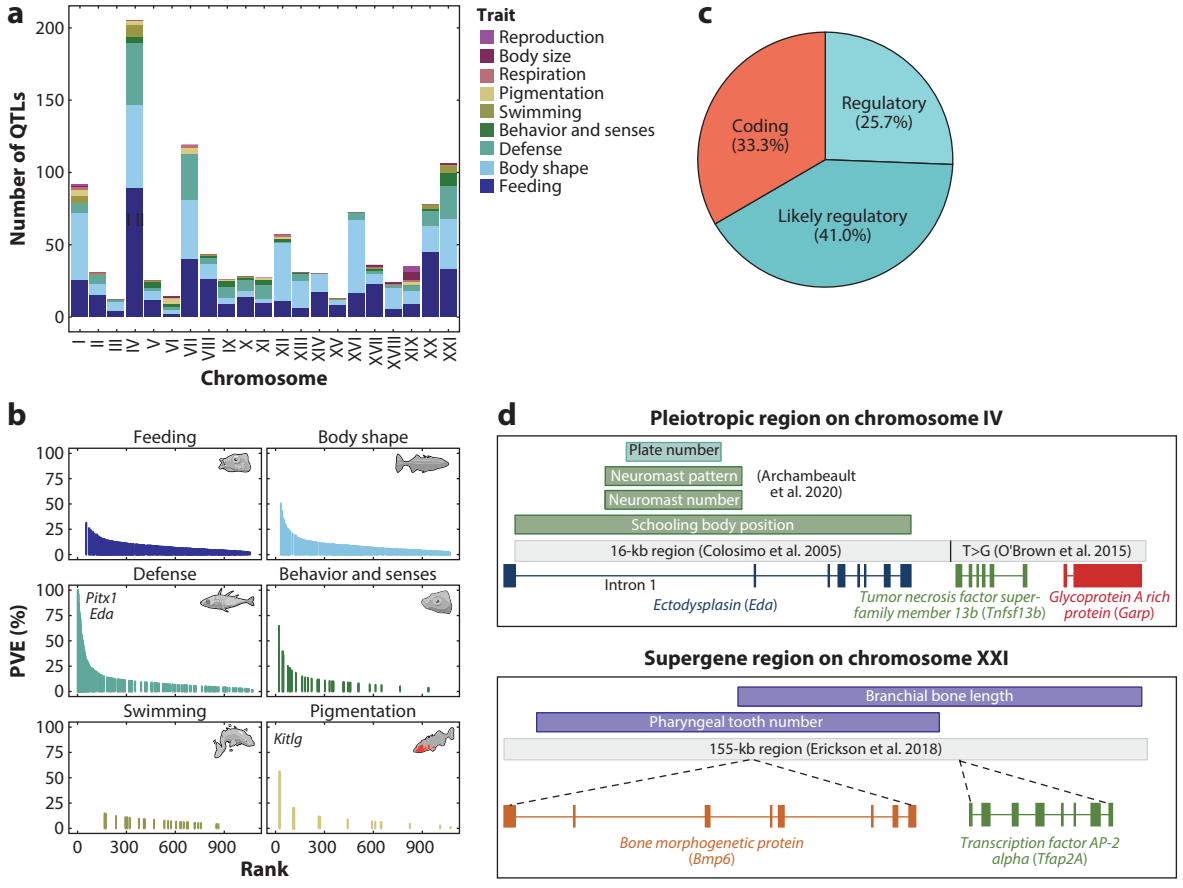

Figure 3

QTL analysis revealing regions of large and small effect as well as supergene regions. (*a*) QTLs identified from various mapping studies reviewed by Peichel & Marques (123), plotted by chromosome. (*b*) Rank-ordered QTLs by PVE for the most prominent categories defined by Peichel & Marques (123). Genes that show a regulatory change of large effect are highlighted by their abbreviation (*Pitx1*, *Eda*, *Kitlg*). (*c*) Percentage of oceanic–freshwater divergent loci in coding and putative regulatory regions identified in the global data set by Roberts Kingman et al. (132). (*d*) Identified pleiotropic and supergene regions on chromosomes IV and XXI, respectively. These diagrams are derived from information provided in References 4, 35, 44, and 117. Gene regions are indicated by boxes representing predicted exons, and the lines connecting them represent introns. Colored boxes above identify the trait associated with the region. Abbreviations: PVE, phenotypic variance explained; QTL, quantitative trait locus.

linkage and association mapping are increasingly able to narrow down the chromosomal regions for loci with PVEs ranging from approximately 10% to 30% (33, 34, 76, 78).

Given the preponderance of small-effect loci from QTL studies of marine and freshwater crosses, combined with the large number (>300) of rapidly evolving loci genome-wide found during scans for freshwater adaptation, it is reasonable to ask to what extent sticklebacks fit existing models of polygenic adaptation. If we consider freshwater adaptation as the high-level phenotype that can be decomposed into a series of lower-level traits, then most adaptations appear to be driven by a few loci of large effect along with a larger number of accompanying smaller-effect loci. An interesting question that arises from this distribution is the degree to which there is any evidence of the genetic redundancy that is a hallmark of polygenic adaptation (8, 9). As discussed

Table 1 Genes relevant to threespine stickleback development

Gene	Chromosome	Stickleback trait	Type of mutation	Mode	Regulatory direction in fresh water	Consequence	Type	PVE	Human homolog disease or trait	Reference(s)
Eda	IV	Plate morphology (number of plates)	Intron mutation	*Cis*-acting	Down	Localized reduction in expression	SGV	78%	X-linked hypohidrotic ectodermal dysplasia	4, 35, 36, 38, 58, 117
		Lateral line patterning						65%		
		Schooling body position						13.5%		
Msx2A	IV	Spine length	Novel splicing enhancer	*Cis*-acting splicing	Truncated	Alternative splice alleles	SGV	30%	NA	76
Pitx1	VII	Pelvic loss	Enhancer deletion	*Cis*-acting regulation	Down	Localized reduction in expression	De novo	~100%	Tibial hemimelia, clubfoot	32, 38, 148, 169
Kitlg	XIX	Gill pigmentation	Unknown	*Cis*-acting regulation	Down	Localized reduction in expression	SGV	56%	Skin and hair color	108
Hps5	XIX	Pigmentation (albinism), bleeding phenotype	Exon frameshift	Coding	NA	Frameshift	De novo	NA	Hermansky–Pudlak syndrome type 5, albinism, bleeding	66
Gdf6	XX	Plate height	Unknown	*Cis*-acting regulation	Up	Localized increase in expression	Unknown	9.9%	Klippel–Feil syndrome	78
Bmp6	XXI	Ventral pharyngeal tooth number	Intron mutation	*Cis*-acting regulation	Down	Localized reduction in expression	Unknown	30%	Hemochromatosis, renal fibrosis	33, 44
Tfap2a	XXI	Branchial bone length	Unknown	Unknown	Down	Localized reduction in expression	Unknown	Unknown	Branchio-oculo-facial syndrome	44

Abbreviations: NA, not applicable; PVE, phenotypic variance explained; SGV, standing genetic variation.

in detail above, regardless of geographic location, there are hundreds of key divergent freshwater loci that consistently evolve in almost all instances of freshwater adaptation. In addition, these loci appear to be at low initial frequencies in the marine population and experience large sweeps toward fixation, rather than the small shifts in intermediate frequency associated with standard (but not all) polygenic models (8, 145). In addition, QTLs significantly overlap with these adaptive divergent loci, particularly QTLs with large effects that are found in multiple populations (45, 55). Thus, there appears to be a subset of large-effect loci that are essential for convergent marine–freshwater adaptation, with much of the underlying genetic variation clustered on certain key chromosomes, such as chromosome IV, that show considerable pleiotropy and tight linkage and thus may be key for rapid adaptation.

There is more uncertainty with regard to the smaller-effect QTLs. There are indications of some level of nonparallelism (56), which might reflect redundancy for smaller-effect loci contributing to adaptation. However, concrete conclusions cannot yet be drawn, given that most QTL studies have had fairly low genomic resolution and that there is still limited power to detect small-effect QTLs (which will further underpower the ability to detect parallelisms). In threespine sticklebacks, it is possible that large-effect QTLs may consist of multiple smaller-effect QTLs (see below) and that such loci may be difficult to detect in selection scans because of their more subtle allele frequency shifts. Though there have been fewer selection and QTL studies, it is possible that adaptation occurring in intra-freshwater comparisons may have a more traditional polygenic basis in favor of smaller-effect loci with redundancy, as there is less observed genomic parallelism in selected divergent loci across studies (see above) as well as evidence of heterogenic QTL use (37), which may reflect the much smaller phenotypic shift associated with this environmental transition compared with marine-versus-freshwater adaptation.

4.2. Localized Gene Regulation Is Important for Vertebrate Adaptation

One limitation of genome-wide QTL studies is that the regions identified are often large (mean size ~10 Mb), spanning multiple genes. High-resolution linkage and association mapping has been conducted in some cases (4, 35, 108, 117) to narrow down the region of interest and identify specific genes associated with trait variation. Interestingly, almost all candidate genes marked by major QTLs thus far have been associated with developmental control genes, though this likely reflects a bias in the selection of the phenotypic variation for study thus far (73). When genes marked by QTLs of large effect have been sequenced among different ecotypes, no prominent causative coding changes have been found that can explain the differences in morphological traits observed in threespine sticklebacks (36). Rather, likely mutations in regulatory elements that alter gene expression appear to play the key role in the stickleback radiation and the rapid adaptation of wild populations uncovered so far (32–34, 84, 108, 117); however, identifying these causative SNPs has been challenging.

Significant allele-specific expression differences between ecotypes have been identified using methods such as reverse transcription polymerase chain reaction (RT-PCR), RNA sequencing (RNA-seq), and in situ hybridization for variation in the number of plates, plate height, pelvic reduction, pigmentation, ventral pharyngeal tooth number, and branchial bone length (32, 34, 76, 78, 108, 117, 148) (**Table 1**). All of these were shown to result from *cis*-acting regulation, with the causative SNPs likely to be in adjacent enhancer or promoter regions. However, a novel exception has recently been described for differences between oceanic and freshwater threespine sticklebacks in the gene controlling spine length (*Msx2A*), where the causal mutation appears to involve a novel splice site that leads to a truncated protein in the freshwater ecotype (76).

Consistent with the predominance of regulatory mutations for adaptation in threespine sticklebacks already experimentally described (4, 32, 33, 38, 78, 108), whole-genome sequencing

studies find that approximately 70% of divergent regions (given specific cutoff criteria decided by the authors) between oceanic and freshwater populations are located in noncoding regions (84, 132) (**Figure 3*c***). Furthermore, Verta & Jones (163) recently examined parallel divergence of gene expression in gill tissue among multiple oceanic–freshwater ecotypic pairs and their F1 progeny in order to dissect the relative roles of *cis*- and *trans*-acting regulatory architecture. Only a few hundred genes were consistently differentially expressed between ecotypes, and these genes were often proximate to the divergent loci in the study by Jones et al. (84). In addition, divergence in *cis*-acting regulatory sequences appeared to be the primary driver of these expression differences in threespine sticklebacks. To summarize, given current evidence, it is hypothesized that adaptation in threespine sticklebacks, particularly for oceanic populations moving to fresh water, depends primarily on localized, tissue-specific gene regulation through mutations in *cis*-acting regulatory regions, such as promoters and enhancers of major developmental genes. However, an important step in the future will be to actually identify causative genetic variants to confirm this pattern (73, 117).

4.3. The Role of Pleiotropy and Supergene Clusters in Threespine Sticklebacks

Genomic studies across a wide range of species are increasingly identifying hot spots of adaptation that contain several tightly linked genes and are often enriched for pleiotropic genes. These regions are known as supergenes (144) and are found predominantly on inversions, which provides a fairly simple mechanism to suppress recombination and thus keep the individual adaptive components in tight linkage (122). In sticklebacks, there are three major inversions linked to adaptation between oceanic and freshwater ecotypes on chromosomes I (442 kb), XI (412 kb), and XXI (1,700 kb) (84). Importantly, there are many more regions in the genome that are not associated with inversions and have features of supergenes, including all of chromosome IV, which has the greatest number of rapidly diverging freshwater-adaptive regions. It is possible that inversions, a general mechanism among other species (166), may not be favored in sticklebacks because of the fitness advantage of disassembling standing genetic variation haplotypes when they reenter oceanic populations.

Peichel & Marques's (123) analysis identified more QTLs than expected by chance for all trait categories on chromosomes IV and XXI, with feeding traits enriched on chromosome XX, body shape on chromosome XVI, and defense on chromosome VII (123) (**Figure 3*a***). Although the initial analyses of QTLs with multiple mapped traits did not elucidate whether pleiotropic genes or linked causative loci within regions led to these highly clustered genome distributions, recent studies have focused on disentangling these effects and trying to break large QTLs into their smaller component parts (4, 44, 111). For example, the haplotype associated with lateral plate number is a 16-kb region on chromosome IV and contains three genes (**Figure 3*d***). Fine-scale mapping localized a 1.4-kb region of intron 1 of the *Eda* gene that associates with three distinct traits that are likely important for freshwater adaptation. In addition, several other traits with much smaller effects map to this larger region, indicating that both pleiotropy and linkage of important loci occur within this supergene-like region (4). Another prominent supergene on chromosome XXI influences two craniofacial traits associated with feeding, increase in numbers of ventral pharyngeal teeth, and extension of branchial bones (33, 34, 109). Although both traits originally mapped to overlapping regions on chromosome XXI, subsequent fine-scale mapping showed that pharyngeal tooth number is associated with downregulation of the freshwater allele of *Bmp6*, while brachial bone length associates with the downregulation of the freshwater allele of *Tfap2a* (44). These findings, coupled with the recent findings of circulating mini-haplotypes, support the hypothesis that these regions of large effect are likely carrying several advantageous mutations.

5. EMERGING FIELDS FOCUS ON ELUCIDATING THE GENOMIC ARCHITECTURE AND REGULATORY MECHANISMS OF COMPLEX TRAITS

5.1. Evolutionary Genomics of Behavior

The field of behavioral genomics is still in its infancy due to the complexity of assessing both individual and group behaviors and the highly polygenic nature of these traits (28). The recent availability of reference genomes and the reduction in cost of generating genomic and transcriptomic data are providing the opportunity to explore the mechanistic links between genes and behavioral traits. Behavioral traits are complex and require carefully designed experiments to quantify and discriminate individual and group behaviors. This problem, coupled with the plastic and continuous nature of behavior and the likelihood that many genes of small effect are involved, makes it difficult to quantify the role of natural genetic variation (23). Genome-wide gene expression studies using microarray and RNA-seq transcriptome techniques are frequently used to quantify common changes in specific tissue types, such as regions of the brain that modulate transcriptional regulatory networks to various stimuli (30, 59, 116, 140). Recently, virus-mediated transgenesis tools (such as brain microinjections) have been developed to facilitate modification of gene regulation directly in relevant tissues, allowing individuals to act as their own control and provide a more detailed mechanistic analysis of behavior (80). Threespine sticklebacks exhibit a range of behaviors that are divergent among ecotypes as well as variable within populations (21). Thus, this species has long been the subject of behavioral studies (21, 77, 161), with a major recent focus on identifying the underlying genetic contribution to these behavioral traits (58, 60).

5.1.1. Ecotypic-level variation in the behavior of threespine sticklebacks. Schooling (coordinated swimming of a group of fish) varies among oceanic and some freshwater ecotypes of threespine sticklebacks, with the former having a strong propensity to school. An apparatus to assess natural schooling responses in threespine sticklebacks consists of eight clustered model sticklebacks that can be moved around a tank to simulate a stickleback school (164). This behavioral assay allowed the investigators to examine both the body position of the individual fish within the school and their tendency to join the school. These two behavioral traits are heritable among oceanic and benthic freshwater threespine sticklebacks (164), while QTL mapping revealed that they were uncorrelated in hybrid offspring and associated with different loci (60). A follow-up transgenic study showed that when benthic sticklebacks had the oceanic *Eda* allele rescued, they exhibited a more oceanic-like body orientation when schooling, directly associating the underlying genomic sequence with part of a social behavior varying among ecotypes (58).

5.1.2. Individual- and population-level variation in the behavior of threespine sticklebacks. Gene expression studies indicate that it is likely that the brain responds rapidly to stimuli by modulating transcriptional regulatory networks (65). Therefore, a common experimental design for examining behavioral variation within populations involves comparing tissue-specific transcriptomic responses among individuals exposed to a specific stimuli to those that are not (23). Threespine sticklebacks have been evaluated for gene expression responses to various encounters (stimuli), such as predation risk (140), territoriality challenges among males (138), social interactions among females (59), and mating opportunities (139). The various encounters appear to lead to tissue-specific responses with dramatic differences in gene expression compared with controls (up to ~10% of genes are up- or downregulated across the tissue-specific transcriptome), providing candidate genes and pathways for further interrogation (23, 138, 140).

For example, to identify candidate genes for male aggression, Bell et al. (13) used an RNA microarray approach in four brain tissues (brain stem, cerebellum, diencephalon, and telencephalon) to measure gene expression differences in a resting state among males showing variation in this behavior. By far the largest number of differentially expressed genes among aggressive and nonaggressive individuals was in the brain stem (625 genes in the brain stem versus 98 across the other three tissues), with several genes that have previously been associated with aggressiveness in other vertebrate species being either significantly up- or downregulated (13). This enrichment in the brain stem contrasted with gene expression differences seen in males after a territorial intrusion, which were more localized to the diencephalon (138). Importantly, the genetic basis of these neuromolecular responses to territorial intrusions also appears to correspond to expression changes observed during social challenges in other species (i.e., mice and honey bees) (131). A time series expression study analyzing differing brain tissues (diencephalon and telencephalon) in male threespine sticklebacks after a territorial intrusion showed that waves of expression lasted for hours after the encounter and evoked different biological processes (such as hormone activity, metabolism, and immune response). Interestingly, when assessing the corresponding epigenome using a chromatin immunoprecipitation followed by sequencing (ChIP-seq) approach during this same time course, Bukhari et al. (30) found that the encounters led to changes in chromatin accessibility that correlated with the observed changes in gene expression and neural functioning, identifying a likely epigenetic mechanism for how species account for social interactions.

Male parental care occurs in phased stages. In stickleback populations more generally, parental care is paternal [with the exception of white sticklebacks in Nova Scotia (81)]. The males build a nest, court gravid females to induce them to deposit their eggs in the nest, guard eggs, and fan them within the nest to provide oxygen. Paternal care (particularly fanning of eggs) varies among individuals (151, 152), and the degree of paternal care is also heritable within populations (14). Increased paternal care appears to provide the offspring with an advantageous epigenetic effect through increased DNA methyltransferase (*Dnmt3a*) expression in the brains of offspring (compared with orphaned offspring), which facilitates de novo methylation. This process may facilitate behavioral development and lower anxiety in offspring when facing predators, conferring increased fitness (101). To assess the regulatory response to paternal care, Bukhari et al. (29) performed a temporal expression study through the various paternal care stages of sticklebacks across differing brain tissues (diencephalon and telencephalon). Comparative genomic analyses indicated that some of the neurogenomic changes described in male threespine sticklebacks were analogous to those experienced during reproduction and pregnancy in mammals (e.g., the upregulation of estrogen and progesterone receptors in the diencephalon during hatching). These two cases (aggression and parental care) show that disentangling the genomic responses to experiences in threespine sticklebacks can reveal conserved pathways that are relevant for understanding behavior across diverse species (141).

5.2. Host–Microbiome Interactions

The study of host–microbiome interactions is a fast-growing field of research, as microbial communities appear to have major influences on host traits and fitness and may have important consequences for human health (3). The first wave of host–microbiome interaction studies have focused primarily on established model organisms (such as inbred mouse strains, *Danio rerio*, *Caenorhabditis elegans*, and *Drosophila*) with noncomplex microbial communities (42). However, several questions cannot be addressed with these host–microbiome models, and there is an increasing need to study these interactions in natural environments using species that more closely resemble humans and show natural population genetic diversity (91). The wealth of behavioral, ecological, and genomic resources is making threespine stickleback a major target for such research.

5.2.1. Factors driving the composition of microbial communities and host traits. The diversity and repeated natural colonizations of threespine sticklebacks in differing environments (marine, estuarine, and freshwater) and the repeated divergence into ecotypes provides the opportunity to address several specific questions about the composition of microbial communities and their relation to hosts. A combined RAD-seq and 16s rRNA analysis of two estuarine and four freshwater populations in Oregon found that gut microbiome composition was better predicted by population genetic divergence than by the local environment or geographic distance between populations (153). To assess the repeatability of changes in gut microbiomes associated with ecotypes, Rennison et al. (130) focused on three sympatric benthic–limnetic species pairs from three lakes in Canada. They identified parallel shifts in the same direction in the gut microbiome composition and function associated with particular ecotypes, indicating that microbiomes, which will be heavily influenced by the contrasting diets of these ecotypes, might play an important role in adaptation and divergence, as they could confer fitness advantages within each ecotype-associated habitat (130).

Larval fish acquire their gut microbiomes from their environment when their digestive system opens after hatching. However, the compositions of both population-level and individual adult stickleback microbiomes result more from their diet and habitat usage than from the microbes available in the surrounding water (26, 150). This parallels the trajectory of the gut microbiome in a human newborn, which initially resembles that of the mother's vagina and then transitions to a standard adult gut microbiome by early childhood (133). Other individual-level factors, such as sex, major histocompatibility complex (MHC) IIb allele diversity, neutral genetic variation (genotype), and helminth infection (see Section 5.3), have been implicated in the microbiome compositions of wild stickleback populations (25–27, 52, 92, 130, 150). An interesting causal chain has also been shown from diet specificity to immune response to changes in microbiome composition, indicating potential immunological control of microbiomes (51).

5.2.2. How does host genetic background influence immune response to microbes? Milligan-Myhre et al. (110) showed that oceanic sticklebacks have a stronger innate immune response to the introduction of microbial communities than freshwater sticklebacks do. The strength of the immune response was measured by counting the number of neutrophils in the intestine (gut) elicited after microbial introduction to the host in controlled experiments raising gnotobiotic fish. This immune response was paralleled in wild-caught fish, with the oceanic ecotype having more neutrophils in the gut than the freshwater ecotype. A follow-up study by Small et al. (149) identified 72 genes that were differentially expressed (several involved in innate immunity) in the developing gut tissue of freshwater and oceanic larvae confronted with artificial introductions of the same microbial communities, providing a list of candidate loci for future targeted studies. This pointed to natural variation within threespine stickleback ecotypes that could be used to further investigate the genomic architecture of the host's immune response. Indeed, a genetic mapping study comparing oceanic and freshwater sticklebacks and examining intestinal neutrophil activity identified two moderate-effect QTLs on chromosome III (15.84% PVE) and chromosome VIII (15.77% PVE) that span several candidate genes (14 genes for the QTL on chromosome III and 13 for the one on chromosome VIII) (12). Intriguingly, some of the same pathways and biological functions for genes identified in the RNA-seq study by Small et al. (149) appeared also to be enriched among the genes at these two QTLs.

5.3. Host–Parasite Interactions

Interactions between threespine sticklebacks and their associated parasites have several cascading effects, including triggering the immune system and changes in appearance (courtship coloration)

(107), reproduction, and several behaviors (foraging, shoaling, diel vertical movement, and reactivity to prey) (6). A wide range of eukaryotic parasites from a diverse range of taxonomic groups [see table 1 in the review by Barber (6)] infect threespine sticklebacks, and these parasites are not ubiquitous throughout the stickleback range. Therefore, different populations and ecotypes of threespine sticklebacks are exposed to different parasite metacommunities in their local environments and differing parasite loads (6), making them a tractable system to study the evolution of host–parasite resistance.

5.3.1. Parasites and host response. Threespine sticklebacks have innate (general) and adaptive (specific) immune responses to parasites and microorganisms (25, 43). MHC genes are among the most diverse genes in vertebrates and play a major role in immunity by presenting antigens derived from pathogens and parasites to T lymphocytes. When threespine sticklebacks are experimentally exposed to specific parasites, the offspring show increased resistance along with rapid shifts in the frequencies of MHC IIb alleles, with as much as a 19% increase for some alleles in just one generation (43). As in the rapid overall genomic change observed across three contemporary lake experiments described in Section 3 (132), importantly, these strongly selected alleles were already common in the ancestral pools as standing genetic variation, so that there is probably a reservoir of immune response alleles among sticklebacks that can respond rapidly to directional selection when new parasites are encountered.

While the selection of immune response genes such as MHC genes is clearly important in responding to metazoan parasites, questions remain about the role of epigenetic change. Sagonas et al. (137) recently began to address these questions by assessing the DNA methylation differences among threespine sticklebacks infected with *Camallanus lacustris* and noninfected fish. Infected fish appear to experience a genome-wide increase in the number of methylated sites, with differential methylation compared with uninfected controls being particularly enriched in genes related to immune response and metabolic processes. In infected fish, a small number of genes for cell turnover and production of novel immune cells (e.g., *itga1* and *npffr2b*) were hypermethylated, indicating that parasites could be repressing the immune response. Epigenetic changes in threespine sticklebacks represent an alternative mechanism for rapid adaptation via plasticity when entering new environments, one that is emerging as an exciting area of research (5, 67).

5.3.2. The threespine stickleback–*Schistocephalus solidus* model of parasite infections. Among the many host–parasite interactions studied in threespine sticklebacks, the greatest focus has been on infection by the tapeworm *Schistocephalus solidus*, the most prevalent parasite in freshwater stickleback populations globally and a model for host–parasite interactions (7). *S. solidus* has a complex life cycle; briefly, eggs hatch in the definitive avian host's intestine, are defecated into the water, and infect copepods. When infected copepods are consumed by a stickleback, the larvae burrow through the gut wall into the body cavity and grow into adults. If the stickleback is later consumed by an endothermic host (typically a bird), competent tapeworms will respond to the elevated temperature by becoming sexually mature and either self-fertilize or outcross if other tapeworms are present in the definitive host's gut. Threespine sticklebacks are an obligate host for *S. solidus*, meaning that the tapeworm cannot complete its life cycle without infecting the intermediate stickleback host.

Variation in resistance to this tapeworm is observed across the species range and among stickleback ecotypes (95, 165). *S. solidus* infection of threespine sticklebacks has several fitness consequences, including negative effects on the fish's body condition, a decrease in energy reserves, and a reduction in gamete production, with the inability to spawn and movement restrictions impacting social interactions (7, 68, 82). The virulence of the parasite, however, appears to differ across the

species range, with several populations in Alaska able to reproduce effectively with high infection rates (68). Infection of the oceanic ecotype is rarely observed in its natural marine environment, likely because *S. solidus* eggs cannot hatch in brackish water. Marine populations along with freshwater populations with a low prevalence of the parasite in their lake environment showed high susceptibility to infection in laboratory experiments, while freshwater populations with a high prevalence of the parasite showed higher resistance to infection (165), strongly suggesting that there is a genetic component to *S. solidus* resistance. A comparison of transcriptomes from threespine sticklebacks from both low- and high-resistance populations identified 64 differentially expressed genes between *S. solidus*–infected and noninfected individuals. As expected, and consistent with the other studies described above, several of these genes were linked to host immunity and responses of both innate and adaptive immunity (MHC). The innate response was correlated with tapeworm establishment, and reactive oxygen species production was correlated with tapeworm growth, indicating that these two key factors are important for *S. solidus* resistance (93). In addition, the *S. solidus* infection in threespine sticklebacks appears to be associated with the composition of gut microorganisms, and interestingly, there appear to be genetic variants that influence the degree of microbial response (92). Recent studies are focusing on characterizing the pathogens carried and transmitted by these tapeworms to the threespine stickleback, providing a new avenue for research on these parasites and their influence on host–microbiome interactions (64).

6. CONCLUSIONS

The threespine stickleback has become invaluable for addressing several of the most fundamental questions in evolutionary genomics. Future research is likely to be increasingly integrative, incorporating not only the topics described here but also other fields, such as neurodevelopment and toxicology, as well as continued integration with ecology. The threespine stickleback's natural history, coupled with high-quality genomic resources, has massively facilitated these insights due to the ease of generating huge amounts of DNA sequence and transcriptomic data that can be leveraged across diverse fields, from evolution to behavior to host–parasite and host–microbiome interactions. As a result, the threespine stickleback system is now strongly positioned to exploit quickly emerging transgenic tools, such as CRISPR/Cas9 (66, 168, 169) and neurosurgical methods (80), to forge strong links between specific genetic variants and phenotypes and provide insights into the molecular basis for heritable human pathology.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Dr. Krista Oke and Dr. Yoel Stuart for helpful discussions. We acknowledge support from National Institutes of Health grant R01GM124330 to K.R.V. and M.A.B.

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Annual Review of Genomics and Human Genetics

Volume 22, 2021

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Errata

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