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The genetic and molecular architecture of phenotypic diversity in sticklebacks

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A major goal of evolutionary biology is to identify the genotypes and phenotypes that underlie adaptation to divergent environments. Stickleback fish, including the threespine stickleback (*Gasterosteus aculeatus*) and the ninespine stickleback (*Pungitius pungitius*), have been at the forefront of research to uncover the genetic and molecular architecture that underlies phenotypic diversity and adaptation. A wealth of quantitative trait locus (QTL) mapping studies in sticklebacks have provided insight into long-standing questions about the distribution of effect sizes during adaptation as well as the role of genetic linkage in facilitating adaptation. These QTL mapping studies have also provided a basis for the identification of the genes that underlie phenotypic diversity. These data have revealed that mutations in regulatory elements play an important role in the evolution of phenotypic diversity in sticklebacks. Genetic and molecular studies in sticklebacks have also led to new insights on the genetic basis of repeated evolution and suggest that the same loci are involved about half of the time when the same phenotypes evolve independently. When the same locus is involved, selection on standing variation and repeated mutation of the same genes have both contributed to the evolution of similar phenotypes in independent populations.

This article is part of the themed issue 'Evo-devo in the genomics era, and the origins of morphological diversity'.

1. Introduction

The modern tools of molecular genetics and genomics have enabled a revolution in evolutionary biology. With these tools, we are now able to investigate long-standing questions about the genetic and molecular changes that underlie phenotypic diversity in natural populations. Recent research in a number of plant and animal systems has started to provide insight into fundamental questions about the genetic and molecular architecture of phenotypic evolution, such as: (i) Does phenotypic evolution occur through mutations of small or large effect?; (ii) Does genetic linkage and/or pleiotropy facilitate phenotypic evolution?; (iii) Does phenotypic evolution occur through changes in coding or regulatory regions of genes?; (iv) When the same phenotype evolves independently, are the same or different genetic changes involved?; and (v) When the same genetic changes are involved, is this due to standing variation or new mutation?

The stickleback family (Gasterosteidae) of fish provides a remarkable opportunity to address these long-standing questions. In particular, the ecology, evolution, morphology, behaviour and physiology of the threespine stickleback (*Gasterosteus aculeatus*) have been intensively studied for decades [1–3]. The ninespine stickleback (*Pungitius pungitius*), which diverged from threespine stickleback approximately 13–16 Ma [4], has more recently been developed as an evolutionary model system [5]. In both species, ancestral marine populations

adapted to diverse freshwater habitats across the Northern Hemisphere at the end of the last glacial period, approximately 15 000 years ago [2]. When comparing extant marine and freshwater populations, extensive phenotypic diversity is observed in morphological, behavioural and physiological traits (figure 1) [5,6]. Within freshwater, threespine sticklebacks have further adapted to different habitats, resulting in phenotypically divergent ecotypes that have been key in the study of speciation [7,8].

With this wealth of natural history studies as a backdrop and the development of genetic and genomic tools for the threespine stickleback, this system has emerged as an evolutionary 'supermodel' [9]. Initially, a genetic linkage map was developed for threespine stickleback using a genome-wide panel of microsatellite markers, which enabled quantitative trait locus (QTL) mapping studies to uncover the genetic architecture of phenotypic differences between populations adapted to different environments [10]. In order to facilitate identification of the genes and molecular changes that underlie QTL for phenotypic differences between populations, a number of additional genomic resources, such as large-insert genomic libraries and physical maps, were developed for the threespine stickleback [11–13]. These genomic tools were fundamental for the high-quality assembly of the genome of a freshwater threespine stickleback female from Alaska [14]. The development of next-generation sequencing technologies has further revolutionized stickleback genetics and genomics. One important technique that has now been widely used in evolutionary genomic studies, restriction site-associated DNA sequencing (RAD-seq), was developed using stickleback as one of the test cases [15,16]. Using such next-generation sequencing approaches to create high-density genetic linkage maps has further improved the initial genome assembly in threespine stickleback [17,18] and enabled comparison of the threespine and ninespine stickleback genomes [19]. Finally, transgenic tools have been adapted for use in sticklebacks [20–22]. These genetic and genomic tools have enabled dozens of QTL studies and the identification of genes and mutations that underlie phenotypic diversity among stickleback populations (electronic supplementary material, tables S1–S3). Here, we summarize how these studies in stickleback have provided new insights into the questions posed above about the genetic and molecular architecture of phenotypic evolution.

2. Identification of quantitative trait loci reveals the genetic architecture of phenotypic diversity in sticklebacks

To date, there have been 28 QTL studies published in threespine stickleback [10,18,23–48] and four in ninespine stickleback [49–52] (electronic supplementary material, tables S1 and S2). Most of these studies (19 in threespine, 3 in ninespine) have focused on divergence between the marine and freshwater ecotypes, with a smaller number of studies investigating divergence between freshwater ecotypes (benthic-limnetic, lake-stream) or between two marine species in Japan (*G. aculeatus*, *G. nipponicus*). Here, we have loosely grouped the phenotypes studied thus far in QTL mapping studies into nine categories based on their putative function (electronic supplementary material, tables S1 and S2). The overwhelming majority of these QTL mapping studies have focused on morphological phenotypes, particularly skeletal and body shape traits that are thought to be important for adaptation to differences in feeding, predation and flow regimes among marine and freshwater habitats. Fewer studies have examined other morphological traits, such as pigmentation, or behavioural traits. Thus, QTL identified in threespine stickleback do not encompass many types of morphological, behavioural and physiological differences among stickleback populations or all axes of habitat divergence. However, this large QTL dataset does provide an opportunity to ask what we have learned so far about the genetic architecture of phenotypic diversity in stickleback, and how this has informed our general understanding of the genetics of adaptation.

(a) Does phenotypic evolution occur through mutations of large or small effect?

A long-standing debate in the field of evolutionary genetics has been whether mutations of large or small effect are more likely to contribute to adaptation [53–56]. In a model that somewhat reconciled the theoretical debate, Orr [56] predicted that a few mutations of large effect and many of smaller effect would contribute to the process of adaptation. Previous analyses of the distribution of effect sizes of QTL identified for body shape and skeletal traits in stickleback provide qualitative support for Orr's model [29,33,42,47]. With data on the per cent variance explained (PVE) for 1034 QTL from the 28 published QTL studies in threespine stickleback, we now have a larger sample size to examine this distribution (figure 2). These distributions look very much like what Orr had predicted, and there are no qualitative differences in the distributions among the nine trait categories. Of course, there are several caveats to this analysis [57]. First, Orr's predictions are specifically about the genetic changes that underlie fitness during adaptation. Although many of these phenotypes studied are predicted to be adaptive, given that they have evolved repeatedly in independent populations, the fitness effects of most of these phenotypes have not been explicitly tested. Second, PVE for small-effect loci near the limit of detection is generally overestimated in crosses with fewer than 500 progeny [58], and many of these QTL studies have analysed crosses with fewer than 500 individuals (electronic supplementary material, table S2). Third, most of the studies have only used one individual per population to establish crosses, and so results may not be representative of the genetic variance within the population. Finally, QTL are large regions that harbour many genes, and we do not know the effect sizes of individual mutations, with the few exceptions discussed below. Despite these caveats, we can conclude that phenotypic evolution of individual traits in stickleback occurs through a few mutations of large effect and many more of smaller effect.

(b) Does genetic linkage and/or pleiotropy facilitate phenotypic evolution?

Theory has suggested that adaptation to new environments can be facilitated by tight linkage of multiple co-selected traits [59–61]. To test whether particular genomic regions are enriched for the presence of QTL, we examined the distribution of 1104 QTL with a known genomic location from the

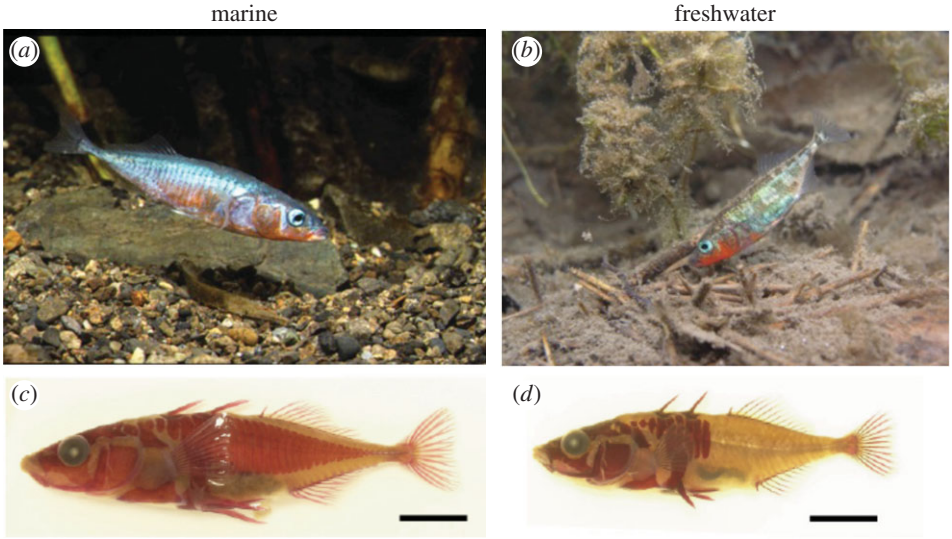

Figure 1. Phenotypic differences between marine and freshwater threespine sticklebacks. The top photos show a representative marine (*a*) and freshwater (*b*) male with the nuptial coloration (red throat, blue eyes) typically found in threespine stickleback males during the breeding season. Differences in overall pigmentation, body shape and body size between marine and freshwater sticklebacks are apparent in these photos; additional morphological, behavioural and physiological differences between marine and freshwater sticklebacks are not depicted. The bottom photos show a representative marine (*c*) and freshwater (*d*) fish stained with alizarin red to highlight the difference in the number of bony lateral plates found along the flank of the fish; scale bar, 10 mm. Photos provided by Jun Kitano and Seiichi Mori.

28 QTL studies in threespine stickleback. The distribution of all QTL across the 21 pairs of stickleback chromosomes is significantly different from the random expectation, based on either the total length of the chromosome or the number of genes on the chromosome (electronic supplementary material, table S4). A significantly biased distribution of QTL is also observed for feeding QTL ($n = 422$), body-shape QTL ($n = 399$) and defence QTL ($n = 175$); there are not enough QTL in other trait categories to perform statistical analyses. Although the current dataset might be biased because particular types of traits have been more well studied than others or mapped in multiple crosses, some interesting patterns emerge when examining which chromosomes have significantly more or fewer QTL than expected (figure 3; electronic supplementary material, table S4). Chromosomes III, VI and XV consistently have fewer QTL than expected. Interestingly, chromosome XIX, which is the sex chromosome in threespine stickleback [62] has somewhat fewer QTL than expected. This is despite theoretical predictions that genes important for adaptation and speciation might be located on sex chromosomes [63], and previous studies demonstrating that QTL for traits important for reproductive isolation between the Japan Sea (*G. nipponicus*) and Pacific Ocean (*G. aculeatus*) sticklebacks map exclusively to the sex chromosomes [30,43]. However, many QTL studies have ignored the sex chromosome or have not properly controlled for sexual dimorphism in their analyses. Thus, asking whether QTL for phenotypic diversity are commonly found on sex chromosomes requires future analyses.

One of the strongest patterns to emerge is that chromosomes IV and XXI have more QTL than expected for all QTL combined, and for all trait classes analysed. Three other chromosomes have more QTL than expected when considering all QTL, but each of these three chromosomes is associated with a single trait category: QTL for feeding traits are enriched on chromosome XX, QTL for body shape traits are enriched on chromosome XVI and QTL for defence traits are enriched on chromosome VII. Although it is predicted that inversions might facilitate clustering of adaptive phenotypes [59–61], only the trait cluster on chromosome XXI is associated with a known inversion between marine and freshwater populations [14], suggesting that other mechanisms might be important for the clustering of QTL in stickleback. This significant clustering of QTL does suggest that linkage and/or pleiotropy facilitates phenotypic evolution in stickleback. However, it is unknown whether the clustering of QTL is because a single genetic change has pleiotropic effects on multiple phenotypes, or because there are many linked genetic changes that each affect one or a few phenotypes. Identification of the genes that underlie these QTL, as discussed in the next section, will allow us to address this question in the future.

3. Identification of genes reveals the molecular architecture of phenotypic diversity in sticklebacks

Knowledge of the genes and mutations underlying phenotypic diversity is integral to understanding the dynamics of evolutionary change. Specific genes have now been identified that contribute to variation in seven phenotypic traits in threespine stickleback and one in ninespine stickleback (electronic supplementary material, table S3) [25,28,38,64–70]. These phenotypes include skeletal traits important for defence (lateral plates, pelvic spines) and feeding (tooth number), pigmentation traits and behaviour and sensory system traits. Although it is more difficult to identify genes that underlie QTL of small effect, the underlying genes have been identified for QTL with effect sizes ranging from 9.9 PVE (plate size) to essentially 100 PVE (pelvic reduction). Because QTL mapping often identifies large genomic intervals containing dozens to hundreds of genes, additional high-resolution linkage mapping or association mapping in wild populations has been used to narrow down the

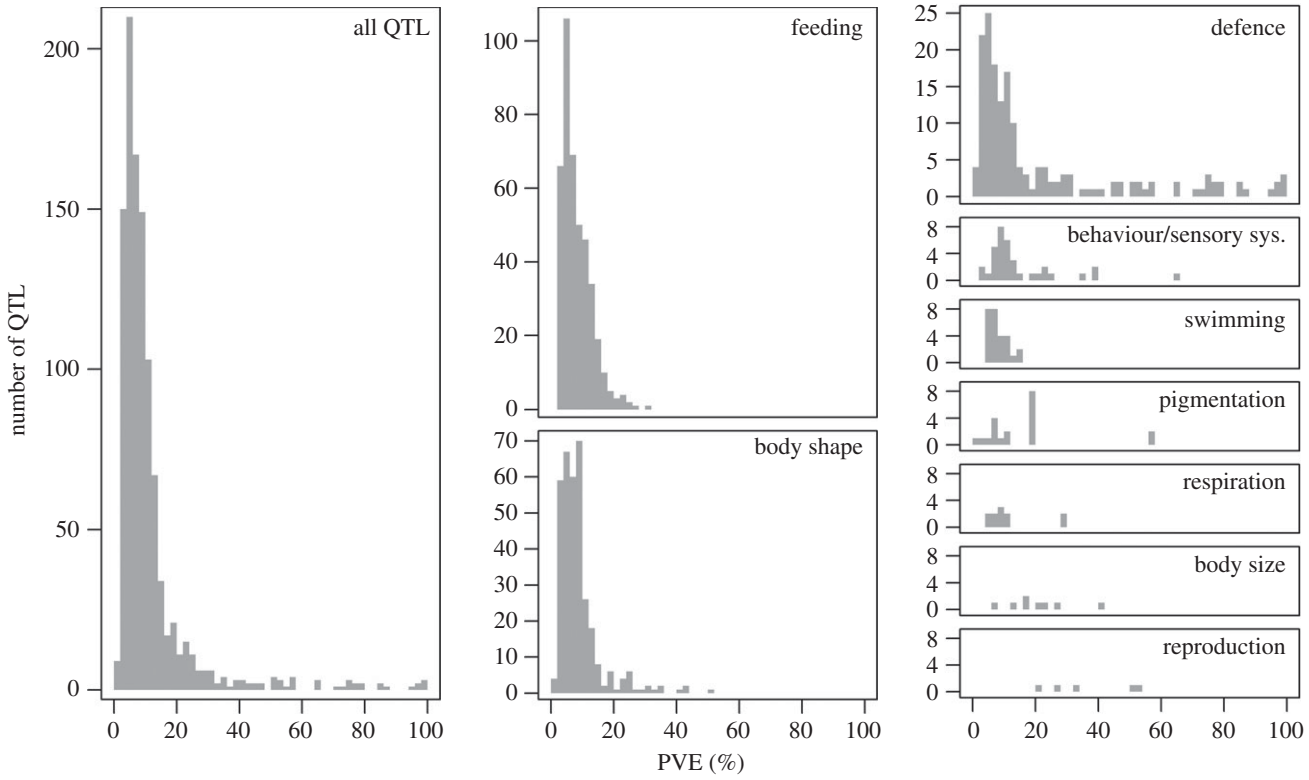

Figure 2. Distribution of effect size for QTL identified in threespine stickleback. Per cent variance explained (PVE) was available for 1034 QTL identified in 28 studies in threespine stickleback across nine trait categories (feeding, $N = 416$; body shape, $N = 342$; defence, $N = 170$; behaviour and sensory system, $N = 35$; swimming, $N = 27$; pigmentation, $N = 20$; respiration, $N = 11$; body size $N = 8$; reproduction, $N = 5$). Data are provided in electronic supplementary material, table S1; 63 QTL identified in the combined scan in Conte *et al.* [44] were redundant and therefore removed from this analysis.

intervals. The presence of interesting candidate genes with known functions in other systems within the QTL regions has also been crucial to the success of these studies. However, it is still challenging to go from an excellent candidate gene to demonstrating that variation in the gene is responsible for variation in the phenotype of interest. Several studies have used allele-specific expression assays to demonstrate that there is differential expression of the candidate gene in a tissue consistent with the phenotypic differences between the populations, and that differential expression is due to changes in *cis* at the gene itself, rather than to changes in *trans* elsewhere in the genome [28,38,65,68–70]. However, the gold standard for demonstrating causality between a genetic change and a phenotypic change is to perform genetic manipulations. Transgenic methods have been used to demonstrate that variation in the *Eda* gene has pleiotropic effects on variation in lateral plate number, lateral line patterning and schooling behaviour [64,66,67]; variation in the *Pitx1* gene leads to loss of pelvic spines [68] and variation in the *Gdf6* gene affects lateral plate size [70].

(a) Does phenotypic evolution occur through changes in coding or regulatory regions of genes?

In all cases so far, the genes identified in stickleback are developmental regulators, often with lethal or highly deleterious effects when the coding regions are disrupted in laboratory organisms. It has been hypothesized that mutations in the tissue-specific regulatory elements of such developmental regulatory genes would be more likely to contribute to phenotypic evolution because such mutations are more likely to avoid the negative pleiotropic consequences of mutations in coding changes in these genes [71,72]. The data in sticklebacks are consistent with this hypothesis, in that regulatory and not coding changes underlie variation in the phenotypes studied thus far (electronic supplementary material, table S3). However, the data are limited to a handful of traits, and it will be interesting to see whether these patterns hold true when genes that underlie many different phenotypes are identified in sticklebacks and other systems.

(b) When the same phenotype evolves, are the same or different genetic changes involved?

The repeated use of similar genes during phenotypic evolution in disparate species suggests that there may be biases and constraints on the types of genes or mutations that can be used during phenotypic evolution [73]. Sticklebacks have repeatedly and independently adapted to similar habitats, thereby providing an opportunity to ask whether the same or different genetic changes underlie similar phenotypic changes. QTL mapping studies can provide a preliminary answer to this question. For example, two studies have performed QTL mapping studies on the same suite of phenotypes in multiple crosses from independent populations adapted to similar habitats. Both studies find that about half of the QTL are shared when independent populations adapt to similar environments [44,47]. Remarkably, a meta-analyses conducted on published studies that examined the genetic basis of repeated evolution also found that the same genes were involved in about half of the cases across a diversity of traits, species and divergence times [73]. Of course, a major caveat of these analyses is that these QTL are large, and the actual genetic changes that underlie a phenotypic change might be

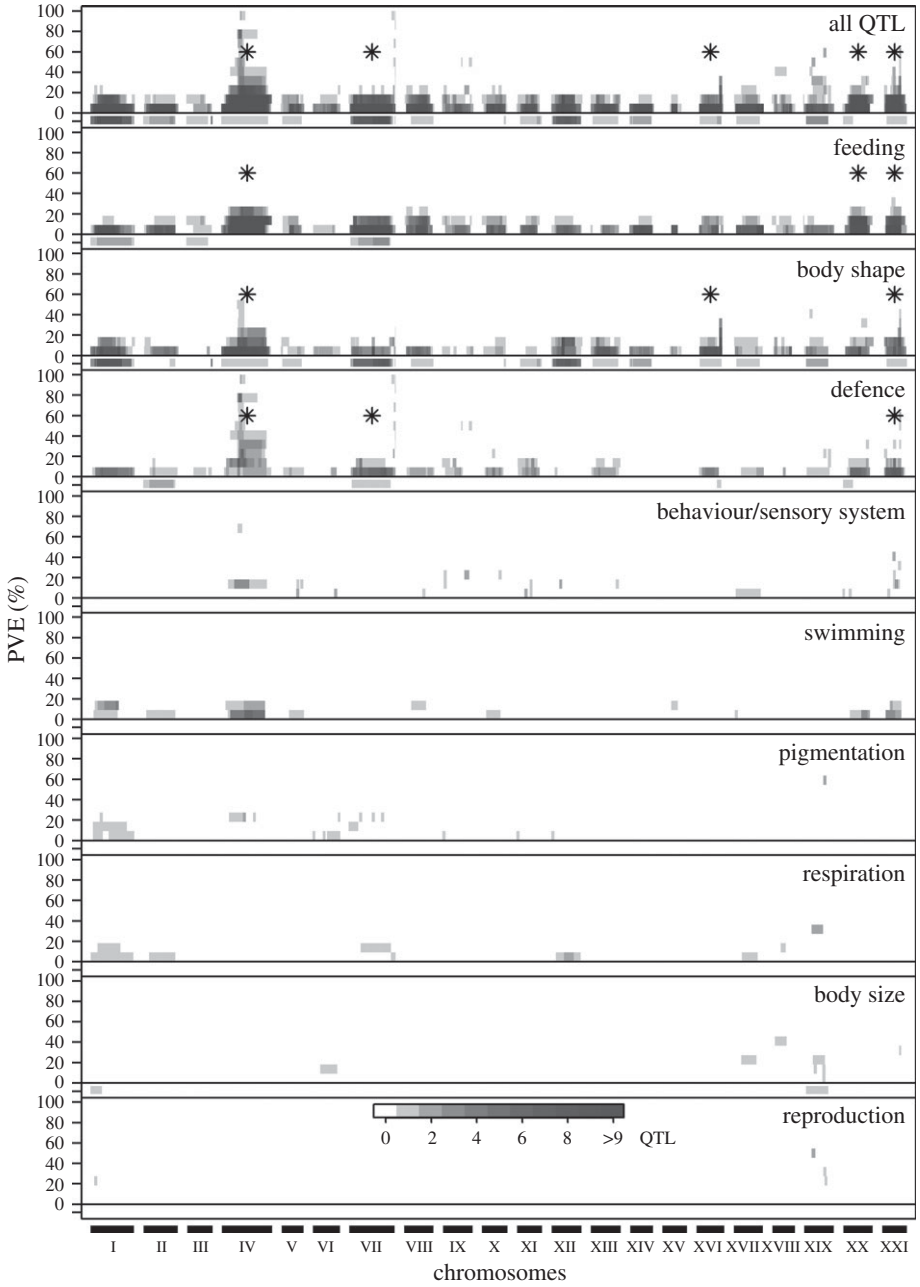

Figure 3. Genomic distribution of QTL identified in threespine stickleback. Genomic position data are based on the Glazer *et al.* [18] genome assembly for 1104 QTL identified in 28 studies in threespine stickleback across nine trait categories (feeding, $N = 422$; body shape, $N = 399$; defence, $N = 175$; behaviour and sensory system, $N = 35$; swimming, $N = 27$; pigmentation, $N = 20$; respiration, $N = 11$; body size $N = 10$; reproduction, $N = 5$). For 71 QTL, PVE estimates are not available and so were plotted in greyscale as values of -8 here. Data are provided in electronic supplementary material, table S1; 63 QTL identified in the combined scan in Conte *et al.* [44] were redundant and therefore removed from this analysis, and one QTL mapped to an unassembled chromosome (chrUN) and was also omitted from this analysis. Chromosomes with more QTL than expected are indicated with an asterisk; full results of statistical analyses are provided in electronic supplementary material, table S4.

different even though the phenotypes map to an overlapping QTL region. When the underlying gene has actually been identified in stickleback, the results are consistent with the QTL studies. There are cases in which the same gene is used in independent populations and cases in which different genes are used (electronic supplementary material, table S3). Interestingly, both mechanisms can be involved when the same trait evolves. For example, the *Pitx1* gene is responsible for the independent loss of pelvic structures in both threespine and ninespine stickleback populations; however, there are also threespine and ninespine populations with pelvic loss that is unlinked to mutations in *Pitx1* [25,49,51,68,69]. Remarkably, it has also been suggested that variation in the *Pitx1* gene contributes to loss of hindlimbs in manatees [69], and variation in other genes and pathways important for phenotypic evolution in sticklebacks (*Kitlg*, *Gdf6*, *Eda*) has also been implicated in phenotypic evolution in humans [28,70,74,75].

(c) If the same genetic changes are found, is this due to standing variation or new mutation?

When the same phenotypes evolve in closely related populations or species, the same genes may be involved due to repeated selection for standing genetic variation or new mutation in these genes [76]. In sticklebacks, selection on standing genetic variation in the marine population clearly plays an important role in adaptation to freshwater. This was first demonstrated with the discovery that the *Eda* gene

is the major locus that underlies lateral plate reduction [64], a phenotype that is one of the hallmarks of most freshwater stickleback populations across the world (figure 1). Sequencing of the region around *Eda* revealed that completely plated marine sticklebacks and low-plated freshwater sticklebacks have very divergent haplotypes, and that the freshwater haplotype is found at low frequency in the marine population [64,65]. Similarly, selection on standing variation in the marine population has also played a role in the evolution of pigmentation [28] and likely plate size [70] in freshwater. However, selection on standing genetic variation is not the only genetic mechanism that underlies phenotypic evolution in sticklebacks, and new mutation is also important. For example, multiple independent deletions of the same enhancer in the *Pitx1* gene underlie the loss of pelvic spines that has occurred repeatedly in freshwater populations [68]. Interestingly, the *Pitx1* gene is located in a region of the stickleback genome that is predicted to be physically fragile, suggesting that repeated evolution might also occur via reuse of the same genes because of inherent differences in mutation rates. Although it is still unclear whether new mutation or selection on standing genetic variation plays a more important role in stickleback evolution, it is clear that these studies have led to new insights about the molecular mechanisms that are likely to contribute to rapid adaptation in many systems.

4. Perspectives and future directions

Much progress has been made in the 15 years since the development of the first genetic linkage map for threespine stickleback [10]. A major challenge for the future is to identify the genes and mutations that underlie additional phenotypic traits in sticklebacks. The development of new genome-editing tools like TALENs and CRISPR/Cas9 for use in sticklebacks [22] will greatly facilitate this research, enabling us to discern whether there are general patterns in the genetic and molecular architecture of phenotypic evolution, at least in sticklebacks. It will also be important to compare results in sticklebacks to those in other systems, like those highlighted in this special issue [77–81], to determine whether the genetic and molecular basis of phenotypic evolution is contingent on the study system or whether general evolutionary patterns will emerge.

This review has focused on recent efforts to identify the links between phenotype and genotype in order to understand phenotypic diversity. However, in most cases, the fitness effects and, therefore, adaptive significance of these genotypes and phenotypes are unknown. With the identification of genes that affect specific phenotypes, we now have the ability to make connections between genotypes and fitness by placing alternative alleles of these genes on a uniform genetic background and following these alleles in semi-natural environments, or by tracking the fitness effects of specific alleles in natural populations [82–85]. A complementary approach to identify links between genotypes and fitness is to conduct population genomic studies, which can identify genomic regions under selection in different habitats [86]. Because these studies are unable to identify the phenotypes that are targets of selection, there is a need to integrate results of QTL studies with the numerous population genomic studies that have now been conducted in sticklebacks (e.g. [14,87–89]). By integrating QTL and genomic data, as has been done in pea aphids and whitefish [90,91], we can identify phenotypes that might be associated with those genomic regions under selection. We can also learn which genomic regions under selection are not associated with any traits mapped to date, potentially revealing additional phenotypes that might be under selection in sticklebacks.

Ultimately, combining these approaches will allow us to make connections between genotypes, phenotypes and fitness, to provide a more holistic understanding of the genetic basis of adaptation [86]. Given the rich history of evolutionary, ecological and ethological research, the excellent genetic and genomic resources present, and the collaborative stickleback community, the stickleback model system is poised to continue to reveal many new insights into the genetic and molecular basis of phenotypic diversity in nature.

Data accessibility. The QTL dataset supporting this article has been uploaded as part of the electronic supplementary material.

Authors' contributions. D.A.M. compiled the database of QTL studies in sticklebacks. C.L.P. and D.A.M. performed the analyses and prepared the figures. C.L.P. wrote the manuscript with input from D.A.M.

Competing interests. We have no competing interests.

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References

1. Wooton RJ. 1976 *The biology of sticklebacks*. London, UK: Academic Press.
2. Bell MA, Foster SA. 1994 *The evolutionary biology of the threespine stickleback*. Oxford, UK: Oxford University Press.
3. Ostlund-Nilsson S, Mayer I, Huntingford FA. 2007 *Biology of the three-spined stickleback*. Boca Raton, FL: CRC Press.
4. Bell MA, Stewart JD, Park PJ. 2009 The world's oldest fossil threespine stickleback fish. *Copeia* **2009**, 256–265. (doi:10.1643/CG-08-059)
5. Merilä J. 2013 Nine-spined stickleback (*Pungitius pungitius*): an emerging model for evolutionary biology research. *Ann. N.Y. Acad. Sci.* **1289**, 18–35. (doi:10.1111/nyas.12089)
6. Hendry AP, Peichel CL, Matthews B, Boughman JW, Nosil P. 2013 Stickleback research: the now and the next. *Evol. Ecol. Res.* **15**, 111–141.
7. McKinnon JS, Rundle HD. 2002 Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.* **17**, 480–488. (doi:10.1016/S0169-5347(02)02579-X)
8. Hendry AP, Bolnick DI, Berner D, Peichel CL. 2009 Along the speciation continuum in stickleback. *J. Fish Biol.* **75**, 2000–2036. (doi:10.1111/j.1095-8649.2009.02419.x)
9. Gibson G. 2005 The synthesis and evolution of a supermodel. *Science* **307**, 1890–1891. (doi:10.1126/science.1109835)
10. Peichel CL, Nereng KS, Ohgi KA, Cole BLE, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM. 2001 The genetic architecture of divergence between threespine stickleback species.

Nature **414**, 901–905. (doi:10.1111/j.1365-294X.2012.05660.x)

11. Kingsley DM *et al.* 2004 New genomic tools for molecular studies of evolutionary change in sticklebacks. *Behaviour* **141**, 1331–1344. (doi:10.10.1038/414901a)
12. Kingsley DM, Peichel CL. 2007 The molecular genetics of evolutionary change in sticklebacks. In *Biology of the three-spined stickleback* (eds S Östlund-Nilsson, I Mayer, F Huntingford), pp. 41–81. Boca Raton, FL: CRC Press.
13. Jones FC *et al.* 2012 A genome-wide SNP genotyping array reveals patterns of global and repeated species-pair divergence in sticklebacks. *Curr. Biol.* **22**, 83–90. (doi:10.1016/j.cub.2011.11.045)
14. Jones FC *et al.* 2012 The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**, 55–61. (doi:10.1038/nature10944)
15. Miller MR, Dunham JP, Amores A, Cresko WA, Johnson EA. 2007 Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Res.* **17**, 240–248. (doi:10.1101/gr.5681207)
16. Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA. 2008 Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* **3**, e3376. (doi:10.1371/journal.pone.0003376)
17. Roesti M, Moser D, Berner D. 2013 Recombination in the threespine stickleback genome—patterns and consequences. *Mol. Ecol.* **22**, 3014–3027. (doi:10.1111/mec.12322)
18. Glazer AM, Killingbeck EE, Mitros T, Rokhsar DS, Miller CT. 2015 Genome assembly improvement and mapping convergently evolved skeletal traits in sticklebacks with genotyping-by-sequencing. *Genes Genomes Genet.* **5**, 1463–1472. (doi:10.1534/g3.115.017905)
19. Rastas P, Calboli FCF, Guo B, Shikano T, Merilä J. 2016 Construction of ultradense linkage maps with Lep-MAP2: stickleback F_2 recombinant crosses as an example. *Genome Biol. Evol.* **8**, 78–93. (doi:10.1093/gbe/ew250)
20. Hosemann KE, Colosimo PF, Summers BR, Kingsley DM. 2004 A simple and efficient microinjection protocol for making transgenic sticklebacks. *Behaviour* **141**, 1345–1355. (doi:10.1163/1568539042948097)
21. Erickson PA, Cleves PA, Ellis NA, Schwalbach KT, Hart JC, Miller CT. 2015 A 190 base pair, TGF-β responsive tooth and fin enhancer is required for stickleback *Bmp6* expression. *Dev. Biol.* **401**, 310–323. (doi:10.1016/j.ydbio.2015.02.006)
22. Erickson PA, Ellis NA, Miller CT. 2016 Microinjection for transgenesis and genome editing in threespine stickleback. *J. Vis. Exp.* **111**, e54055. (doi:10.3791/54055)
23. Colosimo PF, Peichel CL, Nereng K, Blackman BK, Shapiro MD, Schluter D, Kingsley DM. 2004 The genetic architecture of parallel armor plate reduction in threespine sticklebacks. *PLoS Biol.* **2**, 635–641. (doi:10.1371/journal.pbio.0020109)
24. Cresko WA, Amores A, Wilson C, Murphy J, Currey M, Phillips P, Bell MA, Kimmel CB, Postlethwait JH. 2004 Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. *Proc. Natl Acad. Sci. USA* **101**, 6050–6055. (doi:10.1073/pnas.0308479101)
25. Shapiro MD, Marks ME, Peichel CL, Blackman BK, Nereng KS, Jónsson B, Schluter D, Kingsley DM. 2004 Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* **428**, 717–723. (doi:10.1038/nature02415)
26. Kimmel CB, Ullman B, Walker C, Wilson C, Currey M, Phillips PC, Bell MA, Postlethwait JH, Cresko WA. 2005 Evolution and development of facial bone morphology in threespine stickleback. *Proc. Natl Acad. Sci. USA* **102**, 5791–5796. (doi:10.1073/pnas.0408533102)
27. Coyle SM, Huntingford FA, Peichel CL. 2007 Parallel evolution of *Pitx1* underlies pelvic reduction in Scottish threespine stickleback (*Gasterosteus aculeatus*). *J. Hered.* **98**, 581–586. (doi:10.1093/jhered/esm066)
28. Miller CT, Beleza S, Pollen AA, Schluter D, Kittles RA, Shriver MD, Kingsley DM. 2007 *cis*-regulatory changes in *Kit Ligand* expression and parallel evolution of pigmentation in sticklebacks and humans. *Cell* **131**, 1179–1189. (doi:10.1016/j.cell.2007.10.055)
29. Albert AYK, Sawaya S, Vines TH, Knecht AK, Miller CT, Summers BR, Balabhadra S, Kingsley DM, Schluter D. 2008 The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evolution* **62**, 76–85. (doi:10.1111/j.1558-5646.2007.00259.x)
30. Kitano J *et al.* 2009 A role for a neo-sex chromosome in stickleback speciation. *Nature* **461**, 1079–1083. (doi:10.1038/nature08441)
31. Greenwood AK *et al.* 2011 The genetic basis of divergent pigment patterns in juvenile threespine sticklebacks. *Heredity* **107**, 155–166. (doi:10.1038/hdy.2011.1)
32. Malek TB, Boughman JW, Dworkin I, Peichel CL. 2012 Admixture mapping of male nuptial color and body shape in a recently formed hybrid population of threespine stickleback. *Mol. Ecol.* **21**, 5265–5279. (doi:10.1111/j.1365-294X.2012.05660.x)
33. Rogers SM, Tamkee P, Summers B, Balabhadra S, Marks M, Kingsley DM, Schluter D. 2012 Genetic signature of adaptive peak shift in threespine stickleback. *Evolution* **66**, 2439–2450. (doi:10.1111/j.1558-5646.2012.01622.x)
34. Wark AR *et al.* 2012 Genetic architecture of variation in the lateral line sensory system of threespine sticklebacks. *Genes Genomes Genet.* **2**, 1047–1056. (doi:10.1534/g3.112.003079)
35. Greenwood AK, Wark AR, Yoshida K, Peichel CL. 2013 Genetic and neural modularity underlie the evolution of schooling behavior in threespine sticklebacks. *Curr. Biol.* **23**, 1884–1888. (doi:10.1016/j.cub.2013.07.058)
36. Arnegard ME *et al.* 2014 Genetics of ecological divergence during speciation. *Nature* **511**, 307–311. (doi:10.1038/nature13301)
37. Berner D, Moser D, Roesti M, Buescher H, Salzburger W. 2014 Genetic architecture of skeletal evolution in European lake and stream stickleback. *Evolution* **68**, 1792–1805. (doi:10.1111/evo.12390)
38. Cleves PA, Ellis NA, Jimenez MT, Nunez SM, Schluter D, Kingsley DM, Miller CT. 2014 Evolved tooth gain in sticklebacks is associated with a *cis*-regulatory allele of *Bmp6*. *Proc. Natl Acad. Sci. USA* **111**, 13 912–13 917. (doi:10.1073/pnas.1407567111)
39. Erickson PA, Glazer AM, Cleves PA, Smith AS, Miller CT. 2014 Two developmentally temporal quantitative trait loci underlie convergent evolution of increased branchial bone length in sticklebacks. *Proc. R. Soc. B* **281**, 20140822. (doi:10.1098/rspb.2014.0822)
40. Glazer AM, Cleves PA, Erickson PA, Lam AY, Miller CT. 2014 Parallel developmental genetic features underlie stickleback gill raker evolution. *EvoDevo* **5**, 19. (doi:10.1186/2041-9139-5-19)
41. Liu J, Shikano T, Leinonen T, Cano JM, Li M-H, Merilä J. 2014 Identification of major and minor QTL for ecologically important morphological traits in three-spined sticklebacks (*Gasterosteus aculeatus*). *Genes Genomes Genet.* **4**, 595–604. (doi:10.1534/g3.114.010389)
42. Miller CT *et al.* 2014 Modular skeletal evolution in sticklebacks is controlled by additive and clustered quantitative trait loci. *Genetics* **197**, 405–420. (doi:10.1534/genetics.114.162420)
43. Yoshida K *et al.* 2014 Sex chromosome turnover contributes to genomic divergence between incipient stickleback species. *PLoS Genet.* **10**, e1004223. (doi:10.1371/journal.pgen.1004223)
44. Conte GL, Arnegard ME, Best J, Chan YF, Jones FC, Kingsley DM, Schluter D, Peichel CL. 2015 Extent of QTL reuse during repeated phenotypic divergence of sympatric threespine stickleback. *Genetics* **201**, 1189–1200. (doi:10.1534/genetics.115.182550)
45. Ellis NA, Glazer AM, Donde NN, Cleves PA, Agoglia RM, Miller CT. 2015 Distinct developmental genetic mechanisms underlie convergently evolved tooth gain in sticklebacks. *Development* **142**, 2442–2451. (doi:10.1242/dev.124248)
46. Greenwood AK, Ardekani R, McCann SR, Dubin ME, Sullivan A, Bensussen S, Tavaré S, Peichel CL. 2015 Genetic mapping of natural variation in schooling tendency in stickleback. *Genes Genomes Genet.* **5**, 761–769. (doi:10.1534/g3.114.016519)
47. Erickson PA *et al.* 2016 Partially repeatable genetic basis of benthic adaptation in threespine sticklebacks. *Evolution* **70**, 887–902. (doi:10.1111/evo.12897)
48. Yong L, Peichel CL, McKinnon JS. 2016 Genetic architecture of conspicuous red ornaments in female threespine stickleback. *Genes Genomes Genet.* **6**, 579–588. (doi:10.1534/g3.115.024505)
49. Shapiro MD, Summers BR, Balabhadra S, Aldenhoven JT, Miller AL, Cunningham CB, Bell MA, Kingsley DM. 2009 The genetic architecture of skeletal convergence and sex determination in

ninespine sticklebacks. *Curr. Biol*. **19**, 1140–1145. (doi:10.1016/j.cub.2009.05.029)

50. Laine VN, Shikano T, Herczeg G, Vilkki J, Merilä J. 2013 Quantitative trait loci for growth and body size in the nine-spined stickleback *Pungitius pungitius* L. *Mol. Ecol*. **22**, 5861–5876. (doi:10.1111/mec.12526)
51. Shikano T, Laine VN, Herczeg G, Vilkki J, Merilä J. 2013 Genetic architecture of parallel pelvic reduction in ninespine sticklebacks. *Genes Genomes Genet.* **3**, 1833–1842. (doi:10.1534/g3.113.007237)
52. Laine VN, Herczeg G, Shikano T, Vilkki J, Merilä J. 2014 QTL analysis of behavior in nine-spined sticklebacks (*Pungitius pungitius*). *Behav. Genet*. **44**, 77–88. (doi:10.1007/s10519-013-9624-8)
53. Fisher RA. 1930 *The genetical theory of natural selection*. Oxford, UK: Oxford University Press.
54. Kimura M. 1983 *The neutral theory of molecular evolution*. Cambridge, UK: Cambridge University Press.
55. Orr HA. 1998 The population genetics of adaptation: distribution of factors fixed during adaptive evolution. *Evolution* **52**, 935–949. (doi:10.2307/2411226)
56. Orr HA. 2005 The genetic theory of adaptation: a brief history. *Nat. Rev. Genet.* **6**, 119–127. (doi:10.1038/nrg1523)
57. Dittmar EL, Oakley CG, Conner JK, Gould BA, Schemske DW. 2016 Factors influencing the effect size distribution of adaptive substitutions. *Proc. R. Soc. B*. **283**, 20153065. (doi:10.1098/rspb.2015.3065)
58. Beavis WD. 1998 QTL analyses: power, precision and accuracy. In *Molecular dissection of complex traits* (ed. AH Paterson), pp. 145–162. Boca Raton, FL: CRC Press.
59. Kirkpatrick M, Barton N. 2006 Chromosome inversions, local adaptation and speciation. *Genetics* **173**, 419–434. (doi:10.1534/genetics.105.047985)
60. Hoffmann AA, Rieseberg LH. 2010 Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptation and speciation. *Ann. Rev. Ecol. Evol. Syst*. **39**, 21–42. (doi:10.1146/annurev.ecolsys.39.110707.173532)
61. Schwander T, Libbrect R, Keller L. 2014 Supergenes and complex phenotypes. *Curr. Biol*. **24**, R288–R294. (doi:10.1016/j.cub.2014.01.056)
62. Peichel CL *et al.* 2004 The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. *Curr. Biol.* **14**, 1416–1424. (doi:10.1016/j.cub.2004.08.030)
63. Qvarnström A, Bailey RI. 2008 Speciation through evolution of sex-linked genes. *Heredity* **102**, 4–15. (doi:10.1038/hdy.2008.93)
64. Colosimo PF *et al.* 2005 Widespread parallel evolution in sticklebacks by repeated fixation of *Ectodysplasin* alleles. *Science* **307**, 1928–1933. (doi:10.1126/science.1107239)
65. O'Brown NM, Summers BR, Jones FC, Brady SD, Kingsley DM. 2015 A recurrent regulatory change underlying altered expression and Wnt response of the stickleback armor plates gene *EDA*. *eLife* **4**, e05290. (doi:10.7554/eLife.05290)
66. Mills MG, Greenwood AK, Peichel CL. 2014 Pleiotropic effects of a single gene on skeletal development and sensory system patterning in sticklebacks. *EvoDevo* **5**, 5. (doi:10.1186/2041-9139-5-5)
67. Greenwood AK, Mills MG, Wark AR, Archambeault SL, Peichel CL. 2016 Evolution of schooling behavior in threespine sticklebacks is shaped by the *Eda* gene. *Genetics* **203**, 677–681. (doi:10.1534/genetics.116.188342)
68. Chan YF *et al.* 2010 Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer. *Science* **327**, 302–305. (doi:10.1126/science.1182213)
69. Shapiro MD, Bell MA, Kingsley DM. 2006 Parallel genetic origins of pelvic reduction in vertebrates. *Proc. Natl Acad. Sci. USA* **103**, 13 753–13 758. (doi:10.1073/pnas.0604706103)
70. Indjeian VB, Kingman GA, Jones FC, Guenther CA, Grimwood J, Schmutz J, Myers RM, Kingsley DM. 2016 Evolving new skeletal traits by *cis*-regulatory changes in bone morphogenetic proteins. *Cell* **164**, 145–156. (doi:10.1016/j.cell.2015.12.007)
71. King MC, Wilson AC. 1975 Evolution at two levels in humans and chimpanzees. *Science* **188**, 107–116. (doi:10.1126/science.1090005)
72. Carroll SB. 2008 Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* **134**, 25–36. (doi:10.1016/j.cell.2008.06.030)
73. Conte GL, Arnegard ME, Peichel CL, Schluter D. 2012 The probability of genetic parallelism and convergence in natural populations. *Proc. R. Soc. B*. **279**, 5039–5047. (doi:10.1098/rsbp.2012.2146)
74. Kamberov YG *et al.* 2013 Modeling recent human evolution in mice by expression of a selected EDAR variant. *Cell* **152**, 691–702. (doi:10.1016/j.cell.2013.01.016)
75. Guenther CA, Tasic B, Luo L, Beddell MA, Kingsley DM. 2014 A molecular basis for classic blond hair in Europeans. *Nat. Genet*. **46**, 748–752. (doi:10.1038/ng.2991)
76. Barrett RDH, Schluter D. 2008 Adaptation from standing genetic variation. *Trends Ecol. Evol*. **23**, 38–44. (doi:10.1016/j.tree.2007.09.08)
77. Tokita M, Yano W, James HF, Abzhanov A. 2017 Cranial shape evolution in adaptive radiations of birds: comparative morphometrics of Darwin's finches and Hawaiian honeycreepers. *Phil. Trans. R. Soc. B* **372**, 20150481. (doi:10.1098/rstb.2015.0481)
78. Harrison CJ. 2017 Development and genetics in the evolution of land plant body plans. *Phil. Trans. R. Soc. B* **372**, 20150490. (doi:10.1098/rstb.2015.0490)
79. Jiggins CD, Wallbank RWR, Hanly JJ. 2017 Waiting in the wings: what can we learn about gene co-option from the diversification of butterfly wing patterns? *Phil. Trans. R. Soc. B* **372**, 20150485. (doi:10.1098/rstb.2015.0485)
80. Krishnan J, Rohner N. 2017 Cavefish and the basis for eye loss. *Phil. Trans. R. Soc. B* **372**, 20150487. (doi:10.1098/rstb.2015.0487)
81. Xu H-J, Zhang C-X. 2017 Insulin receptors and wing dimorphism in rice planthoppers. *Phil. Trans. R. Soc. B* **372**, 20150489. (doi:10.1098/rstb.2015.0489)
82. Barrett RDH, Rogers SM, Schluter D. 2008 Natural selection on a major armor gene in threespine stickleback. *Science* **322**, 255–257. (doi:10.1126/science.1159978)
83. Rennison DJ, Heilbron K, Barrett RDH, Schluter D. 2015 Discriminating selection on lateral plate phenotype and its underlying gene, *Ectodysplasin*, in threespine stickleback. *Am. Nat*. **185**, 150–156. (doi:10.1086/679280)
84. Kitano J, Bolnick DI, Beauchamp DA, Mazur MM, Mori S, Nakano T, Peichel CL. 2008 Reverse evolution of armor plates in the threespine stickleback. *Curr. Biol*. **18**, 769–774. (doi:10.1016/j.cub.2008.04.027)
85. Marchinko KB, Matthews B, Arnegard ME, Rogers SM, Schluter D. 2014 Maintenance of a genetic polymorphism with disruptive natural selection in stickleback. *Curr. Biol*. **24**, 1289–1292. (doi:10.1016/j.cub.2014.04.026)
86. Barrett RDH, Hoekstra HE. 2011 Molecular spandrels: tests of adaptation at the molecular level. *Nat. Rev. Genet*. **12**, 767–780. (doi:10.1038/nrg3015)
87. Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA. 2010 Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genet*. **6**, e1000862. (doi:10.1371/journal.pgen.1000862)
88. Roesti M, Hendry AP, Salzburger W, Berner D. 2012 Genome divergence during evolutionary diversification as revealed in replicate lake-stream stickleback population pairs. *Mol. Ecol*. **21**, 2852–2862. (doi:10.1111/j.1365-294X.2012.05509.x)
89. Marques DA, Lucek K, Meier JI, Mwaiko S, Wagner CE, Excoffier L, Seehausen O. 2016 Genomics of rapid incipient speciation in sympatric threespine stickleback. *PLoS Genet.* **12**, e1005887. (doi:10.1371/journal.pgen.1005887)
90. Renaut S, Maillet N, Normandeau E, Sauvage C, Derome N, Rogers SM, Bernatchez L. 2012 Genome-wide patterns of divergence during speciation: the lake whitefish case study. *Phil. Trans. R. Soc. B* **367**, 354–363. (doi:10.1098/rstb.2011.0197)
91. Via S, Conte G, Mason-Foley C, Mills K. 2012 Localizing F_{ST} outliers on a QTL map reveals evidence for large genomic regions of reduced gene exchange during speciation-wth-gene-flow. *Mol. Ecol*. **21**, 5546–5560. (doi:10.1111/mec.12021)