The Genetics of Adaptation and Speciation in Threespine Stickleback Species Pairs (*Gasterosteus aculeatus* species complex)

by

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Abstract

Ecological speciation appears to be a common process by which new species arise and the genetics underlying the process can substantially affect its outcome. Replicate 'benthic and limnetic' pairs of threespine stickleback species are an ideal system with which to study the genetics underlying ecological speciation. My first question was: what genetic mechanisms link divergent natural selection to reproductive isolation during ecological speciation? In chapter 2, I present an experiment designed to determine what genetic mechanism links divergent selection on body size to assortative mating by body size in the Paxton Lake species pair. I found that body size functions as a mate signal trait and determines female mate preference via phenotype matching. This implies that genes under divergent selection are the same as those underlying both components of assortative mating, a mechanism that should facilitate ecological speciation with gene flow. My second question was: what is the genetic architecture of adaptation during ecological speciation? In chapter 4, I used QTL mapping to discover the genetic architecture underlying a large number of parallel morphological differences in the Paxton and Priest Lake species pairs and found it to be polygenic and widespread throughout the genome in both. This suggests that many loci underlying ecologically important traits have diverged (and/or divergence has persisted) during ecological speciation despite homogenizing gene flow. My third question was: how predictable are the genetics of adaptation during ecological speciation and in general? Chapters 3 and 4 describe the first studies to quantitatively address this question. In chapter 4, I found that about 50% of QTL for parallel morphological differences are parallel in the two species pairs. Also, on average, the proportional similarity of QTL use underlying individual traits is about 0.4. In Chapter 3, I present the results of a meta-analysis of the genetics underlying repeated phenotypic evolution in natural populations. Using an impartial literature review, I found that the average probability of gene reuse was 0.32 - 0.55. I also found that the probability of gene reuse declined with increasing age of the taxa compared.

Preface

A version of chapter 2 has been published. Conte, G.L., and D. Schluter. 2013. Experimental confirmation that body size determines mate preference via phenotype matching in a stickleback species pair. *Evolution* 67-5: 1477-1484. I conceived of and implemented the experiment, analyzed the data and wrote the manuscript. My supervisor and co-author, Dolph Schluter, advised on and helped with all aspects of the project. Jacob Best, a research assistant, assisted in the set-up and implementation of the experiment.

A version of chapter 3 has been published. Conte, G.L., M.E. Arnegard, C.L. Peichel, and D. Schluter. 2012. The probability of genetic parallelism and convergence in natural populations. *Proc. R. Soc. B* 279: 5039-5047. Dolph Schluter and I conceived of the study, with Catherine Peichel and Matthew Arnegard making important contributions. I conducted the literature survey and led the subsequent detailed investigations of the literature, this task being divided evenly between all co-authors. I organized and analyzed the data with help and supervision from Dolph Schluter. I wrote a large part of the manuscript, but Dolph Schluter made significant contributions to the writing as well.

Chapter 4 is part of a larger study to investigate the genetic architecture of ecological speciation. Catherine Peichel and Dolph Schluter are co-principal investigators of this larger project, and Matthew Arnegard and myself are the leading researchers. For Chapter 4, Dolph Schluter, Catherine Peichel, Matthew Arnegard and myself all contributed the conception of the project. I made the crosses, raised the F_1 hybrids and initiated the pond populations of F_2 hybrids. The F_2 hybrids specimens were collected and preserved by Matthew Arnegard and myself with help from two assistants, Jacob Best and Nicole Bedford. Wild-caught benthic and limnetic specimens were collected and photographed by Richard Svanback and Jennifer Gow. Jacob Best scored morphological phenotypes of all specimens with supervision and guidance from Matthew Arnegard and myself. Jacob Best and I performed all DNA extractions and prepared DNA samples for

genotyping. Genotyping was done at the Fred Hutchinson Cancer Research Center's Genomics Shared Resource by its supervisor, Cassie Sather, under the direction of Catherine Peichel. I curated the data, performed all data analyses, and wrote the manuscript with supervision and help from Dolph Schluter.

I am certified by the Canadian Council on Animal Care (CCAC) / National Institutional Animal User Training (NIAUT) Program. My certificate number is 4061 – 11. Permission for collections of wild threespine sticklebacks (*Gasterosteus aculeatus* species complex) made by my collaborators and I, and used herein was granted by the following permits: BC ministry of the Environment permit numbers NA/SU08-42033 and NA/SU09-51805; Fisheries and Oceans Canada SARA permit number SECT 08 SCI 002 and SARA-116. Permission to care for and use threespine sticklebacks for the studies herein was granted by the University of British Columbia Animal Care Certificate A07-0293.

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Being a member of the Biodiversity Research Centre and the Zoology Department at UBC has been both an honor and a joy. The community, comprised of "power-house" faculty and the impressive students and post-docs they attract, has been inspiring to say the least. Perhaps the most outstanding quality of the community is how interactive its members are, in both scientific and social realms, and for that I am grateful.

My greatest thanks of all go to my family and friends, whose love and support are the very source of my motivation. I thank my mom and dad for, well, nearly everything. I thank my sister for being my closest friend. I thank my husband for his daily love, support, patience and assurance; he is my rock. I thank my extended family for their encouragement and for the tight-knit support group they have always provided. I thank my family in-law for their generous and warm-hearted support and for providing me with the spark of inspiration that set me on an academic track.

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To John, Mary Lynn, Richard and Jessica

1 Introduction

Ecological speciation, whereby ecologically based divergent natural selection leads to the evolution of reproductive isolation, is now thought to be responsible for the origin of a large portion of biological diversity (Schluter 2009; Nosil 2012). Due to the generality and simplicity of its mechanism, ecological speciation can occur in any geographic context and involve a wide variety of reproductive barriers (Schluter 2001; Nosil 2012).

Ecological speciation can be broken down into three necessary components: 1) a source of ecologically-based divergent selection, 2) a form of reproductive isolation and 3) a genetic mechanism linking the two (Kirkpatrick and Ravigné 2002; Rundle and Nosil 2005). In the past couple of decades, we have accumulated a fair amount of data regarding the first two components, while data on the third is still scarce. My PhD work has focused on the genetics underlying the process of ecological speciation. Because ecological speciation is fundamentally based on the process of adaptation, parts of my work also have great relevance to the broader topic of the genetics of adaptation.

The three primary questions addressed herein are: 1) what genetic mechanisms link divergent natural selection to reproductive isolation during ecological speciation?, 2) what is the genetic architecture of adaptation during ecological speciation? and 3) how predictable are the genetics of adaptation during ecological speciation and in general?.

Question 1: what genetic mechanisms link divergent natural selection to reproductive isolation during ecological speciation?

For a form of reproductive isolation to evolve during ecological speciation with gene flow, there must be a genetic mechanism by which it becomes associated with loci under divergent natural selection. Some forms of reproductive isolation accompany divergent natural selection by definition, such as reduced immigrant

and hybrid fitness (a.k.a. extrinsic reproductive isolation) (Schluter 2001; Schluter and Conte 2009; Nosil 2012). This reproductive isolation is, by nature, a result of the same traits that are under divergent selection, and therefore the same underlying loci. Thus, the genetic mechanism linking the two is unambiguous (Schluter and Conte 2009).

Alone, reduced immigrant and hybrid fitness resulting from divergent natural selection may often not be strong enough to result in total reproductive isolation, and speciation may thus involve the evolution of additional forms of reproductive isolation. There are two general genetic mechanisms by which this can occur. First, the very traits under divergent natural selection (and therefore, their underlying loci) may sometimes also cause other forms of reproductive isolation, such as assortative mating, habitat/spatial isolation, temporal isolation or even intrinsic incompatibilities (Mayr 1942; Dobzhansky 1951; Schluter 2001; Nosil 2012). Various sets of terminology exist to explain this phenomenon, including the 'by-product' mechanism (Schluter 2001), pleiotropy (Bolnick and Fitzpatrick 2007), direct selection (Kirkpatrick and Ravigné 2002), the magic trait mechanism (Gavrilets 2004) and the one-allele mechanism (Felsenstein 1981; Servedio 2009). Finding these types of relationships between traits/loci under divergent selection and those involved in additional forms of reproductive isolation is thought to be a 'signature' (though not a requirement) of ecological speciation (Rundle and Nosil 2005).

Alternatively, natural selection may drive the evolution of additional forms of prezygotic reproductive isolation, conferred by loci other than those under divergent natural selection, if they prevent mal-adaptive hybridization; a process known as reinforcement (Kirkpatrick and Ravigné 2002; Servedio and Noor 2003; Bolnick and Fitzpatrick 2007). However, when reproductive isolation is incomplete and individuals adapted to different environments still hybridize, recombination may prevent the build up of linkage disequilibrium between favorable combinations of alleles at different loci in the genome (Kirkpatrick and Ravigné 2002; Gavrilets 2004). A seminal model by Felsenstein (1981) showed that for speciation with gene flow to occur when loci under divergent selection and loci controlling assortative

mating are unlinked, either divergent selection must be very strong or a high level of assortative mating must evolve prior to the initiation of gene flow between populations. Thus, in general, the probability of reinforcement is increased by any mechanism that enhances linkage disequilibrium between the loci under divergent selection and those conferring additional forms of prezygotic reproductive isolation, such as tight physical linkage (Via 2001), regions of low recombination (Butlin 2005) and chromosomal rearrangements (Rieseberg 2001).

The type of genetic mechanism linking divergent natural selection to other forms of reproductive isolation can have a large impact on the probability that ecological speciation will occur (Felsenstein 1981; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999; Doebeli 2005; Servedio 2009). To ultimately understand the relative frequency and importance of different the genetic mechanisms in the origin of new species, we must discover what genetic mechanisms are involved when natural populations undergo ecological speciation.

Question 2: what is the genetic architecture of adaptation during ecological speciation?

The probability of ecological speciation may be influenced by the number of loci underlying the targets of divergent selection (Nosil 2012). Some considerations lead to a prediction that ecological speciation is more likely if few loci underlie divergent adaptations, while others considerations lead us to predict the opposite. If traits under divergent selection are based on fewer loci, then there will be fewer opportunities for interspecific recombination to disintegrate associations between co-adapted complexes (Arnegard and Kondrashov 2004; Gavrilets and Vose 2007). Furthermore, the waiting time to speciation may be shorter in this case, as fewer mutations may be required to produce the variation necessary for divergence (Gavrilets and Vose 2007). Finally, fewer loci imply larger locus effects and therefore stronger selection on each locus (Gavrilets and Vose 2007). This in turn, may increase the probability that selection can overcome migration at a given locus and cause divergence (Yeaman and Otto 2011; Yeaman and Whitlock 2011). On the

other hand, the more loci that are divergent, the higher the probability that other forms of reproductive isolation will arise as a correlated response of their divergence, either via pleiotropic consequences at particular loci and/or by incompatibilities with other loci. Commonly known as the snowball effect, as the number of divergent loci increases, the number of pairwise interactions that can lead to incompatibilities increases rapidly (Orr 1995; Orr and Turelli 2001).

Expectations for the genomic distribution of loci under divergent natural selection during ecological speciation with gene flow also vary. Under certain circumstances, we may expect a clumped genomic distribution of loci, since their cumulative fitness effects may be more likely to outweigh the opposing effects of migration (Yeaman and Whitlock, 2011). According to Yeaman and Whitlock (2011), populations adapt quickly to divergent selection pressures, with the loci initially involved scattered across the genome. Given enough time though, if many loci are capable of producing a phenotype, and/or if chromosomal rearrangements are possible, clumped genetic architectures tend to replace the initial scattered ones, since divergence there is more likely to persist despite the homogenizing effects of gene flow. On the other hand, if only few loci are capable of producing a phenotype, if pleiotropic consequences of rearrangements are too negative, and/or if not very much time has passed, we may expect to see a scattered genomic distribution of loci across the genome (Yeaman 2013).

Clearly, there are several important factors that may differentially influence both the number of divergently selected loci and their genomic distributions during ecological speciation. Studies in natural populations are required to test these predictions and determine under what circumstances we do, in fact, see particular types of genetic architectures.

Question 3: how predictable are the genetics of adaptation during ecological speciation and in general?

One of the most important and instructive questions currently being asked within the field of evolutionary genetics is: 'how predictable are the genetics of

adaptation?' Studying the predictability of the genetics of adaptation leads to understanding the core forces shaping them. A promising way to estimate how predictable the genetics of adaptations are is to look at how commonly repeated phenotypic adaptation is underlain by repeated genetic evolution. This topic has gained increasing popularity in the past several years, and many insightful, though qualitative, reviews have been written (Wood et al. 2005; Arendt and Reznick 2008; Gompel and Prud'homme 2009; Stern and Orgogozo 2009; Christin et al. 2010; Manceau et al. 2010; Elmer and Meyer 2011; Losos 2011; Martin and Orgogozo 2013; Stern 2013). Now, to understand exactly how predictable the genetics of adaptation are in natural populations, quantitative estimates are needed.

Threespine stickleback study system

As a model system of parallel ecological speciation with gene flow, the 'benthic and limnetic species pairs' of threespine sticklebacks (*Gasterosteus aculeatus* species complex) are a system uniquely well suited to addressing my primary questions. Although most lakes containing threespine sticklebacks harbor only one species, there are three lakes (in one case, a pair of connected lakes) in southwestern British Columbia that harbor two sympatric species, a limnetic zone dweller known as the 'limnetic' species and a littoral and benthic zone dweller known as the 'benthic' species (McPhail 1984, 1992, 1994; Schluter and McPhail 1992). (Historically, there were five such lakes (or pairs of connected lakes), but due to introduction of invasive species, one species pair collapsed (Taylor et al. 2006) and another went extinct (Hatfield 2001).) The species pair lakes were formed when glaciers receded in the late Pleistocene (10,000-20,000 years ago) (Schluter and McPhail 1992), and there appears to have been two separate ancestral invasions into each of the lakes (Schluter and McPhail 1992; McPhail 1994; Taylor and McPhail 2000). Subsequently, the evolution of the two forms within each lake has occurred independently from the other lakes (Taylor and McPhail 1999, 2000; Jones et al. 2012a).

The threespine stickleback species pairs are an ideal system in which to study the predictability of the genetics of adaptation. Despite their independent evolution, divergence within each species pair has occurred largely in parallel with the other pairs (Schluter and McPhail 1992; Schluter and Nagel 1995; McKinnon and Rundle 2002; Gow et al. 2008). Benthics and limnetics are consistently distinguished by not only resource and habitat use but by a large number of correlated morphological, behavioral and likely physiological differences (McPhail 1992; Bell and Foster 1994; Rundle et al. 2000; Boughman et al. 2005; Gow et al. 2008). The repeated evolution of these traits in correlation with the environment is interpreted as strong evidence for a role of natural selection in their evolution, because it is very unlikely that these associations arose by stochastic evolutionary forces (Rundle et al. 2000; Schluter 2000). The replicated adaptation across many different traits provides us with an opportunity to accurately gauge the predictability of the genetics of trait adaptation in a single system (i.e. holding other evolutionary parameters constant).

The threespine stickleback species pairs are also an ideal system in which to study the genetics underlying ecological speciation, the third component of ecological speciation. This is in part because we have a relatively solid understanding of the first two components. Regarding the first component of ecological speciation, 'a source of ecologically-based divergent selection', evidence suggests that divergence in ecological traits likely evolved, at least in part, due to resource competition and frequency dependent natural selection (Schluter and McPhail 1992; Schluter 2003). These pressures resulted in ecological character displacement and specialization in the use of either the benthic or the limnetic habitat zone and their respective sets of resources (Schluter and McPhail 1992; Schluter 2003). Regarding the second component, 'a form of reproductive isolation', the fitness trade-offs associated with resource-use specialization has lead to substantial extrinsic reproductive isolation (Rundle 2002; Gow et al. 2007). Morphological differences between benthics and limnetics, including body size and gill raker number, appear to underlie a strong trade-off in feeding efficiency and growth rate along the benthic-limnetic habitat gradient with morphological

intermediates (hybrids) suffering a competitive disadvantage in either habitat (Schluter 1993, 1995; Hatfield and Schluter 1999). Intermediate hybrids are thus, selected against in the wild (Rundle 2002; Gow et al. 2007). An additional form of reproductive isolation separating contemporary populations of benthic and limnetic sticklebacks is behavioral premating isolation, whereby females strongly prefer to mate with males of their own species (Nagel and Schluter 1998; Rundle et al. 2000; Boughman et al. 2005). Body size differences appear to make a predominant contribution to female mate preference (Nagel and Schluter 1998; Albert 2005; Conte and Schluter 2013).

Given our knowledge of the sources of divergent natural selection and the forms of reproductive isolation involved in ecological speciation between the benthic and limnetic species pairs, we now have the opportunity to ask what genetic mechanisms are involved.

Chapter 2:

In chapter 2 of my thesis, I describe a behavioral experiment designed to inform us of the genetic mechanism linking divergent natural selection to assortative mating by body size in a young species pair of benthic and limnetic threespine stickleback (*Question 1*). I ask whether divergent selection on body size may cause assortative mating by body size as an automatic by-product. Specifically, I use experimental manipulation of body size in mate choice trials to determine whether body size, in addition to being under divergent selection, also confers assortative mating by serving as a mate signal trait and determining female preference via phenotype matching. If so, then the genes underlying body size and assortative mating by body size are one and the same, constituting a mechanism that should facilitate ecological speciation with gene flow.

I am also involved in two other studies that are still in progress, and not described herein, that will contribute further to our understanding of the genetics of premating reproductive isolation and how they relate the to the genetics of traits under divergent natural selection (*Question 1*). Specifically, we are attempting to

directly investigate the genetic architecture of female mate choice and male nesting habitat choice using quantitative trait loci (QTL) mapping experiments. These two studies are part of a larger study to investigate the genetics of ecological speciation in the benthic and limnetic species pairs (see Preface and Chapter 4).

Chapters 3 and 4:

In Chapter 4, we investigated how similar, and therefore, how predictable the genetic architecture of divergence is between two benthic and limnetic species pairs. However first, to develop quantitative predictions, which were previously completely lacking in the literature, we conducted a meta-analysis, described in Chapter 3, to estimate the probability of parallel and convergent genetic evolution in natural populations (*Question 3*). Using the results of an impartial literature review, we estimated the probability of gene reuse in natural populations undergoing repeated phenotypic evolution. Furthermore, we asked whether the probability of gene reuse declines with increasing age of the taxa being compared.

Then, in chapter 4, I discuss our investigation of the genetic architectures of parallel differences in a large number of morphological traits in the Paxton Lake and Priest Lake species pairs. Our results are instructive of the number of loci and their genomic distributions during ecological speciation with gene flow (*Question 2*), a topic that will be discussed further in the Conclusions chapter of this thesis. Within Chapter 4, we take a step back and view our results from the more broad perspective of the genetics of adaptation (*Question 3*). We estimate the percent of QTL underlying parallel phenotypic evolution that are parallel and non-parallel. As an additional metric of genetic parallelism, we also estimate the average proportional similarity of QTL use underlying individual parallel traits. Finally, we ask whether genetic parallelism is correlated with the effect size of QTL.

2 Experimental Confirmation That Body Size Determines Mate Preference Via Phenotype Matching in a Threespine Stickleback Species Pair[1]

Introduction

Preference for mates having a similar phenotype to one's self, which we term 'mate choice by phenotype matching' (after 'phenotype matching' in Lacy and Sherman 1983), is interesting in the context of speciation, because of what it implies about the genetics underlying mate signal and mate preference. The evolution of assortative mating between diverging populations that still experience gene flow can be hindered by recombination between alleles for mate preference and those for mate signal (e.g. model 2 in Kondrashov and Kondrashov 1999; Doebeli 2005). When mate choice occurs by phenotype matching, however, such recombination cannot occur, since mate preference is based on one's own signal phenotype (e.g. model 2 in Dieckmann and Doebeli 1999, model 1 in Kondrashov and Kondrashov 1999). This implies that the genes underlying mate signal also determine mate preference values and, therefore, recombination cannot dissociate the two. Mate choice by phenotype matching should thus facilitate the evolution of assortative mating between diverging populations. Furthermore, if mate preference is for matching mate signal traits (mate choice by phenotype matching), and divergent natural selection acts on the mate signal (a.k.a. magic trait; reviewed in Servedio et al. 2011) then ecological speciation with gene flow (Schluter 2001) is greatly facilitated (e.g. model 1 in Dieckmann and Doebeli 1999). This is because a target(s)

[1] A version of chapter 2 has been published. Conte, G.L., and D. Schluter. 2013. Experimental confirmation that body size determines mate preference via phenotype matching in a stickleback species pair. *Evolution* 67-5: 1477-1484.

of divergent selection and both components of mate recognition are all controlled/determined by the same genes and assortative mating may readily evolve as an automatic by-product when divergent selection acts.

Here we test the hypothesis that mate choice by phenotype matching based on body size (hereafter 'size matching') occurs in the benthic and limnetic species pair of threespine stickleback (*Gasterosteus aculeatus* complex) residing in Paxton Lake on Texada Island, British Columbia (BC). For convenience, and following previous practice, we refer to benthics and limnetics as species because they are almost completely reproductively isolated in the wild (Schluter 1993, 1995; McPhail 1994; Nagel and Schluter 1998; Hatfield and Schluter 1999; Vamosi and Schluter 1999; Rundle et al. 2000; McKinnon and Rundle 2002; Boughman et al. 2005; Gow et al. 2007), although they have not been formally designated. The Paxton Lake pair is one of several stickleback species pairs found in small lakes of coastal BC (Schluter and McPhail 1992). In each case, one of the species, the limnetic, is small-bodied and feeds primarily on plankton in the open water whereas the second species, the benthic, is large-bodied and feeds primarily on benthos in the littoral and benthic zones (Schluter and McPhail 1992; Schluter 1993). The lakes formed shortly after the Pleistocene glaciers receded (10,000-12,000 years ago) and likely experienced two separate invasions by ancestral stickleback. Thus, an initial period of allopatry has likely contributed to divergence between the sympatric species (Schluter and McPhail 1992; McPhail 1994; Taylor and McPhail 2000). Nonetheless, since the establishment of sympatry, these pairs have been subject to gene flow (Taylor and McPhail 1999; Gow et al. 2006), yet they have evolved and/or maintained large differences in morphology, ecology and mate recognition (Schluter and McPhail 1992; Schluter 1996; Taylor and McPhail 2000). A conspicuous body size difference is important not only because it represents adaptation to the alternate foraging habitats (big fish have higher foraging efficiency and growth rate in the littoral zone, whereas small fish have the advantage in the open-water; (Schluter 1993, 1995; Hatfield and Schluter 1999)), but because it also appears to be an important mate signal trait involved in assortative mating between the two species (Nagel and Schluter 1998; Albert 2005; Boughman et al. 2005). Previous

observational studies suggest that size matching may occur. Using no-choice mating trials between wild-caught individuals, Nagel and Schluter (1998) found that females hybridized only when placed with males of the opposite species who were similar to her in size, indicating that preference changes with size difference between a mating pair. However, hybridization events tended to occur late in the season when the smallest benthics and the largest limnetics were in breeding condition. Any effect of date on level of discrimination is confounded with differences in body size (Nagel and Schluter 1998). Boughman, Rundle, and Schluter (2005) found the same negative correlation of size difference and mating compatibility in the Paxton lake pair and two other independently evolved species pairs. However, the effects of trial date were not investigated. Furthermore, other variables that tend to be correlated with body size in the wild, such as trophic niche and age, add uncertainty to the conclusions based on observational studies that size matching is occurring.

To overcome this problem, we used experimental manipulation of body size followed by mate choice trails to determine whether size matching contributes to assortative mating between benthic and limnetic sticklebacks. This experimental approach was used previously by McKinnon et al. (2004), who found that in world-wide pairs of stream and marine threespine stickleback populations, female preference changed when female size was manipulated; they were more likely to prefer a male of the opposite ecotype when they were manipulated to be more similar in size to him. This approach, however, has never been used for benthic and limnetic pairs, a study system that has become an important model of species divergence and persistence in the face of gene flow. Here, we compare the propensity of benthic and limnetic females to accept males of the opposite species when they were manipulated by diet either to be more similar or more different in size to males. Importantly, within each species, all females came from the same population and were randomly assigned to treatments. Thus, the effects of genotype, age, early-life experience and other differences potentially affecting mate preferences were randomized. If size matching is not occurring, then manipulation of females' body size should not affect their probability of accepting the

heterospecific male. However, if size matching is occurring, then females manipulated to be similar in size to the heterospecific male will be more likely to accept him than females manipulated to be different in size from him.

Methods

Fish Collection

In the fall of 2009, we collected juvenile fish from two ponds at the UBC Experimental Pond Facility, one containing only Paxton benthics and the other containing only Paxton limnetics. All individuals were thus, naive to the other species. Progenitors of the pond populations had been collected from the wild and introduced to ponds in April of 2008. We collected 400 juveniles of each species and randomly assigned them to tanks of their own species and to size-manipulation groups (either 'abundant-food' or 'reduced-food'). In total, for each of the size manipulation groups there were 10 benthic tanks and 6 limnetic tanks.

Size Manipulation

To generate size differences between groups within a species, we provided them with alternate amounts of food (a mix of blood worms and mysis shrimp). We took measures of standard length, body mass and condition factor (a scaled ratio of body mass to standard length (body mass x 10^5)/standard length^3 (Williams, 2000)) periodically from a sample of the fish from each group. To determine the appropriate amount of food for the abundant-food group that would maximize their growth rate, we periodically gave them *ad libitum* feedings and converted the amount consumed into the percent of the group's estimated average body weight that was consumed per fish on average. We gave each abundant-food tank this amount of food multiplied by the number of fish in the tank, each day. We fed the reduced-food group 2-3 times less (corrected by their groups' estimated average body weight) than that of the abundant-food group of their same species. For any given period of time, the exact reduction in food availability to the reduced-food

group depended on our desired condition and growth rate for them, with the goal being that they be fed as little as possible, while preventing significant reductions in condition factor relative to the abundant-food group.

Over-wintering

We over-wintered the fish in a temperature-controlled chamber to synchronize the onset of breeding condition upon release from winter conditions. Over-wintering took place from February 15th through June 26th 2010. At the start of "winter", temperature was gradually lowered (1 degree/day) from 17C to 8C, and photoperiod was gradually decreased (1 hour/day) from 16:8 L:D to 8:16 L:D. At the end of winter, the temperature and photoperiod were gradually increased, reversing the above changes.

Mating Trials

We conducted no-choice trials, in which a single female and a single male were allowed to interact. No-choice trials result in a score reflecting female acceptance of the male and comparison of such scores between treatments is generally thought to provide a good estimate of the female preference function (Wagner 1998; Bush et al. 2002). Females used in the trials came from both abundant and reduced-food tanks. All benthic males were from the reduced-food group (making them more similar in size to limnetics), and all limnetic males were from the abundant-food group (making them more similar in size to benthics). Thus, the two treatments for each species of female were 'different size' (control) and 'similar size' (experimental) relative to the heterospecific male (Fig A.1). In total, 15 successful trials (i.e. the data was kept) were conducted for benthic females similar in size to a limnetic male and 19 for benthic females different in size to a limnetic male. Thirteen successful trials were conducted for limnetic females similar in size to a benthic male and 10 for limnetic females different in size to a benthic male.

As a result of providing different amounts of food to generate size differences among treatments, the comparison of female mate preference between large and

small experimental fish was confounded with that between abundant-food and reduced-food fish. However, one feature of the experimental design provides a control for the effects of diet manipulation, nutrition and other correlated effects. In the larger benthic species, the control trial involved a large (abundant-food) female and the experimental trial involved a small (reduced-food) female. Conversely, in the smaller limnetic species, the control involved a small (reduced-food) female and the experimental trial involved a large (abundant-food) female. Thus, if diet/nutrition were to bias female mate preference in a predictable direction (e.g. reduced-food fish prefer larger males) then we would expect the direction of effect to be consistent with female food amounts in both species rather than control vs. experimental group.

Mating trials took place in 110 L tanks that were visually isolated from neighboring tanks. Each tank contained limestone gravel covering the bottom, small sprigs of plastic plants located in the two rear corners, a nesting dish filled with sand and soil located in the rear left corner, and a small bunch of java moss anchored next to the right side of the nesting dish. In addition, tanks were regularly replenished with short pine needles, which stickleback males use for structural support in nest building.

We added males showing signs of breeding condition (i.e. red throat color) to trial tanks and gave them five full days to build a nest. A gravid female contained in a jar was placed in the tank of each male for at least 15 mins. per day. These 'motivator females' were not used in mating trials. If a male built no nest within five days, we returned him to his rearing tank and replaced him.

Gravid females were randomly assigned to heterospecific males with nests. At the start of a trial, we released the female into the male's tank, as far from the male as possible. In all trials conducted for this study, the male began to court the female within five minutes. Trials lasted 40 minutes or until spawning. Since spawning occurred only once at around 39 min., all trials were about the same duration. We recorded the following well established courtship behaviors (see Tinbergen 1962; Rowland 1994 for a more complete description) if and when they occurred using the software *Event Recorder* (Berger and Bleed 2003): *male*

behaviors - male approaches female, male bites female, male zigzags towards female, male attempts to lead female to his nest, male performs nest-maintenance behaviors, male creeps-through his nest, *female behaviors* - female exhibits 'head-up' posture, female follows male towards his nest, female approaches male, female inspects male's nest, and female deposits eggs in nest.

Immediately after a trial, we recorded a score to visually qualify males' red nuptial color brightness/intensity, ranging from 1-3. We also measured standard length and body mass for both the female and the male. Finally, if a female did not spawn, we gently squeezed the eggs from her oviduct at the end of the trial to confirm receptivity (Nagel and Schluter 1998). Any female found not ready to mate was excluded (this occurred in 15 out of 72 trials). Among those deemed ready to mate, variation in degree of readiness likely existed. However, this variation was randomized among treatments.

Each female was used in only one trial. However, due to male-limitation, we used some males in a second trial with the opposite type of female one to three days later. For males used twice, we measured standard length and body mass after their second trial. To ensure that using some males twice did not affect the results of the experiment, the data were also analyzed using only the first trial for each male.

We used a dichotomous score to describe female acceptance of the heterospecific male. A score of 0 indicates no acceptance and a score of 1 indicates that some degree of acceptance was shown. More specifically, a score of 1 was assigned if the female reciprocated in courtship with one or more of the following behaviors: head-up posture, female follows male to his nest, female approached male at his nest, female inspects male's nest, female enters male's nest/deposits eggs. Alternatively, a score of 0 was assigned if the female did not reciprocate with any courtship behaviors. We chose this scoring method to maximize the amount of variation available to be analyzed (spawning occurred in only 1 of 57 trials and courtship rarely proceeded to the penultimate step of mating). Female courtship behaviors are known to proceed in a fixed sequence (as listed above) and females may terminate courtship at any point along this sequence. Therefore, it can be inferred that with any given threshold for a score of 1 along this sequence, the

females with a score of 1 would be more likely to mate than those with a score of 0. To demonstrate that our results were not dependent on the particular threshold chosen, we also analyze the data using a score with a more stringent requirement for a score of 1, whereby the female had to at least follow a male to his nest to be assigned a score of 1.

Dates of Trials

For benthic females only, there was a significant difference in the dates of control and experimental trials ($F_{1, 32} = 14.194$, $p = 7x10^{-4}$), because females manipulated to be large began to come into and go out of breeding condition earlier in the season than those manipulated to be small. This difference did not exist for limnetic females ($F_{1,21} = 0.1716$, $p = 0.683$). To completely eliminate trial date as a confounding variable, we also analyzed a dataset containing all trials with limnetic females and only those trials with benthic females that occurred after the first trails involving benthic females from the reduced-food regime ('similar-size' treatment) and before the last trails with the abundant-food regime ('different-size' treatment). This eliminated 10/19 benthic control trials and 3/15 benthic experimental trials. In this dataset, no statistical difference in the dates of control and experimental trails was found ($F_{1, 42} = 0.2275$, $p = 0.601$).

Analysis

We used linear models to test for the effects of size manipulation on standard length, body mass and condition factor. We used logistic regression in a generalized linear model context to test for effects on female acceptance. Our main analysis tested for the effects of experimental treatment, as well as female species and the interaction of treatment and female species. We further examined whether any other measured explanatory variables correlated with female acceptance after accounting for the effects of treatment, by including them one-at-a-time as the second term in a model also including treatment as the first term. These variables included breeding date, male nuptial color and six male courtship behaviors. Finally,

to determine the effects of treatment on male behavior, we used linear models to analyze data from only males' first trials. All analyses were performed in R v2.12.1 (R Core Team 2010).

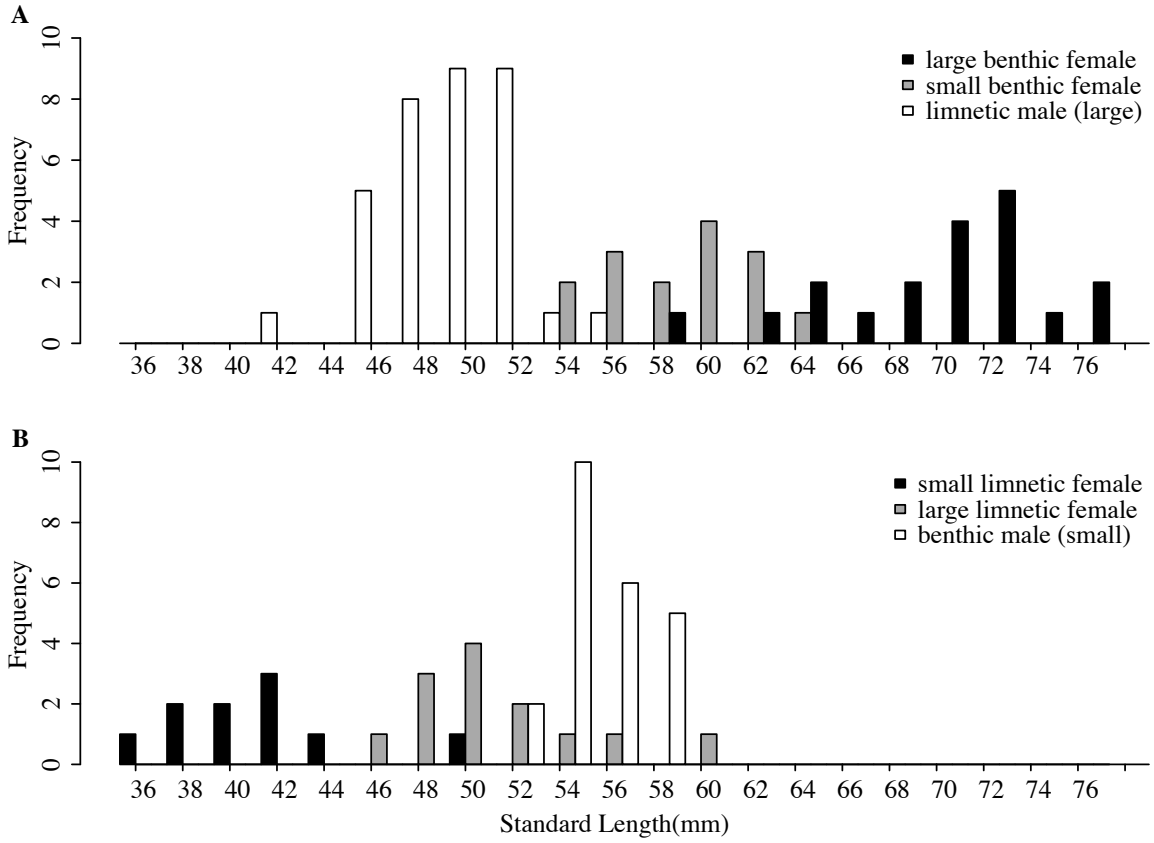
Results

Size Manipulation

The size manipulation resulted in nearly non-overlapping female size distributions between treatments within each species, with highly significant differences in mean for both standard length (Figure 2.1) and body mass (Table A.1). In addition, condition factor was only slightly greater in females manipulated to be large than in those manipulated to be small, but not significantly (Table A.1), suggesting that the manipulation successfully affected the extent of growth without greatly compromising the relative mass for a given size.

Figure 2.1 Standard length distributions after manipulation of body size

(A) Benthic females manipulated to be large and small, compared with the limnetic males (manipulated to be large) that they were paired with. (B) Limnetic females manipulated to be large and small, compared with the benthic males (manipulated to be small) that they were paired with.



Female Acceptance

Treatment (similar size vs. different size) had a highly significant effect on the female acceptance score (Table 2.1). Females paired with a male of the opposite species were more likely to reciprocate courtship behaviors when they were manipulated to be similar in size to him than when they were manipulated to be different in size to him (Figure 2.2). This treatment effect was present in all models and no other explanatory variables significantly correlated with female acceptance either before (tests not shown) or after the effects of treatment were accounted for (Table 2.1). The results from the analysis using 1) the more stringent female acceptance score (Table A.2), 2) only males' first trials (Table A.3), and 3) a subset of the data for which trial date was not a confounding variable (Table A.4), produced the same results as these.

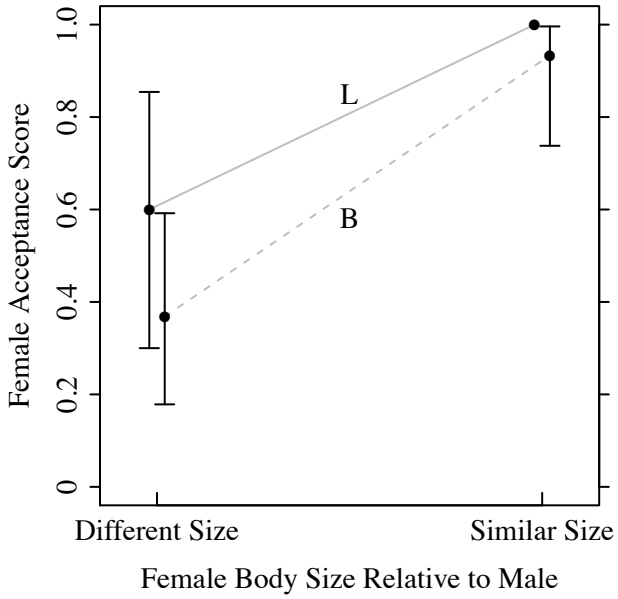
Table 2.1 Effects of explanatory variables on female acceptance scores

Logistic regressions to test for the effects of explanatory variables on female acceptance scores. Each model includes treatment and the additional variable(s) indicated. Treatment was entered first and so has identical effects in all models except model 1i, due to missing data points for male nuptial color. The second explanatory variable in models 1c – 1h are male courtship behaviors. N = 57 mate choice trials.

Model	Explanatory Variable	df	X^2	p
1a - 1h	treatment (similar vs. different size)	1	20.95	$5x10^{-6}$
1i		1	18.48	$2x10^{-5}$
1a	female species	1	2.14	0.14
1a	treatment x female species	1	0.57	0.45
1b	trial date	1	0.58	0.45
1c	no. of approaches	1	0.62	0.43
1d	no. of zig-zags	1	0.10	0.75
1e	no. of bites	1	0.04	0.84
1f	no. of leads to nest	1	0.08	0.78
1g	no. of nest maintenance events	1	0.23	0.63
1h	no. of nest creep-throughs	1	3.28	0.07
1i	male nuptial color	1	0.20	0.29

Figure 2.2 Female acceptance scores

Mean female acceptance score for treatments in which the female was manipulated to be different in body size versus similar in body size to a male of the opposite species, with 95% confidence intervals. Means for limnetic females are connected by a solid line, and for benthic females by a dashed line.



Little–to–no Male Preference

Males did not behave significantly differently towards heterospecific females of different size treatments except in 1 of the 12 comparisons (Table 2.2): limnetic males maintained their nest slightly more often when paired with similar-sized females than when paired with different-sized females (Table 2.2), whereas there was no detectable difference in the other five behaviors. Benthic males did not behave detectably differently towards females in different treatments.

Table 2.2 Effects of female treatment on male behavior

Linear models to test for effects of female treatment (similar vs. different size) on the number of times males exhibited each of six courtship behaviors using data from their first trial only.

Model	Response Variable	Benthic Males		Limnetic Males	
		$F_{(df)}$	p	$F_{(df)}$	p
2a	no. of approaches	$0.06_{(1,14)}$	0.81	$5x10^{-4}{}_{(1,25)}$	0.98
2b	no. of zig-zags	$1.03_{(1,14)}$	0.33	$0.02_{(1,25)}$	0.90
2c	no. of bites	$0.20_{(1,14)}$	0.66	$1.44_{(1,25)}$	0.24
2d	no. of leads to nest	$0.02_{(1,14)}$	0.89	$0.22_{(1,25)}$	0.65
2e	no. of nest maintenance events	$0.80_{(1,14)}$	0.39	$4.23_{(1,25)}$	0.05
2f	no. of nest creep-throughs	$0.02_{(1,14)}$	0.90	$1.10_{(1,25)}$	0.30

Discussion

This study provides experimental evidence that both benthic and limnetic stickleback females prefer mates of the opposite species whose body size more closely matches their own. Males appeared to exhibit little-to-no preference and no effect of male courtship behaviors were detected on female acceptance scores. Unlike previous studies, these results cannot be explained by timing in the mating season, or by any other traits that were not directly affected by manipulation of body size. Our results imply that body size, a trait under divergent natural selection that functions as a mate signal (Schluter 2001; McKinnon and Rundle 2002), also determines a female's preferred size (referred to as simply 'mate preference' below) via phenotype matching.

Along with body size, other traits that distinguish the species, such as shape, color and behavior are likely involved in assortative mating as well. Indeed, Southcott et al. (2013) attribute a large increase in premating reproductive isolation

to interactions of some other species-specific trait(s), with body size and, in benthics, color. Conspicuous shape differences, which have not traditionally been quantified in stickleback mate choice studies, are an interesting candidate. Other studies too have found effects of male nuptial color on female mate preference (Boughman 2001; Boughman et al. 2005). However, note that any traits that were not directly affected by body size were randomized among treatments and therefore cannot explain our results.

Size matching in benthic and limnetic sticklebacks is interesting because of what it implies for the genetics of divergence with gene flow between them. Size-matching implies that the genes underlying a target of divergent natural selection, mate signal trait and mate preference are one and the same and, thus, these traits cannot be dissociated during divergence with gene flow. While the phenomenon of size matching itself may have a separate genetic basis (encoding, for example, a mate choice rule that dictates: 'prefer others whose size is like mine'), such loci effectively transfer the determination of mate preference to body size loci. As a result, divergent selection on body size should lead to assortative mating by body size as a by-product. Thus, the genetics of divergence between the Paxton Lake species is, in this one way, extremely favorable for the speciation process. This finding helps to explain the evolution and persistence of the species pair in the face of gene flow. These results also agree with those of a similar study of marine and stream pairs of threespine stickleback (McKinnon et al. 2004). Because the marine population is ancestral to most freshwater forms, this raises the possibility that the evolution of size-matching predated and subsequently facilitated the evolution of the benthic and limnetic species pair and, indeed, the entire adaptive radiation of threespine sticklebacks into freshwater environments around the northern hemisphere in the late Pleistocene.

How do stickleback females know their own size? They may learn their own size by 'self-referencing' (Hauber and Sherman 2001), for example, by directly judging how well matched their own size (or some other phenotype that changes as a by-product of changing body-size) is with that of others. Termed 'self-referent mate choice', 'self-referent assortative mating', or 'self-referent phenotype matching'

this mechanism has been an important component in many theoretical models of speciation with gene flow (e.g. Gavrilets and Boake 1998; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999; Kirkpatrick and Nuismer 2004; Verzijden, Lachlan, and Servedio 2005; Kisdi and Priklopil 2011). However, as of yet, there have been no tests of self-referencing mechanisms in stickleback. Alternatively, stickleback females may indirectly learn their own size via sexual imprinting or social learning by associating with other stickleback that tend to have the same phenotype as they do, such as their father or siblings (Lacy and Sherman 1983; Hauber and Sherman 2001). This mechanism too has received a good deal of attention in both the theoretical and empirical literature on speciation with gene flow (Laland 1994; Irwin and Price 1999; Owens et al. 1999; Verzijden et al. 2005). Ours and other studies can shed light on the role that sexual imprinting and/or social learning may play in size matching. Two independent studies in benthics and limnetics have failed to find evidence that daughters sexually imprint on their father's body size (Albert 2005; Kozak et al. 2011). Furthermore, in our study, any effects of sexual imprinting were randomized between treatments. However, individuals in our study were raised with conspecifics, and from late adolescence to adulthood were kept in tanks with other individuals in the same size manipulation group. Thus, it is possible that social learning from conspecifics contributes to size matching. Since our experimental sticklebacks were naïve to heterospecifics up until their mate choice trial, our results suggest that experience with heterospecifics is not necessary for the development of size matching. Interestingly, Kozak and Boughman (2008) found that juveniles raised with mostly conspecifics spent more time shoaling with conspecifics of similar size than those of different size, whereas, juveniles raised with mostly heterospecifics spent equal amounts of time shoaling with conspecifics of similar and different sizes. Their results suggest that juvenile experience with conspecifics may be necessary for the development of size matching and thus, a social learning component exists, at least in the case of shoal member preferences. Nonetheless, the relative contributions to size matching of self-reference and learning from siblings or conspecifics, remain to be determined.

In general, phenotype matching has previously been studied extensively as a means of kin recognition, inbreeding avoidance and mate choice for an optimal complementation of major histocompatibility complex (MHC) alleles (Bateson 1978; Lacy and Sherman 1983; Holmes 1986; Heth et al. 1998; Petrie et al. 1999; Hauber and Sherman 2001; Landry et al. 2001; Mateo and Johnston 2001; Milinski 2006; Le Vin et al. 2010). Its potential role in assortative mating and speciation has received less attention. Theoretical models of speciation have implemented various types of mate choice by phenotype matching (Laland 1994; Gavrilets and Boake 1998; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999; Kirkpatrick and Nuismer 2004; Verzijden et al. 2005; Kisdi and Priklopil 2011), but empirical tests are still lacking. Our results along with those of McKinnon et al. (2004) indicate that assortative mating by size matching may have greatly facilitated the rapid and repeated diversification of sticklebacks in freshwater ecosystems where body size is under divergent selection. Other similar examples involve sexual imprinting (reviewed in Irwin and Price 1999). For example, Darwin's finches sexually imprint on their father's song, which is directly affected by beak shape, a trait under divergent selection (Podos 2001; Grant and Grant 2011). Although the signal trait is actually song, a pattern of beak shape phenotype matching emerges because song changes as a by-product of changing beak shape. Divergent selection on beak shape should readily lead to assortative mating by beak shape as a by-product. More studies are greatly needed to determine the prevalence of similar phenomena in nature, as there have been few tests to date. For example while many studies have looked at whether a trait under divergent selection is also a mate signal, whether mate preference is determined by that trait through phenotype matching has rarely been investigated.

3 The Probability of Genetic Parallelism and Convergence in Natural Populations[2]

Introduction

Parallel and convergent evolution of traits in independent populations inhabiting similar environments ("repeated phenotypic evolution") implicates natural selection (Endler 1986; Harvey and Pagel 1991; Schluter 2000; Losos 2011). Processes contributing to phenotypic evolution other than selection, such as mutation and drift, are unlikely to yield the same evolutionary shifts, again and again, in correlation with environment. Conversely, repeated use of the same underlying genes during parallel and convergent phenotypic evolution is thought to reflect biases and constraints on the supply and fixation of beneficial mutations. For example, some genes might contribute to adaptation more often than others because they have more standing genetic variation, higher mutation rates, larger effect sizes, more numerous beneficial mutations, fewer pleiotropic constraints, particular linkage relationships or because they are involved in particular epistatic interactions with the genetic background (Orr 2005; Weinreich et al. 2006; Gompel and Prud'homme 2009; Stern and Orgogozo 2009; Chevin et al. 2010; Christin et al. 2010; Streisfeld and Rausher 2011; Feldman et al. 2012). Knowledge of these underlying effects and constraints might ultimately allow us to predict genetic evolution (Orr 2005; Stern and Orgogozo 2009; Chevin et al. 2010). Instances of parallel and convergent phenotypic evolution provide an opportunity to measure the predictability of genetic changes underlying adaptation.

In some cases, high molecular specificity of a selective agent, such as a toxin in the diet that interferes with the function of particular proteins, drives repeated

[2] A version of chapter 3 has been published. Conte, G.L., M.E. Arnegard, C.L. Peichel, and D. Schluter. 2012. The probability of genetic parallelism and convergence in natural populations. *Proc. R. Soc. B* 279: 5039-5047.

evolution in a small number of genes (Gompel and Prud'homme 2009). For example, resistance to tetrodotoxin (TTX) in puffer fish and several snake species has repeatedly evolved by changes to a few amino acid residues in the outer pore of voltage-gated sodium channel proteins, where the neurotoxin binds to its target, causing paralysis (Jost et al. 2008; Feldman et al. 2012). This specificity explanation fails when many genes influence a trait, and changes to any one may produce similar alterations in phenotype. For example, all known cases of parallel reduction of complete armor plating in freshwater populations of threespine stickleback (*Gasterosteus aculeatus* species complex) involve the same major gene, *Eda* (*Ectodysplasin*) (Colosimo et al. 2005) (for other references see Table B.1), even though mutations in several genes of the *Eda*-signaling pathway in mammals are known to cause similar phenotypic changes in hair, teeth, sweat glands and dermal bones (Knecht et al. 2007). A ready supply of standing genetic variation in *Eda* in the ancestral population likely contributed to almost universal use of the same gene (Colosimo et al. 2005).

Despite a growing number of cases (reviewed in Wood et al. 2005; Arendt and Reznick 2008; Gompel and Prud'homme 2009; Stern and Orgogozo 2009; Christin et al. 2010; Manceau et al. 2010; Elmer and Meyer 2011; Martin and Orgogozo 2013; Stern 2013), the probability of repeated use of the same genes in natural populations has not been estimated. The number of examples of repeated gene use in the published literature gives the impression that this probability might be high. Indeed, repeated use of the same genes is regarded as sufficiently common in evolution that the detection of equivalent genomic signatures of selection between independent natural populations that have adapted to similar environments is a valuable tool for discovering genes involved in adaptation (Hancock et al. 2010; Turner et al. 2010; Chan et al. 2012; Jones et al. 2012b). Yet, the apparent frequency of reuse of genes might be distorted if biased methods are used to detect it or if less attention has been paid to cases in which different genes underlie repeated phenotypic evolution.

Here, we conducted an objective survey of the published literature to make a quantitative estimate of the probability of reuse of genes during repeated

phenotypic evolution in independent lineages of natural populations. We clarify different approaches and biases that may affect estimation of this probability. We focus on repeated changes to the same gene, rather than on reuse of the same mutations, because the mutations are unknown in most cases. We include both protein-coding sequences and associated regulatory regions in our definition of a "gene". We treat paralogous genes as different genes, which is a conservative decision because considering them to be the same gene increases the overall probability of gene reuse.

In addition, we test whether the probability of repeated use of the same genes declines as more distantly related taxa are compared. We would expect the probability to decline if phylogenetically distant taxa use different developmental pathways and networks more often than closely related species when they adapt to similar selection pressures (Futuyma 2009). Another reason to predict a decline is that pleiotropic constraints and the supply of beneficial mutations at a locus are likely to depend on its sequence and on its genetic background, both of which have had more time to diverge between taxa that are more distantly related.

Counterexamples are known in which repeated phenotypic evolution of closely related taxa used different genes, and in which distantly related taxa used the same genes (Arendt and Reznick 2008). Indeed, the frequency of such examples prompted Arendt and Reznick (2008) to conclude that, from a genetic perspective, there is no clear distinction between "parallel evolution" and "convergent evolution". Yet, from a phylogenetic standpoint, it is useful to distinguish cases in which populations derived from the same or closely related ancestors evolved in the same direction (parallel evolution) from cases in which more distantly related, phenotypically differentiated populations evolved a similar trait (convergent evolution). This distinction is also reflected in the design of genetic studies reviewed here. Genetic studies of parallel phenotypic evolution compare multiple derived populations to the *same* ancestor (or to closely related populations representing their common ancestral state), whereas genetic studies of convergent evolution compare each of two or more distantly related, derived populations to *different* ancestral species, rather than to the common ancestor of all the taxa. Using this

distinction, we ask whether the genetics of parallel and convergent evolution differ from one another in the probability of gene reuse.

On genomic approaches for detecting repeated genetic evolution

Genomic approaches hold great promise for detecting repeated use of the same underlying genes in phenotypic evolution. Counting the frequency of signatures of selection in the same genes between replicate natural populations adapting to similar environments, and exhibiting similar phenotypic changes, is straightforward in principle (Turner et al. 2010; Jones et al. 2012b). The approach has the advantage of broad coverage, and it allows the detection of mutations having relatively small effect sizes on fitness. For example, Jones et al. (2012) resequenced one individual from each of 10 marine (ancestral) and 10 phenotypically similar stream populations of threespine stickleback from around the northern hemisphere. Stream and marine populations were consistently distinguished at about 200 loci, the outcome of repeated selection on standing genetic variation. Deeper sequencing of a single stream-marine pair found that 35% of all genomic regions showing evidence of adaptive differentiation between the two populations also separated marine and stream populations globally. The probability of repeated use of the same genes was thus estimated as 0.35 in this study. Genome scans based on genetic markers rather than complete sequences also find evidence for parallel genetic evolution, but to different extents (e.g. Campbell and Bernatchez 2004; Bonin et al. 2006; Egan et al. 2008; Nosil et al. 2008; Bradbury et al. 2010; Hohenlohe et al. 2010).

The main limitation of genomic studies is the lack of information on phenotypic traits affected by genes. Conceivably, separate mutations in the same genes might have divergent, rather than parallel effects on a phenotypic trait, or they might affect different traits. This problem can be partly overcome with functional experiments that determine if independent mutations in the same gene have the same phenotypic effects in different populations. It will be more difficult to identify those cases in which mutations in different genes lead to similar phenotypic

effects. One might argue that fitness itself is the phenotypic trait addressed in genomic studies, since it evolves in parallel as replicate populations adapt to similar environments. On the other hand, fitness evolves in parallel even when phenotypes diverge, and hence estimates of the probability of gene reuse from genomic studies will not necessarily agree with estimates based on the identity of mapped genes underlying phenotypic traits evolving in parallel. For these reasons, it will eventually be interesting to compare results from the two approaches. Here, we chose to focus on genetic studies of repeated phenotypic evolution, which are presently more common than genome sequence comparisons of populations adapted to similar environments.

The genetics of repeated phenotypic evolution

We surveyed published genetic studies to estimate the probability of reuse of the same genes underlying repeated phenotypic evolution in natural populations. These studies used either of two main approaches to assess the genetic basis of phenotypic differences: genetic crosses and analysis of candidate genes.

Under a genetic cross approach, replicate populations that have independently evolved a particular phenotype are crossed to populations representing ancestral phenotypes. Quantitative trait locus (QTL) mapping methods are then used to test markers across the genome for an association with the phenotype of interest in hybrid offspring. Alternatively, mapping is carried out using admixed populations. Ideally, techniques such as fine mapping and functional assays are used subsequently to confirm gene identity (or at least to narrow the identified genomic region). The genetic cross approach has the advantage of genome-wide coverage, allowing multiple loci contributing to the derived phenotype to be discovered and the magnitude of their phenotypic effects to be estimated. For example, this approach was used to map repeated evolution of red wing patterning in *Heliconius erato* and *H. melpomene* butterflies to the *optix* locus in both species (Baxter et al. 2008; Papa et al. 2008; Reed et al. 2011).

More commonly under this approach, researchers carried out genome-wide mapping in one pair of populations representing derived and ancestral phenotypes, and then other methods such as complementation crosses and localized (rather than genome-wide) mapping were used to determine whether the same gene or genomic regions were involved in other instances of repeated evolution. For instance, albinism was mapped to *Oca2* in two separate populations of cavefish, *Astyanax mexicanus*, whereas a complementation cross in a third population indicated that albinism arose through mutations in the same gene (Protas et al. 2006). Or, genetic crosses were used to estimate the number of genes underlying a trait, followed by localized mapping of a candidate gene if Mendelian segregation was observed. For example, crosses between populations of the deer mouse, *Peromyscus maniculatus,* that have evolved on light and dark substrates revealed evidence for a single gene of major effect underlying a coat color phenotype (Linnen et al. 2009). Alleles of the candidate gene *Agouti* were found to segregate perfectly with the phenotype (Linnen et al. 2009; Manceau et al. 2010).

Under the alternative, candidate gene approach, one or a small number of designated genes is tested for association with phenotype. Ideally, the finding of such an association is accompanied by functional assays or other methods to confirm causality. This approach was used to demonstrate that electrical excitability of the myogenic electric organ, which has evolved independently in mormyroid and gymnotiform fishes, involved amino acid substitutions in the same functional regions of the sodium channel gene, *Scn4aa*, in both lineages (Zakon et al. 2006; Arnegard et al. 2010). The candidate gene approach determines whether "this same gene is involved" when independent populations evolve a similar phenotype, but it does not provide estimates of the magnitude of the contributions of all genomic regions affecting a trait. The approach thus only provides a qualitative score of gene reuse. In turn, this may lead to higher estimates of the probability of parallel evolution compared to crossing and mapping studies. For this reason we analyze data from the two approaches separately.

Methods

Literature search

We searched the literature for examples of repeated phenotypic evolution in natural populations that provided evidence on whether the same genes were used. To obtain a representative sample we searched the online Thomson Reuters Web of Knowledge database for all studies in the subject area of evolutionary biology (as of June 17, 2012) that included the topic *gene** and that contained either *parallel** or *converg** in the title (a "*" at the end of a search term includes all words beginning with the preceding letters). We reasoned that these search terms would detect many studies that had tested the genetic basis of parallel or convergent phenotypic evolution regardless of outcome. In support, our search criteria detected multiple studies in which the genetic basis was found not to be the same between independent instances of repeated phenotypic evolution. In total, the search yielded 1612 publications, of which 25 met further criteria for inclusion in the study.

To be included, we required that a study addressed the genetic basis of a repeatedly evolved phenotype in natural populations rather than experimentally evolved or artificially selected populations. While the latter types of studies offer a wealth of information regarding parallel and convergent genetic evolution (e.g. Woods et al. 2006; Kao and Sherlock 2008; Tenaillon et al. 2012) our goal was to better understand repeated genetic evolution in wild populations, which span a greater range of ages and about which less is currently known. We included only studies with original data, rather than reviews. It was also necessary that the phenotypic trait in a study be an organismal-level trait rather than a molecular phenotype, since a protein sequence, expression pattern, or function-based phenotype usually predetermines its underlying gene. We further required that repeated evolution in the phenotypic trait had been discovered prior to the discovery of its genetic basis. This was done to avoid an obvious bias accompanying a reverse discovery sequence in which the phenotypes were investigated only after repeated genetic changes had been found. Finally, we included only instances in

which the direction of evolution was known or strongly suspected in independent populations, to exclude populations that might instead represent reversions to the ancestral state. This criterion meant that we could not include studies of the genetics of abdominal pigmentation in *Drosophila*, where the direction of evolution could not be verified (Wittkopp et al. 2003), and the evolution of increased pigmentation in native American peoples, which involved evolution in the opposite direction compared to pigmentation changes in other human populations (Quillen et al. 2012).

Data from the 25 papers meeting our criteria were arranged according to phylogeny of taxa and similarity of traits (Table B.1). We then carried out an exhaustive search for all other publications on the same traits in the same species to ensure that we had the most up-to-date information on the genetic basis of the phenotypic traits in the examples originally identified by our objective search. We did not pursue citations found in papers included in our study that described other cases of parallel or convergent evolution not detected in our primary Web of Knowledge search. We felt that including them might produce a citation bias that would inflate the apparent probability of gene reuse. Adhering to this objective criterion forced us to leave out some well-known studies of the genetics of phenotypic evolution. For example, our primary search turned up three study systems in which repeated evolutionary loss of pigmentation involved the gene, *Mc1r*: beach mice (Steiner et al. 2009), White Sands lizards (Rosenblum et al. 2010) and Mexican cavefish (Gross et al. 2009). However, the search did not turn up other known cases of pigmentation evolution involving *Mc1r* (Ritland et al. 2001; Theron et al. 2001; Eizirik et al. 2003; Nachman et al. 2003; Mundy et al. 2004; Römpler et al. 2006). We stress that our aim was to estimate the probability of repeated genetic evolution, which demanded an impartial survey. We do not claim to have eliminated all sources of bias, especially publication bias and the difficulty of detecting and identifying genes of small effect.

With the help of TimeTree (Hedges et al. 2006) we obtained phylogenies and node age estimates for all relevant taxa, including those from different study systems that had independently evolved a similar phenotype (Table B.1). This

allowed us to compare probability of gene reuse with estimated node age of common ancestors. In cases of parallel phenotypic evolution (i.e., independently derived forms are crossed, or compared, to the same, recent ancestral form), node ages are also approximate times of onset of phenotypic divergence. In most cases of convergent evolution (i.e., different derived forms are compared to different ancestral forms), the onset of phenotypic divergence occurred within each lineage long after the divergence of the lineages themselves. Hence good estimates of the timing of phenotypic shifts (e.g., Lavoué et al. 2012) were often difficult to obtain, and we are unable to analyze the additional effects of trait origin times on the probability of gene reuse.

Calculating the probability of repeated gene use

We analyzed data from genetic crosses and candidate gene studies separately. Studies that employed genetic cross methods provided effect sizes (percent variance explained or magnitude of effect) that we used to compute the relative contribution of identified genes or QTL (if causal genes had not yet been identified) to the evolved phenotypic change in a particular cross. These effects were rescaled so that the contributions represented proportions and summed to 1.0 (see bar graphs at the tips of the hypothetical phylogenetic tree in Figure 3.1). In a single case, effect sizes for two out of five mapped genes were not available, so the unexplained variance was split evenly between the two. Genes that were confirmed to have a major effect, either by complementation tests of shared use of a gene of major effect or localized mapping of a candidate gene in a cross in which the trait showed simple Mendelian segregation, were assigned an effect size of 1.

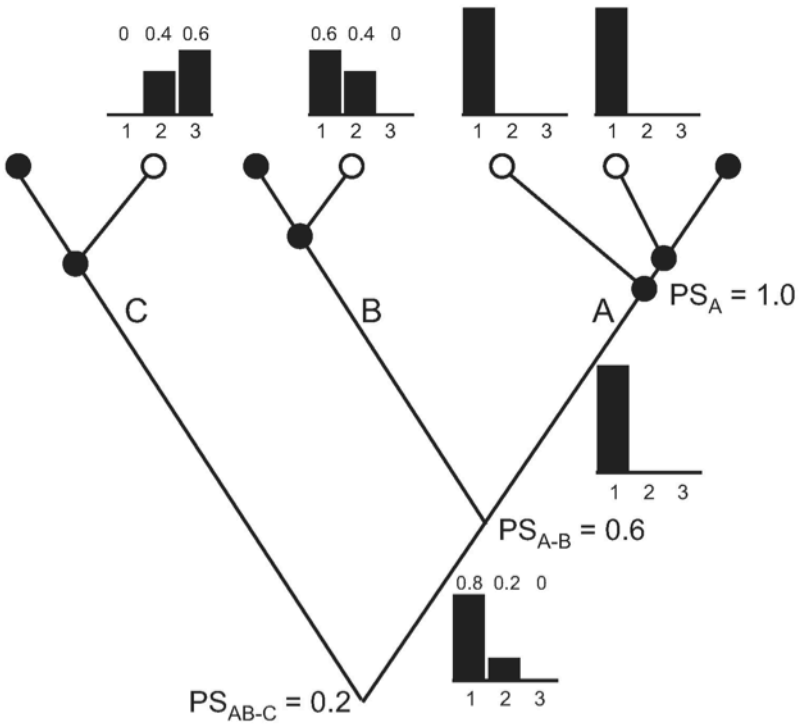
Probability of gene reuse between a pair of taxa was quantified using proportional similarity (Whittaker 1952), calculated as $\mathrm{PS} = \sum_{\mathrm{i}} \min(p_{i1}, p_{i2})$, where p_{i1} and p_{i2} are the proportional contributions of gene i in the two taxa (Figure 3.1). This quantity treats the distribution of contributions by genes in each of the two taxa as a frequency distribution and measures their intersection. When causative genes within QTL were not known, co-localizing QTL were considered to represent

repeated use of the same "gene". It is possible that different genes within co-localizing QTL are responsible in different cases of repeated phenotypic evolution. However, at this point, QTL represent the best available information for many taxa. Future studies will be better able to estimate the prevalence of different but tightly linked genes underlying repeated phenotypic evolution.

When data were available on multiple derived populations for a given named species that independently evolved the same phenotype (e.g., two populations above the node for species A in Figure 3.1), PS was calculated between all population pairs and then averaged, yielding a single PS estimate for the species. The species value for the relative contributions of different genes was then calculated by averaging the relative contributions of its multiple populations (see bar graph below node A in Figure 3.1). Our justification for using just one data point per species for a given trait was that frequency of gene use of derived populations within a species is expected to be greater than that between populations of different species, even after accounting for age differences. This is because populations within a species are typically crossed to the same ancestral form and so are not independent. They are also more likely to share standing genetic variation. This decision is conservative, because treating separate populations within species as independent replicates in the overall analysis raises the probability of repeated gene reuse. Proportional similarity was then calculated separately between each sister pair in the phylogeny (e.g., PS is calculated between taxa A and B in Figure 3.1). After calculating PS between two sister taxa at a given node, the relative contributions of genes in the two taxa were averaged (see bar graph below the node connecting A and B in Figure 3.1). This average was then used to calculate proportional similarity between sister taxa at the next node down the tree (e.g., between C and the node connecting taxa A and B in Figure 3.1).

Figure 3.1 Calculating proportional similarity

Hypothetical example to illustrate calculation of proportional similarity (PS) to measure probability of gene reuse between sister taxa. A, B, and C represent species having one or more populations that independently evolved a similar change in phenotype (open circles) compared with an ancestral phenotype (filled circles). Bar graph above each derived population indicates the relative contributions of each gene *i* to the phenotype (here, *i* is 1, 2, or 3). PS is calculated between a pair of taxa as $\mathrm{PS} = \sum_i \min(p_{i1}, p_{i2})$, where p_{i1} and p_{i2} are the proportional contributions of gene *i* in the two taxa. Within a species, proportional similarity is measured between all pairs of derived populations and averaged. Relative contributions of genes are then averaged among populations (illustrated for species A by the bar graph immediately below node A). $\mathrm{PS}_{\mathrm{A\text{-}B}}$ compares the relative contributions of the three genes in species B with the average for species A ($\mathrm{PS} = 0.6 + 0 + 0 = 0.6$). $\mathrm{PS}_{\mathrm{AB\text{-}C}}$ compares the relative contributions of the three genes in species C with the average of A and B, shown in the bar graph below the node connecting A and B ($\mathrm{PS} = 0 + 0.2 + 0 = 0.2$).



In our analysis of candidate gene studies, a given population or species received a score of 1 if use of the candidate gene was confirmed and a 0 if the assay used produced no evidence that the gene contributed to the trait. We recognize that

assays were not always exhaustive and often could not completely rule out an effect of the gene on the trait. Proportional similarity between two taxa was calculated as $\mathrm{PS} = \min(p_1, p_2)$, where p_1 and p_2 are the proportional uses of the candidate gene in the two taxa. When data on multiple derived populations were available for a given named species, the species value for candidate gene reuse was calculated by averaging the values (0's and 1's) across the populations. Proportional similarity was then calculated separately between each sister pair in the phylogeny. After calculating PS between two sister taxa at a given node, the proportional use values for candidate genes was averaged between the two taxa. This average value was then used to calculate proportional similarity between sister taxa at the next node down the tree. When more than one informative candidate gene was available at any given node, the above process was repeated for each gene and their PS values were averaged. Comparisons between two sister taxa, one of whose data was obtained using the candidate gene method, and the other of whose data came from genetic crosses, were included in the analysis of candidate genes. In such cases only, we treated the mapping data as though a candidate gene approach had been applied, assigning a score of 0 or 1 for candidate gene use. In some cases the same node is used in both mapping and candidate gene analyses. However, the populations being compared in such cases are always mutually exclusive.

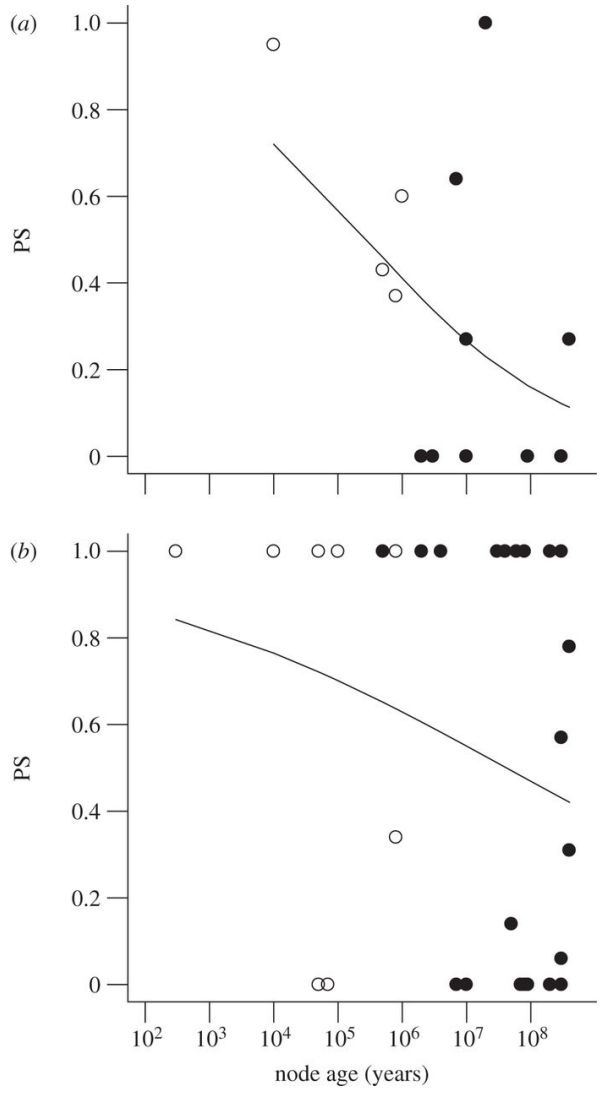
We repeated all analyses using a second, multiplicative measure of overlap of gene contributions between taxa, $O = \sum_i (p_{i1} * p_{i2}) / \sqrt{\sum (p_{i1})^2 \sum (p_{i2})^2}$, where p_{i1} and p_{i2} are the proportional contributions of gene *i* in the two taxa (Pianka 1973). O represents the probability that a random draw from the proportional use distributions of each species results in the same gene, scaled so that the measurement is insensitive to the number of genes in the distribution. Applying this measure led to virtually identical results as proportional similarity, and so we present only proportional similarity. Throughout, standard errors and *P*-values should be regarded as heuristic because of uncertainty about the degree of independence of observations in the meta-analysis.

Results

Results are plotted separately for measurements based on genetic cross methods (Figure 3.2a) and candidate gene methods (Figure 3.2b). Each point represents the mean of proportional similarity (PS) between pairs of populations of a single species, if multiple populations were available (parallel evolution - open symbols), or between sister species or other sister taxa at deeper nodes of the phylogenetic trees (convergent evolution - filled symbols). Approximate ages of nodes are given along the horizontal axis. In the case of species values (open symbols), node age represents the approximate time at which repeated trait divergence began between ancestral and derived populations. Ages of sister species and more distantly related sister taxa (filled circles) only indicate the age of their common ancestor, since repeated phenotypic evolution typically occurred long afterward (cf. Figure 3.1).

Figure 3.2 Probability of gene reuse by node age

Measurements of the probability of gene reuse based on (a) data from genetic crosses and (b) candidate gene data. Open symbols represent average of proportional similarity between all pairs of derived populations within the same species (i.e., parallel evolution). Filled symbols represent similarity measurements between sister taxa at deeper nodes in the phylogenetic trees (i.e., convergent evolution). Curves are best-fit logistic regressions to the data.



On the basis of data from genetic crosses, estimated similarity of gene usage between taxa undergoing repeated phenotypic evolution in a trait is 0.32 ± 0.10 SE on average. The probability of gene reuse based on candidate gene data is 0.55 ± 0.08 SE.

The results showed the predicted tendency for the probability of gene reuse between sister taxa to decline with the age of the node of their common ancestor (Figure 3.2). Also, across the span of ages represented, estimated probability of gene reuse tended to be higher in candidate gene data than in data from genetic crosses. The predicted probability of gene reuse was high (around 0.8) in both data sets at the youngest nodes. This probability declined to about 0.10 by about 10^8 y for mapping data, but remained higher (about 0.40) at the same node age for candidate gene data (Figure 3.2).

To test these trends we used logistic regression to model the relationship between proportional similarity, node age, and genetic method (genetic cross vs. candidate gene). Results indicate that the decline in the probability of gene reuse with node age is real (χ_1^2 = 3.04, P = 0.04, one-tailed test). The effect of genetic method was not statistically significant in a two-tailed test (χ_1^2 = 2.64, P = 0.10), and so these data do not fully resolve the difference between mapping and candidate gene data. These tests are conservative because many values of PS lie between 0 and 1 (Figure 3.2), and so have lower residual variance than assumed by logistic regression. Reanalysis using quasi-binomial errors (McCullagh and Nelder 1989) slightly strengthen the above findings. Finally, a randomization test confirmed the overall correlation between PS and node age (r = –0.25, P = 0.04, one-tailed test).

Points obtained from comparing multiple populations within a species to the same ancestral form (open symbols in Figure 3.2, representing parallel phenotypic evolution) are younger than points obtained by comparing species and higher taxa to different ancestors (filled symbols, representing cases of convergent phenotypic evolution). The decline in proportional similarity with older nodes thus implies that the probability of repeated use of the same underlying genes is indeed lower for convergent evolution than parallel evolution, as we defined these terms. Specifically, when estimated using data from genetic crosses, the probability of reuse of the same genes is 0.47 ± 0.15 SE on average in taxa undergoing parallel phenotypic evolution and 0.24 ± 0.12 SE on average in taxa undergoing convergent phenotypic evolution. Likewise, using candidate gene data, the probabilities of reuse of the same genes are

0.67 ± 0.17 SE and 0.51 ± 0.09 SE on average for parallel and convergent phenotypic evolution, respectively.

Discussion

Our results based on data from genetic crosses indicate that when similar traits evolve independently in different lineages, the probability that the same genes are used is estimated to be 0.32, on average. The probability estimated from candidate gene studies is 0.55, on average. One explanation for such a high probability of repeated use of the same genes is that at the time of adaptation, effect sizes and availability of beneficial mutations were strongly biased toward a small number of genes. This result can be summarized by the "effective" number of genes, equivalent to the number of loci available if all have effects of equal magnitude and the same probability of fixation. For example, imagine that in two populations there are n equivalent genes underlying a trait under selection in which new advantageous mutations might occur and fix. If a major effect mutation occurs and fixes in one gene in one of the populations, the probability is $1/n$ that a second population experiencing similar selection fixes a mutation in the same gene. In this case, a probability of gene reuse of 0.32 corresponds to an n of $1/0.32 = 3.1$ effective genes. A probability of gene reuse of 0.55 corresponds to an n of $1/0.55 = 1.8$ effective genes. This rough calculation is simplistic, because real genes do not have equivalent effects. In addition, it does not indicate the cumulative number of genes that might contribute if parallel or convergent evolution is repeated many times. Nevertheless, the high probabilities of gene reuse estimated from published data indicate that the effective number of genes used in parallel and convergent phenotypic adaptation is typically small. If the causes of this low number can be elucidated, then genetic evolution may indeed be somewhat predictable (Gompel and Prud'homme 2009; Stern and Orgogozo 2009; Streisfeld and Rausher 2011).

It is difficult to judge how surprising these estimates of effective number of genes are without knowing the total number of genes available in which mutations would cause similar phenotypic changes. Some data are available to assess this. Of 6

genes of the *Eda* signaling pathway, mutations in most of which produce a similar phenotype in mammals, only two have been found to be associated with lateral plate variation in threespine stickleback: *Eda* and the receptor *Edar* (Knecht et al. 2007). Similarly, Streisfeld and Rausher (2011) noted that changes to any of the nine enzymes of the anthocyanin biosynthetic pathway would alter pathway flux and produce a change in intensity of flower pigmentation. In accord, 37 spontaneous mutations affecting floral pigment intensity have been detected in 5 of these 9 genes, predominantly in coding regions (another 32 mutations affecting floral pigment intensity occurred in transcription factors). However, in all 7 cases in which evolved differences in pigment intensity were mapped, the fixed changes mapped to transcription factors that regulate the pathway genes rather than to the genes themselves, indicating a strong fixation bias away from coding mutations in pathway proteins (Streisfeld and Rausher 2011). In 5 of these 7 cases, the changes occurred in a gene encoding an *R2R3 Myb* transcription factor. Such findings suggest that the number of genes used and reused in adaptive evolution is a small subset of available genes. A host of factors may lead to much higher probabilities of certain genes being involved in phenotypic adaptation than others, including amounts of standing genetic variation, differences in mutation rates or mutation effect sizes, pleiotropic constraints, linkage relationships and epistatic interactions with the genetic background (Orr 2005; Weinreich et al. 2006; Gompel and Prud'homme 2009; Stern and Orgogozo 2009; Chevin et al. 2010; Christin et al. 2010; Streisfeld and Rausher 2011; Feldman et al. 2012).

Any explanations for such a high probability of repeated use of the same genes must also explain why this probability declines as more distantly related taxa are compared. First, the high probability of repeated use of the same genes by young, closely related populations might result in part because they have access to the same pool of standing genetic variation (Colosimo et al. 2005; Barrett and Schluter 2008), an option not available to more distantly related taxa. Second, as lineages diverge, not only do the specific genes that affect the phenotypic trait diverge in sequence, but the genetic backgrounds with which they interact diverge as well. Hence, the biases that favor use of some genes over others during repeated

phenotypic evolution themselves should evolve, in which case we would expect the probability of repeated use of the same genes to decline with time and genetic divergence. The probability that changes to the same genes produce similar phenotypic changes is also likely to be reduced the more widely divergent the lineages (Zhang 2003), unless gene functions are highly conserved.

Repeated evolution can be divided into two types: parallel evolution, whereby evolution begins from the same starting point, and convergent evolution, whereby evolution begins at different starting points. Arendt and Reznick (2008) argued that from a genetic perspective there is no clear distinction between parallel and convergent evolution. We found that average proportional similarity of genes underlying parallel phenotypic evolution was greater than that underlying convergent evolution (Figure 3.2). The reasons are likely similar to those described for the effect of node age, since points representing parallel evolution have younger node ages than points representing convergent evolution. If evolution is biased toward some genes over others, populations beginning from the same ancestral genome will more likely share these biases than populations beginning from divergent genomes. However, there is no sudden break in the probability of gene reuse between parallel and convergent evolution (Figure 3.2). The distinction is one of degree rather than of kind.

Our estimates based on candidate genes are higher than those based on genetic crosses. Although not statistically significant in our analysis, the difference suggests that the calculated probability of repeated use of the same gene depends on the methods used to detect it. If the difference is real, what are the possible reasons? Whereas genetic cross methods allow us to estimate the contributions of all genes (or at least all genes of moderate to major effect) to repeated phenotypic evolution, the candidate gene approach allows us to determine only whether a specific gene of interest makes a contribution in each case. This essentially lowers the bar for a positive outcome in the case of candidate genes, because the probability of reuse of a gene of interest between two taxa is likely to be higher than the proportional shared use of genes when all mapped genes are considered. Another reason is that the candidate gene method might be more strongly affected

by publication bias than estimates based on genetic crosses. We suspect that studies that fail to confirm a role for a candidate gene are more likely to go unreported than results from mapping studies, which produce noteworthy findings if evidence for genes is found anywhere in the genome. On the other hand, estimates of proportional similarity that take magnitude of effect into account (i.e., those based on genetic crosses) are prone to higher sampling error, which will tend to cause a downward bias in estimates for the probability of gene reuse.

Caution is warranted when interpreting our results because of numerous judgments and uncertainties inherent to a meta-analysis involving heterogeneous data collected from various organisms, traits, and genes. In some cases, we considered overlapping QTL to be reuse of the same "gene", though we may eventually learn that different genes within the QTL underlie repeated phenotypic evolution. While this and other factors already described, such as publication bias, may cause us to overestimate the probability of gene reuse, still other factors may cause an underestimation. For example, our definition of repeated genetic evolution treats paralogous genes as different (several examples were present in our dataset, including the paralogous genetic basis of convergent evolution of caerulein skin toxin in frogs (Roelants et al. 2010), of digestion of foregut-fermenting bacteria in leaf-eating colobine monkeys and ruminant artiodactyls (Zhang 2003, 2006), and of red flower color in *Mimulus* spp. (Cooley and Willis 2009; Cooley et al. 2011)). In addition, multiple populations within a single named species were represented by only a single data point in our analysis (the average) to prevent rampant parallel genetic evolution within any one species from unduly affecting the results. Finally, the number of studies on which we have based our analyses is not large, which also adds uncertainty to these results. Despite these uncertainties, our aim here has been to stimulate thinking about these issues and to move towards a quantitative understanding of repeated genetic evolution, which we have attempted with the best available information.

As we accumulate more studies of the genetics underlying repeated phenotypic evolution in natural populations we will be better able to estimate the probability of the same genes being used. In turn, this will enhance our ability to ask

what factors explain variability in genetic parallelism and convergence. For example, broader sampling may allow us to ask whether there is a difference in probability of gene reuse between loss-of-function and gain-of-function traits, or between genes of major and minor effect. Improved knowledge of the biochemical functions and pathway positions of genes will allow us to address whether genes that influence a greater number of other genes in developmental pathways are more or less likely to underlie repeated phenotypic evolution than genes acting at terminal points in the pathways (Stern and Orgogozo 2008). Knowledge of mutations will allow us to address how properties such as dominance contribute to the probability they will repeatedly underlie evolution of a phenotype (Rosenblum et al. 2010). Further tests are required of the mechanisms proposed to underlie the high rate of reuse of the same genes, such as pleiotropy and mutation bias (Streisfeld and Rausher 2011). In the future, it will be interesting to compare our estimates with probabilities of gene reuse from whole-genome sequences of populations adapting to similar environments. We feel that studies starting from purely genetic and genomic approaches must incorporate steps to understand the phenotypic effects of the genetic changes detected. This will be important to determine whether parallel genomic signatures resulted from selection on the same phenotypic traits in different populations, and to determine the mechanisms of selection. Likewise, studies of phenotypic evolution should be followed through to its genetic basis to gain a better understanding of the consequences of repeated phenotypic evolution at the level of genes and mutations. With solid connections between phenotypes and genotypes, repeated phenotypic evolution provides a powerful way to study the predictability of genetic changes underlying adaptive evolution.

4 The Extent of Parallel Genetic Evolution Underlying Parallel Phenotypic Evolution in Pairs of Benthic and Limnetic Threespine Stickleback Species

Introduction

It is now clear that the genetics of adaptation are to some extent predictable, but we still have a poor understanding of how predictable they are (Stern and Orgogozo 2008; Conte et al. 2012; Martin and Orgogozo 2013; Stern 2013). Studying the genetics of repeated phenotypic evolution allows us to estimate the predictability of the genetics adaptation. When organisms independently evolve similar phenotypes in response to similar selection pressures, we can ask 'how similar is the genetic 'solution' underlying those phenotypes?' The more similar the genetic basis of repeatedly evolved phenotypes, the more predictable we may conclude the genetics of adaptation are.

The predictability of the genetics of adaptation should be affected by how many genetic 'solutions' are available. If mutations in only a few genes tend to be capable of producing a similar phenotypic change then the genetics of adaptation should be more predictable than if mutations in many genes tend to be capable of doing so. The predictability of the genetics of adaptation may be further affected if some of the available genes are more likely to underlie phenotypic adaptation than others. Thus far, evidence indicates that the genes used and reused in adaptive evolution are a small subset of available genes (Conte et al. 2012; Stern 2013). A recent meta-analysis based on published studies of natural populations estimates that across taxa separated by a wide range of divergence times, the average probability of gene reuse is 0.32 - 0.55 (depending on the type of data used to calculate it) (Conte et al. 2012). By taking the inverse of this probability, Conte et al. (2012) calculated a simplistic estimate of the *effective* number of genes available for adaptive evolution of a phenotype, if all genes have equal effects; the effective number of genes was 2 - 3. While it is very difficult to estimate the *actual* number of

genes in which mutations may lead to a particular phenotype, some methods are available, albeit imperfect. Counting the number of genes in biosynthetic pathways that may affect a trait when mutated (Knecht et al. 2007; Streisfeld and Rausher 2011), or counting the number genes in which mutations affecting a trait occur in mutagenesis screens (Breidenstein et al. 2008; Yeung et al. 2009; Stern 2013) or in breeding or horticultural records (Streisfeld and Rausher 2011), suggest that the number of genes in which mutations are capable of producing a particular phenotype is often much larger than the estimated 2-3 effective genes that are used in adaptive evolution of a phenotype on average (Conte et al. 2012; Stern 2013). These observations suggest that the probability of repeated genetic evolution is higher than we would expect if mutations in all genes potentially affecting a trait had equal chances of occurring and going to fixation. Rather, some genes might contribute to adaptation more often than others if, for example, they have fewer pleiotropic constraints, higher mutation rates or more standing genetic variation (Streisfeld and Rausher 2011; Martin and Orgogozo 2013; Stern 2013). To ultimately understand what factors affect the predictability of the genetics of adaptation and to what extent, we must start by estimating just how predictable they are.

Studies employing forward genetic approaches, such as quantitative trait loci (QTL) mapping and association mapping are still the gold standard for investigating the genetic basis of repeated phenotypic evolution (Conte et al. 2012). Compared to other common approaches, including reverse genetic techniques and genome scans for signatures of selection, the advantages of QTL and association mapping are the ability to link genotypes to the phenotypes they affect, the ability to discover the entire detectable genetic architecture of phenotypes via genome-wide scans, and the resulting ability to estimate the relative effect sizes of multiple QTL underlying phenotypes (Lynch and Walsh 1998; Broman and Sen 2009).

Several cases exist in which a repeatedly evolved phenotype has been mapped in multiple populations (Conte et al. 2012) and from these we can get an idea of how commonly repeated phenotypic evolution is underlain by repeated genetic evolution. However, there are several ways in which the data are lacking.

First, mapping studies so far have tended to focus on a few, discrete traits. Since biological traits appear to vary continuously more often than they vary discretely, discrete traits are probably not representative of most traits involved in population differentiation (Mackay 2001) and thus, it is difficult to make generalizations based on such studies. A second area in which mapping studies to date are still lacking is that, to our knowledge, no single studies have used crosses from multiple populations to explicitly test for repeated use of the same QTL. Rather multiple mapping studies have been done at disparate times and in disparate ways (Conte et al. 2012), potentially introducing confounding variables.

We investigated two pairs of threespine stickleback species (hereafter called 'species pairs'), one pair from Paxton Lake and the other from Priest Lake on Texada Island, British Columbia. Both pairs are comprised of a limnetic ecotype that specializes on use of resources in the lake's limnetic zone, and a benthic ecotype that specializes on use of resources in the lake's littoral and benthic zones (McPhail 1984, 1992, 1994; Schluter and McPhail 1992). Repeated genetic evolution seems relatively probable in the species pairs for several reasons. First, phenotypic divergence within each pair has occurred largely in parallel among the replicate pairs, and individuals of the same ecotype from different lakes strongly resemble one another (Schluter and McPhail 1992; Schluter and Nagel 1995; McKinnon and Rundle 2002; Gow et al. 2008). Second, the species pairs appear to have originated in the past 10,000-12,000 years, following double invasions by ancestral populations into the post-glacial lakes (Schluter and McPhail 1992; McPhail 1994; Taylor and McPhail 2000). Thus, the species pairs are young and the biases potentially causing some genes to underlie adaptive evolution more frequently than others are likely to still be shared by them. Finally, evidence suggests that the evolution of many freshwater threespine stickleback populations involved repeated natural selection for shared standing genetic variation (Colosimo et al. 2005; Miller et al. 2007; Kitano et al. 2010; Jones et al. 2012b). Some genetic variation showing signatures of natural selection has been found to be shared by the species pair ecotypes, and this too may be explained by repeated use of standing variation (Jones et al. 2012a). Given these considerations, we expect to find a relatively high amount

of genetic parallelism underlying parallel phenotypic evolution between the species pairs.

We used QTL mapping to map the genetic architecture of many continuously varying, quantitative traits, as well as a few discrete traits that repeatedly differentiate the species within separate pairs. We chose to focus on morphological traits, since morphological divergence has been largely parallel between the species pairs. We used identical methods for crossing, raising, phenotyping, genotyping, linkage mapping, QTL mapping, and analyzing QTL in both species pairs. Thus, our results are directly comparable. We raised our F_2 hybrids in controlled, semi-natural ponds allowing natural expression of the focal phenotypes.

To measure the extent of genetic parallelism, we developed and implemented a model selection technique, using the Akaike information criterion (corrected for finite sample size; AICc) to compete alternative models of the effects of single chromosomal regions (identified as being QTL in at least one of the lakes) in the two lakes. From these we determined whether individual chromosomal regions had parallel phenotypic effects (i.e. effects in the same direction, though not necessarily of equal magnitude) or non-parallel phenotypic effects in the two lakes, whereby two forms of non-parallel phenotypic effects were an effect in only one of the lakes or effects in opposite directions in the two lakes. We note that QTL are (often large) chromosomal regions of association with phenotypic traits that usually contain many genes in addition to the causative one(s). However, at the time being, they represent our best available information. As a second and distinct measure of genetic parallelism, we measured the overlap in the proportional contributions of all QTL effects underlying each individual trait in the two pairs, using proportional similarity as in Conte et al. (2012) (hereafter called 'proportional similarity of QTL use'). While the first measure of genetic parallelism reflects how commonly QTL have phenotypic effects in the same direction in both lakes, the second measure reflects to what extent QTL make equal contributions to the phenotypic difference in the two lakes.

Finally, since our dataset contained a relatively large number of traits and QTL, we were able to ask whether the phenotypic effect sizes of QTL predict

whether their effects are parallel or non-parallel. All else equal, mutations (that underlie QTL) of larger beneficial effect are more likely to fix than those of smaller beneficial effect (Fisher 1930; Kimura 1983), and thus, they are more likely to fix repeatedly. However, mutations with larger phenotypic effects may be less likely to be beneficial if the same mutations affect other traits as well (Fisher 1930). Looking at the pattern in our data may help guide our intuition regarding how the phenotypic effect size of QTL may affect the probability of genetic parallelism in natural populations.

Results

Parallel Phenotypic Evolution

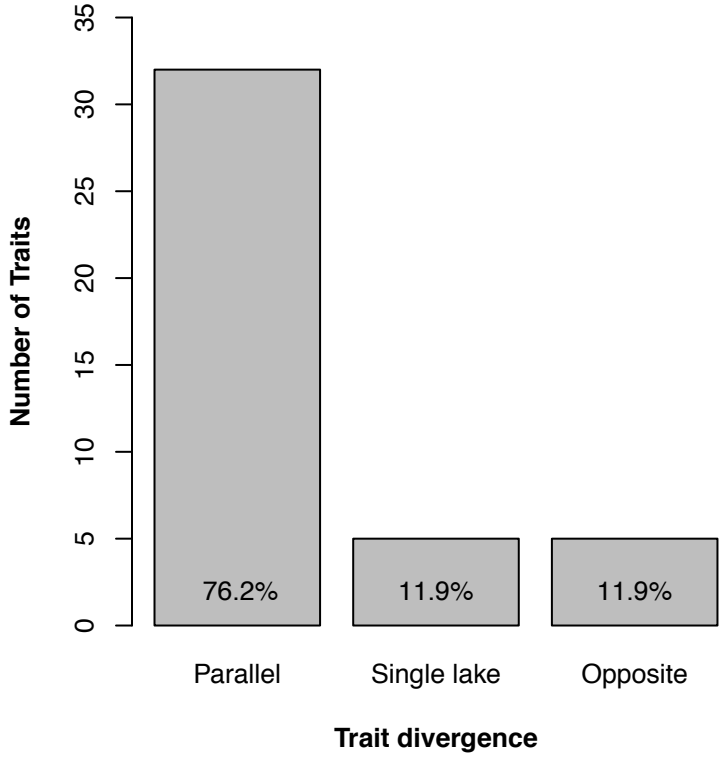
We began by determining which of our 58 focal phenotypic traits (Figure C.1) had diverged in parallel in the two lakes. We considered trait divergence to be 'parallel' when it was in the same direction in the two lakes (i.e. benthics had a higher mean than limnetics in both lakes, or vise versa), though not necessarily of the same magnitude. There were two ways in which trait divergence was considered non-parallel. First, trait divergence was considered to be only in a 'single lake' if it occurred in one lake but not the other (i.e. benthic and limnetic trait means differed in only one of the two lakes). Second, trait divergence was considered 'opposite' if it was in opposite directions in the two lakes (i.e. benthics had a higher mean than limnetics in one lake and a lower mean than limnetics in the other). These definitions describe alternative linear models that we fitted to the data for each trait separately. We then used AICc to determine which model was best supported by the data for each trait.

We found that 76.2% (n=32) of traits diverged in parallel in the two lakes. Divergence in the remaining 23.8% of traits was non-parallel, whereby 11.9% (n=5) diverged in only a single lake and 11.9% (n=5) diverged in opposite directions in the two lakes (Figure 4.1 and Table C.1). Fifteen traits for which more than one trait divergence category fit the data nearly equally well were left out. Also, the total

number of traits considered here was 57 rather than 58, since rather than long gill raker count and short gill raker count on the first gill arch, only the total gill raker count on the first gill arch was included. This result corroborates claims that that the majority of morphological traits have diverged in parallel between the species pairs, though also demonstrates that evolution in a substantial number of traits has been non-parallel. For the remainder of the study we focused on only the genetics underlying traits that diverged in parallel, thereby allowing us to estimate the predictability of the genetics of adaptation.

Figure 4.1 Trait divergence categories

The frequency and percentage of traits determined to have diverged in parallel in the two lakes (n=32), in only a single lake (n=5) and in both lakes but in opposite directions (n=5). Fifteen traits for which more than one trait divergence category fit the data nearly equally well were left out.



Parallel genetic evolution:

We detected a total of 58 QTL that had an effect in one or both lakes underlying 26 (of the 32) traits that diverged in parallel (Figure C.3). Examples of

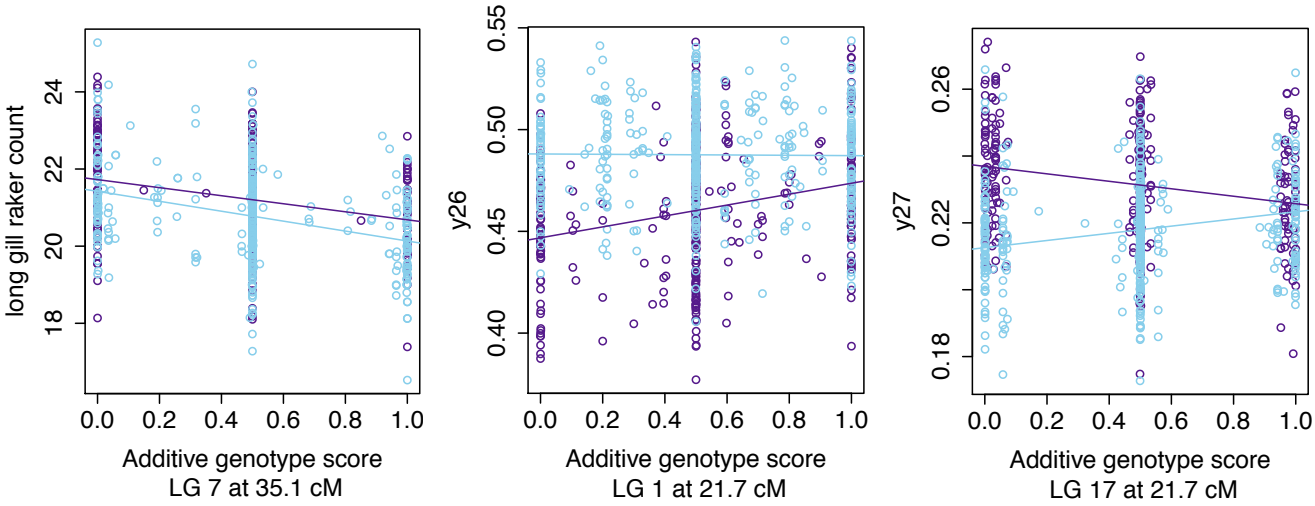
QTL scan results are shown in Figure C.2. All detected QTL are shown in Tables C.3 - C.5. We considered these 58 QTL as candidate chromosomal positions at which to test for genetic parallelism and hereafter, refer to them as 'candidate QTL'.

We considered the effects of a candidate QTL to be 'parallel' when the phenotypic effects of genotypes at the candidate QTL were in the same direction in the two lakes, though not necessarily of identical magnitudes (i.e., F_2 hybrids with benthic genotypes had a higher mean phenotype than F_2 hybrids with limnetic genotypes in both lakes, or vise versa). A candidate QTL was considered to have an effect in only a 'single lake' if the genotypes at the candidate QTL had a phenotypic effect in one lake but not the other. The effects of a candidate QTL were considered 'opposite' if phenotypic effects of genotypes at the candidate QTL were in opposite directions in the two lakes (i.e. F_2 hybrids with benthic genotypes had a higher mean phenotype than F_2 hybrids with limnetic genotypes in one lake but the opposite in the other lake). Linear models of QTL effects representing the three possibilities were fitted to the data at each candidate QTL, and we used AICc to determine which model was best supported by the data (Table C.6).

Figure 4.2 shows examples that illustrate our definitions. In each panel, an additive genotype score of 0 indicates the limnetic genotype, 1 the benthic genotype and 0.5 the heterozygote (values in between indicate uncertain genotypes, with score reflecting genotype probability). The left panel of Figure 4.2 shows an example of a QTL with parallel effects: in F_2 hybrids, the number of long gill rakers decreases with an increasing additive genotype score at a candidate QTL on linkage group 7, in both lakes. In contrast, the middle panel of Figure 4.2 shows an example of a QTL with an effect in only a single lake: the trait 'landmark y26' (the y-coordinate of a landmark placed on the dorsum of the trunk over the pectoral fin mid-point) changes with the genotype of a candidate QTL on linkage group 1 in Priest Lake but not in Paxton Lake. Finally, the right panel of Figure 4.2 shows an example of a QTL with opposite effects: the trait 'landmark y27' (the y-coordinate of a landmark placed at the posterior insertion of the dorsal fin at the first soft ray) changes with the genotype of a candidate QTL on linkage group 17 in both lakes, but in opposite directions.

Figure 4.2 Examples of QTL with parallel, single-lake and opposite effects

Examples of phenotype by genotype relationships at candidate QTL in F_2 hybrids from the Paxton Lake cross (light blue) and the Priest Lake cross (purple). Phenotypes are shown on the *y*-axes. The *x*-axes show the additive genotype score at the candidate QTL with 0 indicating the limnetic genotype, 1 the benthic genotype and 0.5 the heterozygote (values in between indicate uncertain genotypes, with score reflecting genotype probability). Lines represent the fitted values of linear models fitted to the phenotype and genotype data for each lake separately (light blue: Paxton Lake cross, purple: Priest Lake cross), using family identity and sex as covariates. Phenotypic measurements shown here are corrected for family identity.

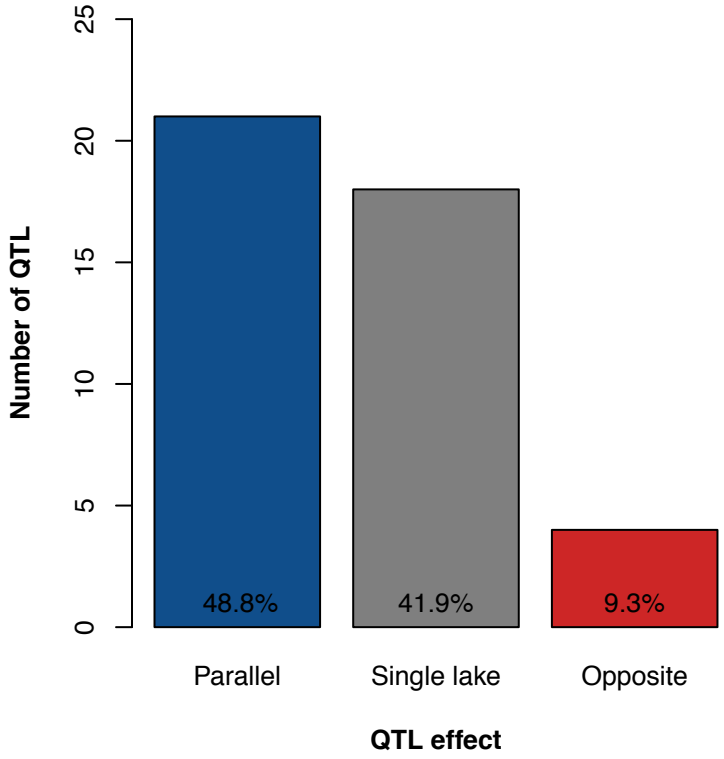


For 15 of the 58 candidate QTL, more than one QTL effect category fit the data nearly equally well, so they were not included in the following calculations. Of the remaining 43 candidate QTL for parallel traits (n=23), 48.8% (n=21) had parallel effects in the two lakes, 41.9% (n=18) had an effect in only a single lake and 9.3% (n=4) had opposite effects in the two lakes (Figure 4.3). That is, almost 50% of all QTL detected that underlie parallel phenotypic evolution had phenotypic effects in the same direction in crosses from both lakes, though not necessarily of identical magnitudes. About 40% of QTL had a phenotypic effect in one lake's cross but not the other, and the remaining approximately 10% of QTL had phenotypic effects in opposite directions in crosses from the two lakes. To ensure that including multiple QTL that map to the same genomic regions (and therefore are either based on same loci or separate tightly linked loci) did not bias our result, we also calculated the

average proportion of QTL effects per chromosome, whereby each chromosome is a data point, rather than each QTL. The results of this more conservative approach (Figure C.4) are very similar to those presented above (Figure 4.3).

Figure 4.3 QTL effect categories

The frequency and percentage of candidate QTL underlying traits that evolved in parallel that were determined to have parallel effects (n=21; blue), an effect in only a single lake (n=18; grey) and opposite effects (n=4; red). 15 QTL for which more than one QTL effect category fit the data nearly equally well were left out.



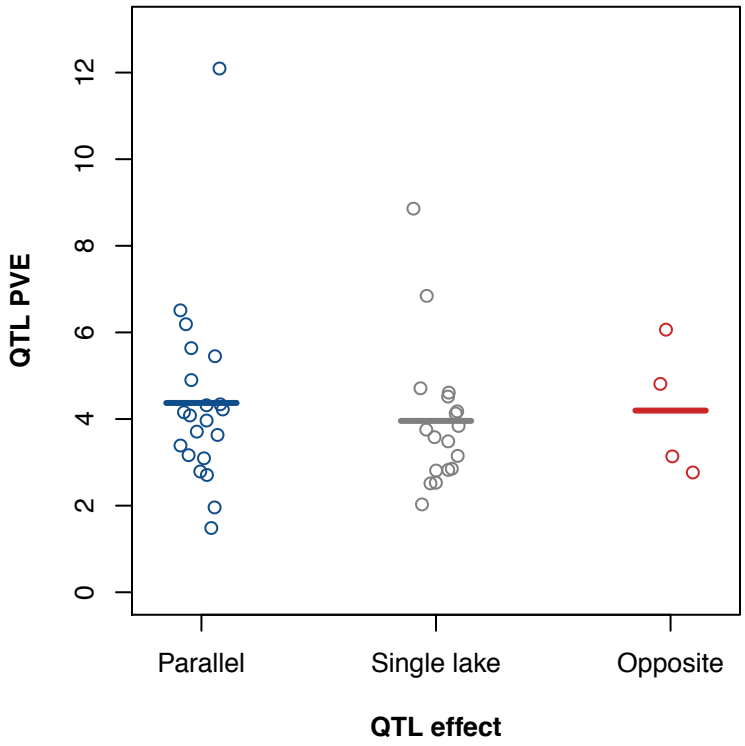
As a distinct measure of genetic parallelism, we calculated the proportional similarity of QTL use for each of the 26 traits that had undergone parallel evolution and for which candidate QTL were detected (Table C.7). Here, proportional similarity is the overlap in the proportional contributions (i.e. proportional phenotypic effects) of all QTL affecting a trait (more details in Methods). Across parallel traits, we found that the average proportional similarity of QTL use was 0.38 with a standard deviation of 0.34.

Correlates of genetic parallelism

We found no effect of QTL PVE (the percent of the phenotypic variance explained by QTL; a measure of a QTL's phenotypic effect size) on whether QTL were parallel or non-parallel (where non-parallel includes QTL with an effect in only a single lake and those with opposite effects) (df=1, X^2=0.43, p=0.51) (Figure 4.4). Samples sizes are the same as those shown in Figure 4.3.

Figure 4.4 QTL PVE by QTL effect category

Percent of the phenotypic variance explained (PVE) by QTL with parallel effects (blue), an effect in only a single lake (grey) and opposite effects (red). For each candidate QTL, the PVE of its associated phenotype was calculated separately for the Paxton Lake cross and the Priest Lake cross, and the higher of the two is plotted here. Solid lines represent means for each group. Samples sizes are the same as those shown in Figure 4.3.

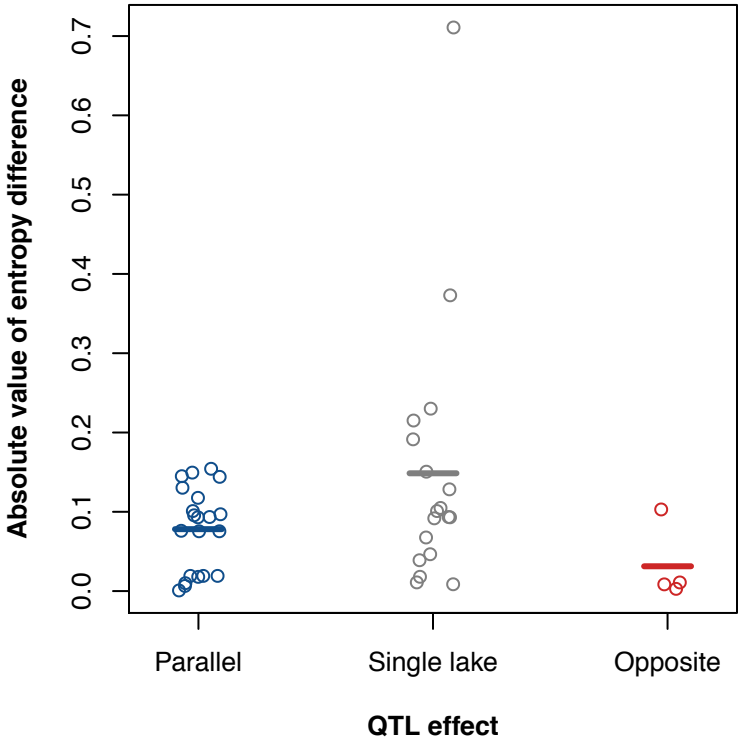


Since our markers were differentially informative in the crosses from the two lakes, we also tested whether differences in genotype information content, measured as genotype entropy (Broman and Wu 2013), explained variation in

whether QTL had an effect in both lakes (parallel and opposite effects) or in only a single lake. We, indeed, found evidence that entropy differences between the crosses affected whether QTL were detected in both lakes or not (df=1, X^2=5.58, p=0.02) (Figure 4.5).

Figure 4.5 Entropy difference between crosses by QTL effect category

The absolute value of the difference in entropy (where entropy is an index of genotype information content) between the Paxton Lake cross and the Priest Lake cross at candidate QTL that were determined to have parallel effects (blue), an effect in only a single lake (grey) and opposite effects (red). Solid lines represent means for each group. Sample sizes are the same as those in Figure 4.3.



Discussion

We found that almost 50% of QTL underlying parallel phenotypic differences between the Paxton and Priest Lake species pairs were parallel, meaning their phenotypic effects were in the same direction. When considering only QTL that actually had an effect in the crosses from both lakes, 84% were parallel, while the

other 16% had effects in opposite directions. We also found that the average proportional similarity of QTL use underlying traits that evolved in parallel in the two lakes was about 0.4. These are the first comprehensive estimates of the extent of genetic parallelism underlying repeated phenotypic evolution in a single system. Because our focal phenotypes have repeatedly evolved in correlation with the environment and are therefore likely to be adaptive (Endler 1986; Harvey and Pagel 1991; Schluter 2000; Losos 2011), our study addresses the predictability of the genetics of adaptation. Since we mapped many, continuously varying quantitative traits as well as a few discrete traits, our results may be more characteristic of the genetics of adaptation than estimates based only on few, discrete traits.

Our estimate of the average proportional similarity of QTL use underlying parallel traits, 0.4, is not far off from the estimate by Conte et al. (2012), where proportional similarity of gene/QTL use between young populations belonging to the same species and evolving in parallel was 0.47. Conte et al. (2012) also showed that the probability of repeated genetic evolution appears to decrease with increasing age of the taxa being compared (Conte et al. 2012). Repeated genetic evolution may be more likely to occur in younger taxa if the biases potentially causing some genes to be used for the adaptive evolution of a phenotype more frequently than others diverge over time themselves. Younger taxa are also likely to share more standing genetic variation than older taxa, increasing the probability that the same loci are repeatedly involved in adaptive evolution of a phenotype. Given the young age of the threespine stickleback species pairs being compared here and the fact that shared standing genetic variation has probably been involved in the evolution of the species pairs, our estimates likely fall on the high end of the distribution of estimates that we can expect to find among other natural populations.

Our estimates may be affected by several biases stemming from the use of QTL to study repeated genetic evolution, rather than genes or mutations. Because QTL are genomic regions that are usually large and contain many genes in addition to the causal one(s), we may have overestimated the amount of genetic parallelism if different loci within/linked to QTL are responsible for parallel phenotypic

changes. Shared local genomic landscape (Renaut et al. 2014) in addition to weak selection for the clustering of loci involved in local adaptation (Yeaman 2013) may, indeed, increase the probability that different loci within the same genomic regions may underlie parallel phenotypic evolution. On the contrary, QTL detection limitations may have caused us to underestimate the amount of genetic parallelism. First, the traits we mapped were mostly continuously varying, quantitative traits, underlain by mostly small effect loci. The closer the effect of a QTL was to our detection threshold (either slightly above or below it), the less likely it became that we would be able to detect that same QTL twice, due to sampling error (Beavis 1998). For this reason, we may have miss-categorized some QTL of small effect as having an effect in only a single lake. Interestingly though, if this were often the case, we would expect to have an enrichment of very small effect QTL in the 'single lake' QTL effect category, which we do not see (see Figure 4.4). Second, we only used one cross per lake and therefore, one individual per species. If the effects of any morphological QTL are polymorphic within species, and our F_0 progenitors from one lake happened to be lacking those effects, we may have concluded that such QTL had an effect in only one lake, when, in fact, we just did not sample the effect in the other lake. Third, we may have missed some parallel (and non-parallel) QTL if there was simply too little genotype information in the QTL region in one of the crosses. We did, in fact, find evidence for an enrichment of large differences in genotype information content (entropy) between the crosses at candidate QTL that were found to have an effect in only a single lake. It is, thus, possible that some 'single-lake' QTL actually belong in the parallel or non-parallel QTL effect categories, but that genotype information content was too low in one of the crosses to detect their effect. While that may be, it seems that entropy differences were high enough to have potentially mislead us in a relatively small number of cases (see Figure 4.5).

We found no evidence that the phenotypic effect sizes of QTL affect how likely they are to be parallel. On the one hand, we might have expected to see that larger effect QTL were more often parallel than smaller effect QTL. This is because mutations (which underlie QTL) have an increased probability of fixation with an increased beneficial effect (Fisher 1930; Kimura 1983). If phenotypic effect sizes of

mutations are positively correlated with their fitness effect sizes, then mutations of large phenotypic effect should be on average more likely fix and therefore, more likely to fix repeatedly. On the other hand, if mutations affect multiple phenotypes and this results in a weak correlation between phenotypic effect sizes and fitness effect sizes, our predictions regarding parallelism may differ. For instance, according to Fisher's geometric model, mutations with a large phenotypic effect infrequently contribute to adaptation in new or changing environments (Fisher 1930; Orr 2006) and typically occur early in an adaptive walk on a fitness landscape, before deleterious side-effects on other traits are too severe (Orr 2006) with distance from the optima also affecting how frequently they occur (Rogers et al. 2012a). Consequently, it is possible that mutations of larger phenotypic effect are equally or less likely to fix repeatedly than mutations of smaller phenotypic effect.

In our study, it is possible that opposing influences of the phenotypic effect size of QTL on the probability that their effects are parallel (such as those just discussed) result in no net effect. Another possibility is that even if a clear trend does exist, it could only be seen with greater variation in effect size and/or a larger sample size than was involved in our study. More work is clearly needed in this area. For example, studies that directly measure the fitness effect sizes of QTL will provide useful insights, as predictions regarding fitness effect sizes are more straightforward than those regarding phenotypic effect sizes. Note that, if the number of traits that map to a genomic region is a good proxy for the cumulative fitness effect size of that genomic region, then we might expect to see a relationship between this number and how parallel the genomic region is (the proportion of traits with a parallel QTL in the region). However, we saw no such relationship in our data and since that may be due to a weak correlation between number of traits that map to a region and the fitness effect size of the region, we do not formally present that result here.

Conclusions

Here we present the first comprehensive estimate of the extent of parallel genetic evolution underlying parallel phenotypic evolution, in a single system. These estimates reflect the predictability of the genetics of adaptation in the system. In young species pairs of threespine stickleback, we find that almost 50% of QTL have parallel effects and on average about 40% of QTL use underlying individual traits is shared. We find no evidence that the phenotypic effect size of QTL affects how likely they are to be parallel. Future studies may improve upon our estimates by using a greater number of genetic markers that are informative in both species pairs, multiple crosses per species pair and even larger samples sizes of F_2 hybrids.

As we obtain more and better estimates of the predictability of the genetics of adaptation we will be better able to understand what factors influence predictability. Future studies should aim to estimate the *actual* number of genes in which mutations may lead to a particular phenotype, and then begin to dissect the deterministic factors that we predict will cause the *effective* number to be lower.

Methods

Genetic Crosses and Experimental Ponds

In 2009, we used wild-caught adult fish to make two *in vitro* interspecific crosses, one using fish from Paxton Lake and the other using fish from Priest Lake. Both crosses involved a limnetic female and a benthic male. We stored their bodies in 95% ethanol for DNA analysis. We reared the resulting F_1 hybrids in the laboratory. On May 2, 2010 we randomly selected 35 F_1 hybrid adults (19 female and 16 male) from the Paxton cross and 25 F_1 hybrid adults (12 female and 13 male) from the Priest cross. We took a sample of caudal fin tissue from each individual F_1 hybrid for DNA analysis and then released them into separate experimental ponds at the UBC pond facility. These ponds (25 x 15 m) were designed to harbor both benthic and limnetic habitat, containing a sloping shallow zone and a deep open-water zone (6 m deep) (Arnegard et al. in press). To establish a natural prey base,

we inoculated the ponds with macrophytes, sediments and water full of aquatic insects, mollusks and plankton from Paxton Lake. We did this once in the spring of 2009 (a year before releasing our F_1 hybrids) and once in the spring of 2011. We additionally added 1.25kg of a 25.5:1 mix of 50% pure KNO_3 : KH_2PO_4 in the spring of 2009 and again in the spring of 2010. After release, the F_1 hybrids were allowed to mate freely with their full-siblings in the ponds throughout the breeding season. The following year, on September 14, 2011, we collected 407 adult F_2 hybrids from the Paxton Lake cross and 324 adult F_2 hybrids from the Priest Lake cross. We euthanized F_2 hybrids using buffered MS222 and then took a sample of caudal fin tissue from each individual F_2 for DNA analysis. Then, we fixed each F_2 hybrid body in 10% formalin for morphological measurements. During the same summer, we collected an additional 180 F_2 hybrids from the Paxton Lake cross and 92 F_2 hybrids from the Priest Lake cross for a separate study. These F_2 hybrids were not included in the QTL mapping stage of this study but were used to construct the linkage map.

Wild-caught benthic and limnetic samples

To enable us to determine whether or not our focal phenotypes diverged in parallel, we obtained high quality photos of Alizarin Red-stained, wild-caught benthic and limnetic specimens from Paxton and Priest Lakes. From these collections, made in the spring of 2005 (Ingram et al. 2012), we used 25 benthics and 21 limnetics from Paxton Lake and 36 benthic and 22 limnetics from Priest Lake. Since the Priest limnetic sample contained no females, we supplemented the collection with 23 additional Alizarin red stained wild-caught Priest limnetics (10 female) that were collected, stained and photographed in 1999 by J. Gow.

Phenotype Measurements

We stained the F_2 specimens with Alizarin Red, following the methods of Peichel et al. (2001), and then took lateral high-resolution photographs, containing a ruler in each photograph for scale. All of the following steps were done separately for the wild-caught benthic and limnetic collection and for the F_2 hybrid collection.

Using tpsDig (Rohlf 2010) we digitized and scaled 26 morphological landmarks (Figure C.1) on the photos of the specimens in randomized order. We measured centroid size as the square root of the sum of squared distances of the 26 landmarks from their centroid. We then performed Generalized Procrustes Superimposition on the x and y coordinates of the scaled landmarks using the R package 'shapes' (Dryden 2013), resulting in 52 'landmark traits'. To correct for specimen bending, we followed the approach of Albert et al. (2008).

We scored five meristic traits (i.e. countable quantitative traits) (Figure C.1) using the fixed and stained F_2 specimens themselves. In the absence of the wild-caught reference fish specimens, we scored meristic traits using their photos. However, since photos do not show the gill rakers, we could not count the long and short gill rakers on the first gill arch for the wild-caught benthic and limnetic samples, as was done for the F_2 hybrids. Instead we used counts taken by Ingram et al. (2012) of the total gill raker number on the first gill arch for the same individuals. For the 23 additional Priest limnetic fish, no gill raker counts were available, and thus, they were left out of the test of parallelism in gill raker divergence. We tested for and removed significant outlier data points for all traits using the function 'outlierTest' in the R package 'car' (Fox et al. 2013). F_2 hybrids that were standard length outliers were dropped from the study (4 Paxton individuals and 1 Priest individual).

Identifying parallel phenotypic evolution

We classified a trait's divergence as 'parallel' when the species difference in the trait was in the same direction in both lakes (i.e. benthics had a higher mean than limnetics in both lakes, or vise versa), though not necessarily of the same magnitude. We classified a trait's divergence as 'opposite' when the species difference in the trait was in opposite directions in the two lakes (i.e. benthics had a higher mean than limnetics in one lake and a lower mean than limnetics in the other). If the species differed in a trait in only one of the two lakes, we classified the trait's divergence as only in a 'single lake'. Finally, if the species did not differ in a

trait in either lake, we classified the trait's divergence as in 'neither lake'. For each trait, we tested these scenarios by fitting 5 linear models to phenotypes of our wild caught benthics and limnetics from both lakes and then deciding which fit the data best (Table C.1).

Model 1: 'same effect', included the main effect of species (benthic vs. limnetic). Model 2: 'different effect' included the main effects of both species and its interaction with lake (allowing the effect of species to differ between the lakes). In Model 3: 'effect in Paxton only', the main effect of species was manipulated so that all Priest individuals were treated as the same species. The reverse was done in Model 4: 'effect in Priest only'. Finally in Model 5: 'no effect', the effects of species were dropped completely. All models included the main effect of sex.

We then grouped traits into the four 'Trait divergence' categories based on the model with lowest AICc value, which we term the "best model". Trait divergence was considered to be parallel if either model 1 was the best, or model 2 was the best and the effect of species was in the same direction in the two lakes. Traits were considered to be divergent in only a single lake if either model 3 or 4 was the best. Trait divergence was considered to be opposite if model 2 was the best and the effect of species was in opposite directions in the two lakes. Finally, a trait was considered to be divergent in neither lake if model 5 was the best.

For 15 traits, more than one trait divergence category fit the data nearly equally well. That is, the delta AICc value between the best model and the second best model was less than 2 and the second best model called for a different trait divergence category than the best model did (Table C.1). These 15 traits were, therefore left out of all calculations and analyses in which trait divergence category was a variable. Finally, because the gill raker counts for our wild-caught reference fish were of the total number on the first gill arch, rather than the subdivided counts of long and short rakers on the first arch, as was scored in our F2 hybrids, the trait divergence category determined for the total number of gill rakers, was inferred to be the trait divergence category of the subdivided counts as well. However, for calculating the proportion of traits that diverged in parallel, in a single lake and in

opposite directions (Figure 4.1), only the total gill raker count was considered (therefore, n=57 rather than n=58 traits for those calculations).

SNP Genotyping

We isolated genomic DNA from caudal fin tissue of our 4 F_0 progenitors, 60 F_1 hybrids and 735 F_2 hybrids using either Proteinase K digestion, phenol-chloroform extraction, ethanol precipitation and re-suspension of the precipitated DNA in 30 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), or the DNeasy 96 Blood and Tissue Kit (QIAGEN), using only 30µL of Buffer AE for the first elution (leading to a relatively high DNA concentration in the eluant). We then diluted an aliquot of each sample using TE buffer to a DNA concentration between 3 ng/µL and 150 ng/µL, based on the PicoGreen® assay (Life Technologies).

We genotyped all F_0, F_1, and F_2 individuals using Illumina's GoldenGate assay and a custom multiplex oligonucleotide pool developed for a recently published collection of SNPs (Jones et al. 2012a). We found 430 of these SNPs to be polymorphic in at least one of our crosses (246 were polymorphic in the Paxton cross and 318 were polymorphic in the Priest cross, 134 of which were polymorphic in both). See Table C.2 for the identities, genomic locations, and U.S. National Center for Biotechnology Information (NCBI) identification numbers for all 430 SNP markers. The Illumina Sentrix Array Matrices used for genotyping were processed at the Genomics Shared Resource of the Fred Hutchinson Cancer Research Center (Seattle, WA, USA). We scored genotypes from the raw data using GenomeStudio software (Illumina Inc.).

Linkage Mapping

We created a linkage map with JoinMap ver. 3.0 (Ooijen and Voorrips 2002), coding F_2 hybrid genotypes according to the 'cross pollinator' population code for outbred crosses between two diploid parents. To determine the identity of each F_2 hybrid's F_1 parents, we used the R package 'MasterBayes' (Hadfield 2013) to reconstruct pedigrees based on the full SNP dataset. For creating the linkage map,

we included F_1 x F_1 families containing at least 10 F_2 hybrids. This included 268 F_2 hybrids from the Paxton cross and 261 F_2 hybrids from the Priest cross, some of which were not used for QTL mapping, as noted above.

We used JoinMap ver. 3.0 to compute all obtainable pairwise recombination frequencies and associated LOD (logarithm (base 10) of odds) scores between SNP makers for each F_1 x F_1 family (hereafter 'family') separately. We then combined them into a single pairwise data file (*pwd-file*) for all families and used JoinMap to produce a single linkage map derived from the combined results of the Paxton and Priest Lake crosses. We also created separate linkage maps for each lake. We found that we detected all the same QTL when we analyzed each separate lake's cross with its own map as we did when we analyzed each separate lake's cross with the combined map. Therefore, we proceeded with the use of the combined map for the remaining analyses.

Identifying candidate QTL

To identify a set of chromosomal regions at which to conduct tests of genetic parallelism, we carried out a round of QTL scans to identify those chromosomal regions that had a phenotypic effect in at least one of the lakes (which we termed 'candidate QTL'). We constructed three QTL maps for each trait by interval mapping using Haley-Knott regression via the 'scanone' function in R/qtl, and F_2 cross genotype coding (Broman and Wu 2013). 'scanone' includes additive and dominance components of genotypes when testing for QTL. The first QTL map was made using only the F_2 hybrids from the Priest Lake cross (n=323) and the second QTL map was made using only the F_2 hybrids from the Paxton Lake cross (n=403). The third QTL map was made using the F_2 hybrids from both crosses, and the scan included a genotype x cross interaction covariate. The main effects of family identity and sex were used as covariates in all three of the scans. We lumped all F_2 hybrids with no full siblings into a single 'pseudo-family'. This pseudo-family consisted of 20 F_2 hybrids in the Priest Lake cross and 24 F_2 hybrids in the Paxton Lake cross. We found that use of these pseudo-families presented no artifacts. For each trait, we

performed 10,000 permutations to determine the genome-wide LOD threshold for significant QTL at the $\alpha = 0.05$ level.

Our candidate QTL dataset included only QTL underlying traits that were determined to have diverged in parallel. Some of these QTL were discovered in both the combined scan and one or both of the single-lake scans, as indicated by overlapping 1.5 LOD confidence intervals (i.e. confidence intervals for the position of QTL that extend on either side of the peak LOD score position to the position at which a 1.5 LOD drop relative to the peak LOD score is seen). In this case, we used the QTL position from the combined scan in our candidate QTL dataset and discarded the result(s) from the single-lake scan (though all detected QTL are shown in Tables C.3 - C.5).

Testing for parallel genetic evolution

We classified a QTL's effect as 'parallel' when its phenotypic effect was in the same direction in both lakes (i.e. F_2 hybrids with benthic genotypes had a higher mean than F_2 hybrids with limnetic genotypes in both lakes, or vise versa), though not necessarily of the same magnitude. We classified a QTL's effect as 'opposite' when its phenotypic effect was in opposite directions in the two lakes (i.e. F_2 hybrids with benthic genotypes had a higher mean than F_2 hybrids with limnetic genotypes in one lake and the opposite in the other lake). If the phenotypic effect of a QTL was present in only one of the lakes, we classified the QTL's effect as only in a 'single lake'. For each candidate QTL, we tested these scenarios by fitting 5 linear models to the phenotypes of F_2 hybrids from both lakes combined, and then deciding which fit the data best (Table C.6).

When present, the main effects of QTL genotype (at the peak marker position; the position showing the strongest association with the phenotype), included both the additive and dominance components of genotypes (referred to as additive score and dominance score, respectively). The additive score, reflecting the estimated proportion of the genotype that is from the benthic grandparent, was calculated as $0.5 - P(AA)/2 + P(BB)/2$, and the dominance score, reflecting the

estimated probability that the genotype is heterozygote, was calculated as, P(AB)/2, where A and B represent the alternative alleles at the SNP marker, and P(AA), P(AB) and P(BB) represent an individual's probability of having the respective genotype. Genotype probabilities were calculated using the R/qtl function 'calc.genoprob' (Broman and Wu 2013). Model 1: 'same effect', included the main effects of QTL genotype. Model 2: 'different effect' included both the main effects of QTL genotype and the interaction between its additive score with lake (allowing the additive genotypic effect to differ between the lakes). In Model 3: 'effect in Paxton only', the main effect of QTL genotype was manipulated so that the genotypes of Priest F_2 hybrids were fixed. The reverse was done in Model 4: 'effect in Priest only'. Finally in Model 5: 'no effect', the effects of QTL genotype were dropped completely. All models included family identity and sex as covariates.

We then grouped the 58 candidate QTL into the three 'QTL effect' categories based on which model had the lowest AICc value, which we term the 'best model'. QTL we considered to have parallel effects if either model 1 was the best, or model 2 was the best and the effect of the QTL was in the same direction in the two lakes. QTL were considered to have an effect in only a single lake if either model 3 or 4 was the best. QTL were considered to have opposite effects if model 2 was the best and the effect of the QTL was in opposite directions in the two lakes. Since model 5 was never the best, no QTL were considered to have an effect in neither lake. For 24 candidate QTL, more than one QTL effect category fit the data nearly equally well. That is, the delta AICc value between the best model and the second best model was less than 2 and the second best model called for a different QTL effect category than the best model did (Table C.6). These 15 candidate QTL were, therefore, left out of all calculations and analyses in which QTL effect category was a variable.

As a second, distinct measure of genetic parallelism, we estimated the proportional similarity of QTL use underlying each trait represented in our candidate QTL dataset that had undergone parallel phenotypic evolution (n=26) (Conte et al. 2012). We accomplished this by fitting multiple QTL linear models using the R/qtl function 'fitqtl' (Broman and Wu 2013) (see Table C.7). We did this separately for the two lakes. Of the full set of candidate QTL for any given trait, only

those having a significant effect (α=0.05) in an ANOVA comparing a full 'single QTL, single lake linear model' to a reduced model from which QTL genotype terms were dropped were included in the multiple QTL model for that lake. The 'single lake, single QTL linear models' contained the main effects of QTL genotype, including the additive and dominance score terms, as well as the main effects of family identity and sex. The multiple QTL models included, the main effects of those QTL genotypes, as well as the main effects of family identity and sex.

We calculated the proportional similarity of QTL use underlying each trait following the methods of Conte et al. (2012). For each lake separately, the PVEs of all QTL included in the multiple QTL model for a given trait, calculated using R/qtl's 'fitqtl' function via 'drop one QTL at a time ANOVAs' (Broman and Wu 2013), were scaled so that their sum was equal to 1, resulting in proportional contributions of each QTL to the phenotype (Table C.7). We then calculated proportional similarity as the minimum overlap in the distribution of proportional contributions; $PS = \Sigma_i \min(p_i1, p_i2)$, where p_i1 and p_i2 are the proportional contributions of QTL_i in the two taxa.

Correlates of genetic parallelism

We asked whether QTL PVE (the percent of the phenotypic variance explained by QTL; a measure of a QTL's phenotypic effect size) had an effect on whether QTL were parallel or non-parallel. We determined PVE for each candidate QTL in each lake separately, using 'single lake, single QTL linear models' (described above) and then ANOVAs comparing the full model to a reduced model from which QTL genotype terms were dropped. We calculated PVE as the absolute value of the difference in the residual sum of squares explained by the full and reduced models divided by the total sum of squares explained by the full model. Using the higher PVE value of the two lakes, we then asked whether the mean PVE of QTL with parallel effects was different from the mean PVE of QTL with non-parallel effects (including QTL with effects in only one lake and opposite effects), using binomial logistic regressions. 15 candidate QTL were left out of this analysis because more

than one QTL effect category fit the data nearly equally well. The analysis included the remaining 43 candidate QTL underlying traits that evolved in parallel.

We tested whether differences in genotype information content explained any variation in whether QTL were determined to have an effect in both lakes (parallel and opposite effects) or an effect in only a single lake. As a measure of genotype information content, we used the function 'plot.info' in R/qtl to calculate an 'entropy' score at each marker whereby, a lower value indicates greater genotype information content (Broman and Wu 2013). When QTL peak markers were real SNP markers, we simply extracted the entropy score of each cross at that peak marker. When the 'peak marker' fell between two real SNP markers, we calculated entropy using linear interpolation. We then asked whether QTL with an effect in only a single lake were associated with larger differences in entropy between the lakes, than QTL with an effect in both lakes, using a binomial logistic regression. The same candidate QTL were included in this analysis as were included in the analysis of the effect of QTL PVE on QTL parallelism (described directly above).

5 Conclusions

The origin of species may quite often occur via the process of ecological speciation (Schluter 2001; Nosil 2012) and the genetics underlying the process may substantially affect its outcome (Schluter and Conte 2009). The studies herein have made contributions to our understanding of the genetics of ecological speciation and of adaptation in general. In this chapter, I revisit the primary questions outlined in the introduction and discuss how our work has contributed to answering them.

Question 1: what genetic mechanisms link divergent natural selection to reproductive isolation during ecological speciation?

In chapter 2, we show that body size, a trait under divergent natural selection, also functions as a mate signal and determines female mate preference, via phenotype matching in the Paxton Lake threespine stickleback species pair. This implies that the genes controlling body size are the same as those that cause assortative mating by body size, constituting a genetic mechanism that is thought to greatly facilitate ecological speciation with gene flow. As a result, divergent selection on body size should lead to assortative mating by body size as an automatic by-product.

We see support for similar mechanisms in many natural populations. For example, divergent adaptations may often lead to spatial and/or temporal isolation that limit the probability of differentially adapted reproductive individuals encountering one another. Populations of pea aphids (*Acyrthosiphon pisum*) that are adapted to different host plant species experience spatial isolation as a byproduct of living and mating on their respective host plants (Via 1999). Populations of apple maggot flies (*Rhagoletis pomonella*) that are adapted to different host plant species experience both spatial isolation as a result of living and mating on their host plants and temporal isolation as a byproduct of the different fruiting times of their respective host-plant species (and their own co-evolved timing of emergence) (Bush 1969). Lord Howe palms (Arecacea) that are adapted to different soil types are

temporally isolated by differences in flowering time that are thought to be caused by physiological changes induced by the soil type (Savolainen et al. 2006). Populations of sunflower (*Helianthus petiolaris*) that are differentially adapted to dune and non-dune habitats experience some spatial isolation due to the distance between their habitats (Andrew et al. 2012). Populations of yellow monkeyflower (*Mimulus guttatus*) that are differentially adapted to costal vs. inland habitats are isolated both by distance between the habitats and differences in flowering time that are associated with climatic regimes within the habitats (Lowry et al. 2008b).

Like in our study, divergent adaptations have also been shown to affect assortative mating decisions in natural populations. In most of these cases they have been found to function as a mate signal, these constituting 'classic magic traits' (reviewed in Servedio et al. (2011)). Though, some examples also exist in which traits under divergent natural selection have been shown to affect mate preferences, for example in cases of sensory drive (reviewed in Boughman (2002)).

Our results make an important contribution to this literature because we provide one of the first examples of a trait under divergent natural selection functioning as a mate signal *and* determining mate preference, via phenotype matching. The issue of building linkage disequilibrium between assortative mating and targets of divergent natural selection is not eliminated unless the genes under divergent selection control both mate signal and mate preference. This fact has been underappreciated until recently (Doebeli 2005; Maan and Seehausen 2012; Conte and Schluter 2013) and thus, the rarity of examples is perhaps not due to rarity in nature but rather a lack of appropriate testing. These observations beg more work in the area to fill in the apparent gap.

Question 2: what is the genetic architecture of adaptation during ecological speciation?

In Chapter 4, we identified a large number of QTL for morphological divergence that are widely distributed across the genome in both the Paxton and Priest Lake species pairs of threespine stickleback. Likewise, in a recently published

study (Arnegard et al. in press) that was part of the same larger project to discover the genetic architecture of ecological speciation (see Preface), we showed that the genetic architecture of juvenile niche divergence in the Paxton Lake species pair is explained by multiple, unlinked QTL. Interestingly, this genetic architecture appeared to be largely additive, with each benthic allele making an approximately equal contribution to an overall benthic niche phenotype. Our findings in the stickleback species pairs are interesting because they suggest that many loci underlying ecologically important traits have diverged (and/or divergence has persisted) during ecological speciation despite the homogenizing effects of gene flow.

In other natural populations, we see examples ranging from few to many loci underlying traits under divergent natural selection. For example, in a review of ecological speciation in phytophagous insects, three of eight studies found 1-2 loci underlying divergently selected traits, three studies supported 3-5 such loci and two concluded polygenic control (Matsubayashi et al. 2010). Also, in a review of speciation in flowering plants, anywhere from 1-17 loci were found to underlie reproductive isolation, which in many cases was a result of traits under divergent natural selection (Lowry et al. 2008a). Based on a large number of genome scans (e.g. Hohenlohe et al. 2010; Michel et al. 2010; Jones et al. 2012a,b; Renaut et al. 2012; Andrew and Rieseberg 2013, to name a small few) we also see that the genomic distributions of divergent loci can be anywhere from highly clustered to widespread. Although we cannot determine the role of these regions in divergence without accompanying studies to associate them with the phenotypes they affect (as is currently the case for many genome scans, but certainly not all), we nonetheless see that snapshots of genetic divergence across the genome may involve anywhere from few and/or clustered loci to many and/or widespread loci.

While both theory (see Introduction) and empirical observations suggest that the number of loci underlying traits under divergent selection may range from few to many and their genomic distributions may range from clustered to widespread, more work is needed to determine whether theoretical explanations for this

variation, such as the strength of selection, the amount of gene flow and progress towards speciation, can predict what we find in nature.

Question 3: how predictable are the genetics of adaptation during ecological speciation and in general?

In Chapter 3, we estimated the probability of gene reuse in natural populations undergoing repeated phenotypic evolution, informing us of the predictability of the genetics of adaptation across a wide range of taxa. We found this estimate to be 0.32-0.55, depending on the genetic methods employed by individual studies. Furthermore, we found that the probability of gene reuse declines with increasing age of the taxa being compared. In Chapter 4, we estimated the extent of genetic parallelism underlying parallel morphological divergence between the Paxton and Priest Lake species pairs, providing insight to the predictability of the genetics of adaptation in a single system. We found that about 50% of QTL for parallel morphological differences are parallel, and on average, the proportional similarity of QTL use underlying individual morphological traits is about 0.4. In addition, we find no evidence that the phenotypic effect size of QTL predicts whether or not they are parallel.

Studying the genetics of repeated phenotypic evolution is important because it informs us of the predictability of the genetics of adaptation. Repeated phenotypic evolution appears to be a common phenomenon and hundreds of examples exist in the literature. In a growing number of these cases, the genetic basis is being or has been discovered, providing us with the opportunity to directly and empirically estimate the predictability of the genetics of adaptation. While this topic is of considerable interest to evolutionary biologists and has been the subject of many recent studies and reviews, our study in chapter 3 is the first to provide a quantitative estimate of the probability of repeated genetic evolution across a wide range of taxa. Furthermore, our study in chapter 4 is the first to comprehensively provide said estimates in a single system.

Concluding Remarks

The studies contained herein have relevance to a variety of fundamental topics in evolutionary biology. Chapter 2 and Chapter 4 both make significant contributions to answering controversial questions regarding the genetics of ecological speciation. In addition, Chapter 3 and Chapter 4 are the first studies to provide quantitative estimates of the predictability of the genetics of adaptation during ecological speciation and in general.

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Appendices

Appendix A: Chapter 2 Supplementary Material

Figure A.1 Experimental Design

Grey cartoon fish represent individuals for which size was manipulated to be similar to that of the opposite species. Black cartoon fish represent individuals for which size was manipulated to be different from that of the opposite species.

Treatment	**Female**	**Male**	**Predictions**	
			if phenotype matching by size:	***if no phenotype matching by size:***
Different Size (Control)	Large Benthic 	Large Limnetic 	Mating more likely in similar-size treatment than in different-size treatment	Mating equally likely in similar-size and different-size treatments
Similar Size (Experimental)	Small Benthic 	Large Limnetic 		
Different Size (Control)	Small Limnetic 	Small Benthic 		
Similar Size (Experimental)	Large Limnetic 	Small Benthic 		

Table A.1 Effects of body size manipulation

Effects of body size manipulation on mean standard length (cm), body mass (g) and condition factor (g $10^5 cm^{-3}$) of benthic and limnetic females randomly assigned to abundant-food and reduced-food groups. Standard are deviations in parentheses.

Female	Measure	Abundant-food	Reduced-food	F-value	df	p
Benthic	Standard Length (cm)	70.10 (4.72)	59.72 (3.17)	53.45	1	$2.6x10^{-8}$
	Body Mass (g)	5.3 (1.0)	2.8 (0.5)	72.05	1	$1.1x10^{-9}$
	Condition Factor	1.24 (0.14)	1.17 (0.12)	2.22	1	0.146
Limnetic	Standard Length (cm)	50.06 (3.61)	42.23 (3.82)	39.89	1	$2.9x10^{-6}$
	Body Mass (g)	1.8 (0.3)	0.8 (0.2)	62.38	1	$1.0x10^{-7}$
	Condition Factor	1.04 (0.10)	0.97 (0.15)	1.46	1	0.244

Table A.2 Effects of explanatory variables on stringent female acceptance scores

Logistic regressions to test for the effects of explanatory variables on the more stringent female acceptance scores, for which females had to at least follow the male to his nest to be assigned a score of 1. Each model includes treatment and the one additional variable indicated. Treatment was entered first and so has identical effects in all models except model 1i, due to missing data points for male nuptial color. The second explanatory variable in models 1c – 1h are male courtship behaviors. N = 57 mate choice trials.

Model	Explanatory Variable	df	X^2	p
1a – 1h	treatment (similar vs. different size)	1	6.44	0.01
1i		1	4.75	0.03
1a	female species	1	2.14	0.14
1a	treatment x female species	1	0.14	0.70
1b	trial date	1	2.23	0.14
1c	no. of approaches	1	0.79	0.37
1d	no. of zig-zags	1	2.30	0.13
1e	no. of bites	1	0.46	0.50
1f	no. of leads to nest	1	0.26	0.61
1g	no. of nest maintenance events	1	0.001	0.98
1h	no. of nest creep-throughs	1	11.22	0.001
1i	male nuptial color	1	0.70	0.40

Table A.3 Effects of explanatory variables on female acceptance scores using males' first trial

Logistic regressions to test for the effects of explanatory variables on female acceptance scores using data from only the first trial of each male (N = 43 trials total). Each model includes treatment and the one additional variable indicated. Treatment was entered first and so has identical effects in all models except model 1i, due to missing data points for male nuptial color. The second explanatory variable in models 1c – 1h are male courtship behaviors.

Model	Explanatory Variable	df	X^2	p
1a – 1h	treatment (similar vs. different size)	1	15.17	$1x10^{-4}$
1i		1	12.73	$4x10^{-4}$
1a	female species	1	2.44	0.12
1a	treatment x female species	1	0.38	0.54
1b	trial date	1	0.93	0.34
1c	no. of approaches	1	0.02	0.90
1d	no. of zig-zags	1	0.37	0.54
1e	no. of bites	1	0.89	0.35
1f	no. of leads to nest	1	0.30	0.58
1g	no. of nest maintenance events	1	1.67	0.20
1h	no. of nest creep-throughs	1	0.42	0.52
1i	male nuptial color	1	0.53	0.47

Table A.4 Effects of explanatory variables on female acceptance scores using restricted date range

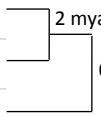
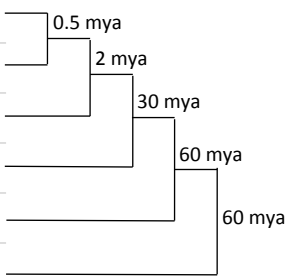
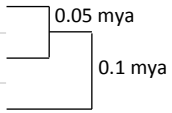
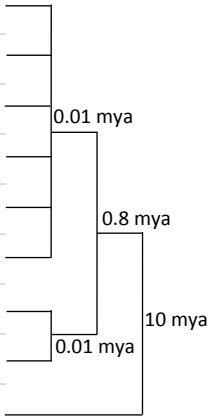
Logistic regressions to test for the effects of explanatory variables on female acceptance scores using data from all limnetic female trials and only those benthic female trials that occurred during the restricted date range in which both 'large' and 'small' category benthic females were in breeding condition (N = 44 trials total). Each model includes treatment and the one additional variable indicated. Treatment was entered first and so has identical effects in all models except model 1i, due to missing data points for male nuptial color. The second explanatory variable in models 1c – 1h are male courtship behaviors.

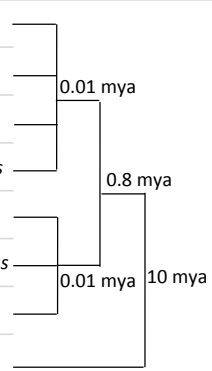
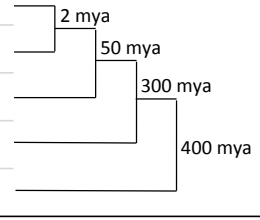
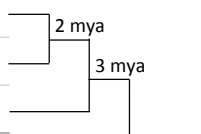
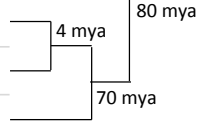
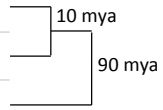
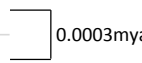
Model	Explanatory Variable	df	X^2	p
1a – 1h	treatment (similar vs. different size)	1	14.80	$1x10^{-4}$
1i		1	13.79	$2x10^{-4}$
1a	female species	1	2.29	0.13
1a	treatment x female species	1	0.59	0.44
1b	trial date	1	0.0001	0.99
1c	no. of approaches	1	1.12	0.29
1d	no. of zig-zags	1	0.04	0.85
1e	no. of bites	1	2.54	0.11
1f	no. of leads to nest	1	0.72	0.40
1g	no. of nest maintenance events	1	0.27	0.61
1h	no. of nest creep-throughs	1	3.02	0.08
1i	male nuptial color	1	0.32	0.57

Appendix B: Chapter 3 Supplementary Material

Table B.1 Summary of cases detected by our literature search

(Starts on next page) Listed node ages are the means of all available estimates, rounded to the nearest highest significant figure. Node ages are only shown between taxa that evolved a similar phenotype (individual taxa with ancestral phenotypes are not shown). Initial genetic methods: m = genome-wide mapping; ct = complementation test; mcg = mapping of a candidate gene; cg = candidate gene. The column “Informative Candidate Gene(s) Confirmed” refers to those candidate genes confirmed to contribute to the repeatedly evolved phenotype in the taxa specified in the same row, for which there is also evidence to draw upon regarding its contribution in the other taxa that evolved the same phenotype. A candidate gene was considered to have no effect if the assay used produced no evidence that the gene contributed to the trait. We recognize though, that assays were not always exhaustive and could not completely rule out an effect of the gene on the trait. Candidate genes considered to have no effect are listed (as "not ___") only when nothing more is known about the genetic basis of the phenotype.

Phenotype	Independent Origins In	Mean Estimated Node Ages (rounded to the nearest highest significant figure)	Initial Genetic Method	Estimated Relative Contribution of Genes or QTL	Informative Candidate Gene(s) Confirmed	References
Larval trichome loss	*Drosophila montana*		cg	––	*svb*	Dickinson et al. 1993[3]; Sucena and Stern 2000[3]; Sucena et al. 2003[1]; Tamura et al. 2004[2]; Morales-Hojas et al. 2011[2]
	Drosophila borealis		cg	––	*svb*	
	Drosophila sechellia		m	*svb*: 1	––	
Skin toxin - caerulein	*Xenopus laevis*		cg	––	*xlcae3p*	Hedges et al. 2006[2]; Roelants et al. 2010[1]; Roelants et al. 2011[3]
	Litoria splendida		cg	––	*lscae1p*	
Tetrodotoxin resistance	*Thamnophis couchii*		cg	––	*Nav1.4*	de Queiroz et al. 2002[2]; Wüster et al. 2008[2]; Wood et al. 2011[2]; Feldman et al. 2012[1]
	Thamnophis atratus		cg	––	*Nav1.4*	
	Thamnophis sirtalis		cg	––	*Nav1.4*	
	Amphiesma pryeri		cg	––	*Nav1.4*	
	Rhabdophis tigrinus		cg	––	*Nav1.4*	
	Liophis epinephelus		cg	––	*Nav1.4*	
Lactase persistance	European *Homo sapiens*		m	*LCT*: 1	––	Tishkoff et al. 2007[1]; Enattah et al. 2008[3]; Ingram et al. 2009[3]
	Saudi Arabian *H. sapiens*		cg	––	*LCT*	
	Kenyan and Tanzanian *H. sapiens*		cg	––	*LCT*	
Reduction in lateral plate number	Paxton benthic *Gasterosteus aculeatus*		m	*Eda*: 0.83; *LG7*: 0.04; *LG10*: 0.08; *LG21*: 0.05	––	Orti et al. 1994[2]; Hatfield 1997[3]; Colosimo et al. 2004[3]; Cresko et al. 2004[1]; Schluter et al. 2004[3]; Colosimo et al. 2005[1]; Cano et al. 2006[3]; Kitano et al. 2008[3]; Bell et al. 2009[2]; Shapiro et al. 2009[1]; Le Rouzic et al. 2011[3]; Rogers et al. 2012[3]; Rogers pers. comm.[3]
	Cranby *G. aculeatus*		m	*Eda*: 0.98; LG7: 0.02	––	
	Paq; Graham; Bear Paw *G. aculeatus*		m	*Eda*: 1	––	
	Boot Lake; Whale Lake *G. aculeatus*		ct	*Eda*: 1	––	
	Nakagawa Creek *G. aculeatus*		ct	*Eda*: 1	––	
	6 Pacific freshwater populations of *G. aculeatus*		cg	––	*Eda*	
	5 Atlantic populations of *G. aculeatus*		cg	––	*Eda*	
	5 Scandinavian populations of *G. aculeatus*		cg	––	*Eda*	
	Fox Hole *Pungitius pungitius*		m	LG12: 1	––	

Phenotype	Independent Origins In	Mean Estimated Node Ages (rounded to the nearest highest significant figure)	Initial Genetic Method	Estimated Relative Contribution of Genes or QTL	Informative Candidate Gene(s) Confirmed	References
Pelvic spine and girdle reduction	Paxton benthic *G. aculeatus*		m	*Pitx1*: 0.731; *LG1*: 0.073; *LG2*: 0.122; *LG4*: 0.074	––	Orti et al. 1994[2]; Cresko et al. 2004[3]; Shapiro et al. 2004[3]; Marks 2006[3]; Shapiro et al. 2006[1]; Coyle et al. 2007[3]; Bell et al. 2009[2]; Shapiro et al. 2009[1]; Chan et al. 2010[3]
	Boulton *G. aculeatus*		m	LG4: 1	––	
	7 Alaska populations of *G. aculeatus*		cg	––	*Pitx1*	
	Dolomite; Orphia *G. aculeatus*		cg	––	not *Pitx1*	
	Loch Fada *G. aculeatus*		m	*Pitx1*: 1	––	
	Loch Vifilsstadavat *G. aculeatus*		ct	*Pitx1*: 1	––	
	Loch Scadavay *G. aculeatus*		cg	––	not *Pitx1*	
	Fox Hole *Pungitius pungitius*		m	LG4: 1	––	
Inability to use galactose	West African strains *Saccharomyces cerevisiae*		m	*GAL3*: 1	––	Cliften et al. 2003[2]; Hittinger et al. 2004[1]; Beltrao and Serrano 2005[2]; Warringer et al. 2011[3]
	27361N strain *S. cerevisiae*		ct	*GAL1*: 1	––	
	Saccharomyces kudriavzevii		cg	––	*Gal 1 - Gal 7*	
	Candida glabrata		cg	––	*Gal 1 - Gal 7*	
	Eremothecium gossypi and Kluyveromyces waltii		cg	––	*Gal 1 - Gal 7*	
Red floral pigmentation (system 1)	*Mimulus l. variegatus*		mcg	*Pla2:* 1	––	Nie et al. 2006[2]; Cooley & Willis 2009[1]; Cooley et al. 2011[1]; Grossenbacher and Whittall 2011[2]
	Mimulus naiandinus		ct	*Pla2:* 0	––	
	Mimulus cupreus		mcg	*Pla1:* 1	––	
Red floral pigmentation (system 2)	*Ipomoea Mina* clade		cg	––	*F3'h*	Stefanovic et al. 2002[2]; Hedges et al. 2006[2]; Streisfeld & Rausher 2009[1]; Des Marais et al. 2010[3]; Smith & Rausher 2011[1]
	Ipomoea horsfalliae		cg	––	*F3'h*	
	Iochroma gesnerioides		cg	––	*Dfr*	
Electrical activity of myogenic electric organ	*Mormyroids*		cg	––	*Scn4aa*	Hedges et al. 2006[2]; Zakon et al. 2006[1]; Arnegard et al. 2010[3]; Lavoué et al. 2012[2]
	Gymnotiforms		cg	––	*Scn4aa*	
Red wing patterns	*Heliconius melpomene*		m	*optix*: 1	––	Baxter et al. 2008[3]; Papa et al. 2008[3]; Pohl et al. 2009[2]; Quek et al. 2010[3]; Reed et al. 2011[1]
	Heliconius erato		m	*optix*: 1	––	
Digestion of foregut-fermenting bacteria	*Pygathrix nemaeus*		cg	––	*RNase1B*	Zhang et al. 2002[3]; Zhang 2003[1]; Hedges et al. 2006[2]; Sterner et al. 2006[2]; Zhang 2006[3]; Fabre et al. 2009[2]
	Colobus guereza		cg	––	*RNase1beta; RNase1gamma*	
	ruminant artiodactyls		cg	––	*bovine pancreatic RNase gene*	
Life history (latitudinal clines*)	Australian *D. melanogaster*		cg	––	*InR*	David and Capy 1988[2]; Paaby et al. 2010[1];
	North American *D. melanogaster*		cg	––	*InR*	

Phenotype	Independent Origins In	Mean Estimated Node Ages (rounded to the nearest highest significant figure)	Initial Genetic Method	Estimated Relative Contribution of Genes or QTL	Informative Candidate Gene(s) Confirmed	References
Ultrahigh-frequency hearing for echolocation	Yangochiroptera and Yinpterochiroptera◆	80 mya	cg	––	*Prestin, Tmc1, Pjvk, Cdh23, Pcdh15, Otof*	Hedges et al. 2006[2], Liu et al. 2010[3], Davies et al. 2012[1], Shen et al. 2012[3]
	Tursiops truncatus		cg	––	*Prestin, Tmc1, Pjvk, Cdh23, Pcdh15, Otof*	
Rapid development rate	Clearwater *Oncorhynchus mykiss*	1 mya	m	OC8: 0.80; OC9: 0.03 ;OC10: 0.02; OC14: 0.04; OC24: 0.04; Ocb: 0.07	––	McCusker et al. 2000[2]; Robison et al. 2001[3]; Sundin et al. 2005[3]; Nichols et al. 2007[3]; Miller et al. 2012[1]
	Swanson *O. mykiss*		m	tthR13 (same as OC8 above): 0.60; tthR9: 0.19; tthR6:0.21	––	
Reduced pigmentation (system 1)	European *H. sapiens*	0.05 mya	m	❖*Kitlg*: 0.23; *SLC45A2*: 0.0175; *SLC45A5*: 0.315; *TYR*: 0.14; *Oca2*: 0.14	––	Shriver et al. 2003[3]; Graf et al. 2005[3]; Lamason et al. 2005[3]; Soejima et al. 2006[3]; Miller et al. 2007[1]; Norton et al. 2007[3]
	East Asian *H. sapiens*		cg	––	not *SLC45A2;* not *SLC45A5*	
Reduced pigmentation (system 2)	Gulf-coast *Peromyscus polionotus*	0.07 mya	m	✚*Agouti*: 0.64; *Mc1r*: 0.33; *LG14*: 0.03	––	Hedges et al. 2006[2]; Hoekstra et al. 2006[3]; Degner et al. 2007[2]; Steiner et al. 2007[3]; Linnen et al. 2009[3]; Steiner et al. 2009[1]; Manceau et al. 2011[3]
	Atlantic coast Anastasia Island *P. polionotus*	0.07 mya	cg	––	not *Mc1r*	
	Atlantic coast Sotheastern *P. polionotus*	7 mya	cg	––	not *Mc1r*	
	Sand Hill *Peromyscus maniculatus*	90 mya	mcg	*Agouti*: 1	––	
Reduced pigmentation (system 3)	White sands *Sceloporus undulatus*	40 mya	cg	––	*Mc1r*	Schulte et al. 2000[2]; 2003[2]; Vidal and Hedges 2009[3]; Rosenblum et al. 2010[1]
	White sands *Holbrookia maculata*	200 mya	cg	––	*Mc1r*	
	White sands *Aspidoscelis inornata*	300 mya	cg	––	*Mc1r*	
Reduced pigmentation (system 4)	Paxton benthic *G. aculeatus*	0.01 mya	m	*Kitlg*: 1	––	Miller et al. 2007[1]
	Fishtrap Creek *G. aculeatus*	400 mya	cg	––	*Kitlg*	
	Gasterosteus williamsoni		cg	––	*Kitlg*	
Reduced pigmentation (system 5)■	Pachón cavefish *Astyanax mexicanus*	0.5 mya	m	*Oca2*: 0.57; *Mc1r*: 0.43	––	Sadoglu and McKee 1969[3]; Jeffrey 2001[2]; Wilkens and Strecker 2003[3]; Protas et al. 2006[1]; Gross et al. 2009[1]
	Molino *A. mexicanus*	300 mya	m	*Oca2*: 1	––	
	Yerbaniz/Japonés *A. mexicanus*		ct	*Oca2*: 1	––	
	Curva *A.mexicanus*		ct	*Mc1r*: 1	––	
	Chica *A. mexicanus*		ct	*Mc1r*: 1	––	
	Piedras *A.mexicanus*		ct	*Mc1r*: 1	––	

Notes:

★Clines are bidirectional.

◆Yinpterochiroptera includes all members except the family Pteropodidae, as they do not exhibit high-frequency hearing. Yangochiroptera and Yinpterochiroptera are conservatively grouped together here, as it is not yet known whether high frequency hearing evolved independently in each lineage or only once and then was lost in Pteropodidae.

❖Light pigmentation alleles at *Kitlg* are shared between European and East Asian humans. For *Kitlg* and *SLC45A2* effect size in melanin units was converted to an estimated PVE using a linear conversion factor based on the these values for *SLC45A5*. After doing so, the remaining unexplained variance was split evenly for *Tyr* and *Oca2*.

✚Average relative contributions for all body regions phenotyped.

■Both albino and brown phenotypes were considered "reduced pigmentation". The Pachón population is polymorphic for these phenotypes, but individuals are either one or the other. Therefore, we set the relative contributions of the underlying genes to the frequency with which each gene would underlie "reduced pigmentation" in a hypothetical cross between heterozygotes at both loci. The Japonés population is fixed for the albino phenotype, but also harbors a "brown mutation". Since this mutation is not the current cause of reduced pigmentation, it was assigned a relative contribution of 0.

[1]Reference found by objective literature search; [2]Additional reference used to estimate node age; [3]Additional reference for genetics underlying trait

Table B.2 Node numbers

Nodes corresponding to data in Table B.3. Node ages are given in Table B.1.

Phenotype	Independent Origins In	Node Number
Larval trichome loss	*Drosophila montana*	1
	Drosophila borealis	2
	Drosophila sechellia	
Skin toxin - caerulein	*Xenopus laevis*	3
	Litoria splendida	
Tetrodotoxin resistance	*Thamnophis couchii*	4
	Thamnophis atratus	5
	Thamnophis sirtalis	6
	Amphiesma pryeri	7
	Rhabdophis tigrinus	8
	Liophis epinephelus	
Lactase persistance	European *Homo sapiens*	9
	Saudi Arabian *H. sapiens*	10
	Kenyan and Tanzanian *H. sapiens*	
Reduction in lateral plate number	Paxton benthic *Gasterosteus aculeatus*	11
	Cranby *G. aculeatus*	
	Paq; Graham; Bear Paw *G. aculeatus*	
	Boot Lake; Whale Lake *G. aculeatus*	
	Nakagawa Creek *G. aculeatus*	13
	6 Pacific freshwater populations of *G. aculeatus*	
	5 Atlantic populations of *G. aculeatus*	14
	5 Scandinavian populations of *G. aculeatus*	12
	Fox Hole *Pungitius pungitius*	

Phenotype	Independent Origins In	Node Number
Pelvic spine and girdle reduction	Paxton benthic *G. aculeatus*	15, 16, 17, 18
	Boulton *G. aculeatus*	
	7 Alaska populations of *G. aculeatus*	
	Dolomite; Orphia *G. aculeatus*	
	Loch Fada *G. aculeatus*	
	Loch Vifilsstadavat *G. aculeatus*	
	Loch Scadavay *G. aculeatus*	
	Fox Hole *Pungitius pungitius*	
Inability to use galactose	West African strains *Saccharomyces cerevisiae*	19, 20, 21, 22
	27361N strain *S. cerevisiae*	
	Saccharomyces kudriavzevii	
	Candida glabrata	
	Eremothecium gossypi and Kluyveromyces waltii	
Red floral pigmentation (system 1)	*Mimulus l. variegatus*	23, 24, 27
	Mimulus naiandinus	
	Mimulus cupreus	
Red floral pigmentation (system 2)	*Ipomoea Mina* clade	25, 26, 27
	Ipomoea horsfalliae	
	Iochroma gesnerioides	
Electrical activity of myogenic electric organ	*Mormyroids*	28
	Gymnotiforms	
Digestion of foregut-fermenting bacteria	*Pygathrix nemaeus*	29, 30
	Colobus guereza	
	ruminant artiodactyls	

Phenotype	Independent Origins In	Node Number
Red wing patterns	*Heliconius melpomene*	31
	Heliconius erato	
Life history (latitudinal clines)	Australian *D. melanogaster*	32
	North American *D. melanogaster*	
Ultrahigh-frequency hearing for echolocation	Yangochiroptera and Yinpterochiroptera	33
	Tursiops truncatus	
Rapid development rate	Clearwater *Oncorhynchus mykiss*	34
	Swanson *O. mykiss*	
Reduced pigmentation (system 1)	European *H. sapiens*	35
	East Asian *H. sapiens*	
Reduced pigmentation (system 2)	Gulf-coast *Peromyscus polionotus*	36
	Atlantic coast Anastasia Island *P. polionotus*	37
	Atlantic coast Sotheastern *P. polionotus*	38
	Sand Hill *Peromyscus maniculatus*	39
Reduced pigmentation (system 3)	White sands *Sceloporus undulatus*	40
	White sands *Aspidoscelis inornata*	41
	White sands *Holbrookia maculata*	42
Reduced pigmentation (system 4)	Paxton benthic *G. aculeatus*	43
	Fishtrap Creek *G. aculeatus*	
	Gasterosteus williamsoni	
Reduced pigmentation (system 5)	Pachón cavefish *Astyanax mexicanus*	44
	Molino *A. mexicanus*	
	Yerbaniz/Japonés *A. mexicanus*	45
	Curva *A.mexicanus*	
	Chica *A. mexicanus*	46
	Piedras *A.mexicanus*	

Table B.3 Proportional similarity at each node

Proportional similarity at each node in the data set. Methods: 'cross' = genetic cross, 'cg' = candidate gene. Node numbers are identified in Table B.2. Some nodes are represented twice (once under method 'cross' and again with method 'cg'); in such cases there is no overlap between the populations used in the two analyses.

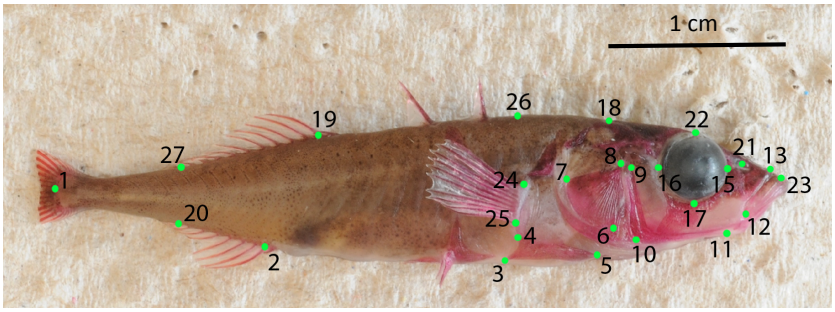
Phenotype	Node Number	Average Proportional Similarity	Rounded Average Estimate of Node Age (years)	Species-level?	Method
Reduction in lateral plate number	11	0.95	10000	y	cross
Reduction in lateral plate number	14	0.00	10000000	n	cross
Pelvic spine and girdle reduction	17	0.37	800000	y	cross
Pelvic spine and girdle reduction	18	0.27	10000000	n	cross
Inability to use galactose	19	0.00	2000000	y	cross
Red floral pigmentation	23	0.00	2000000	n	cross
Red floral pigmentation	24	0.00	3000000	n	cross
Red wing patterns	31	1.00	20000000	n	cross
Rapid development rate	34	0.60	1000000	y	cross
Reduced pigmentation	38	0.64	7000000	n	cross
Reduced pigmentation	39	0.00	90000000	n	cross
Reduced pigmentation	44	0.43	500000	y	cross
Reduced pigmentation	45	0.00	300000000	n	cross
Reduced pigmentation	46	0.27	400000000	n	cross
Larval trichome loss	1	1.00	2000000	n	cg
Larval trichome loss	2	1.00	60000000	n	cg
Skin toxin - caerulein	3	0.00	200000000	n	cg
Tetrodotoxin resistance	4	1.00	500000	n	cg
Tetrodotoxin resistance	5	1.00	2000000	n	cg
Tetrodotoxin resistance	6	1.00	30000000	n	cg
Tetrodotoxin resistance	7	1.00	60000000	n	cg
Tetrodotoxin resistance	8	1.00	60000000	n	cg
Lactase persistance	9	1.00	50000	y	cg
Lactase persistance	10	1.00	100000	y	cg
Reduction in lateral plate number	13	1.00	800000	y	cg
Reduction in lateral plate number	14	0.00	10000000	n	cg
Pelvic spine and girdle reduction	17	0.34	800000	y	cg
Pelvic spine and girdle reduction	18	0.00	10000000	n	cg
Inability to use galactose	20	0.14	50000000	n	cg
Inability to use galactose	21	0.57	300000000	n	cg
Inability to use galactose	22	0.78	400000000	n	cg

Phenotype	Node Number	Average Proportional Similarity	Rounded Average Estimate of Node Age (years)	Species-level?	Method
Red floral pigmentation	25	1.00	4000000	n	cg
Red floral pigmentation	26	0.00	70000000	n	cg
Red floral pigmentation	27	0.00	80000000	n	cg
Electrical activity of myogenic electric organ	28	1.00	300000000	n	cg
Digestion of foregut-fermenting bacteria	29	0.00	10000000	n	cg
Digestion of foregut-fermenting bacteria	30	0.00	90000000	n	cg
Life history (latitudinal clines)	32	1.00	300	y	cg
Ultrahigh-frequency hearing for echolocation	33	1.00	80000000	n	cg
Reduced pigmentation	35	0.00	50000	y	cg
Reduced pigmentation	37	0.00	70000	y	cg
Reduced pigmentation	38	0.00	7000000	n	cg
Reduced pigmentation	39	0.00	90000000	n	cg
Reduced pigmentation	40	1.00	40000000	n	cg
Reduced pigmentation	41	1.00	200000000	n	cg
Reduced pigmentation	42	0.06	300000000	n	cg
Reduced pigmentation	43	1.00	10000	y	cg
Reduced pigmentation	45	0.00	300000000	n	cg
Reduced pigmentation	46	0.31	400000000	n	cg

Appendix C: Chapter 4 Supplementary Material

Figure C.1 All phenotypes scored

Landmark numbers were made consistent with those in Arnegard et al. (in press).



Landmarks (x and y coordinates)

1. posterior midpoint of the caudal peduncle
2. anterior insertion of the anal fin at the first soft ray
3. posteroventral corner of the ectocoracoid bone
4. posterodorsal corner of the ectocoracoid bone
5. anterior-most corner of the ectocoracoid bone
6. anteroventral corner of the opercle
7. posterodorsal corner of the opercle
8. dorsal edge of the opercle-hyomandibular boundary
9. dorsal-most extent of the preopercle
10. posteroventral corner of the preopercle
11. anterior-most extent of the preopercle along the ventral silhouette
12. posteroventral extent of the maxilla
13. anterodorsal extent of the maxilla
14. *No landmark*
15. anterior margin of the orbit in line with the eye's midpoint
16. posterior margin of the orbit in line with the eye's midpoint
17. ventral margin of the orbit in line with the eye's midpoint
18. posterior extent of neurocranium (i.e., supraoccipital) along dorsal silhouette
19. anterior insertion of the dorsal fin at the first soft ray
20. posterior insertion of the anal fin at the first soft ray
21. edge of the lachrymal at the naris
22. dorsal margin of the orbit in line with the eye's midpoint
23. anterior-most extent of the premaxilla
24. dorsal insertion of the pectoral fin
25. ventral insertion of the pectoral fin
26. dorsum of the trunk over the pectoral fin midpoint
27. posterior insertion of the dorsal fin at the first soft ray

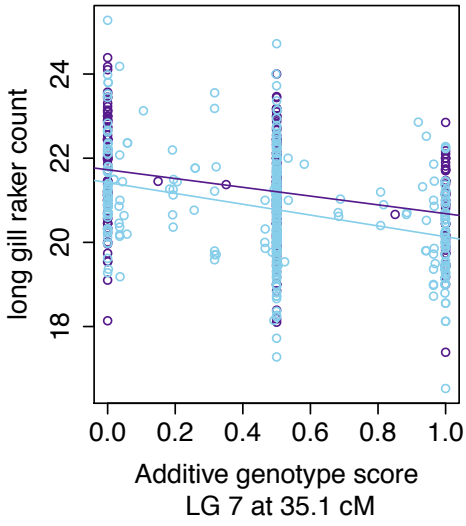
- centroid size (square root of the sum of squared distances of the 26 landmarks from their centroid)

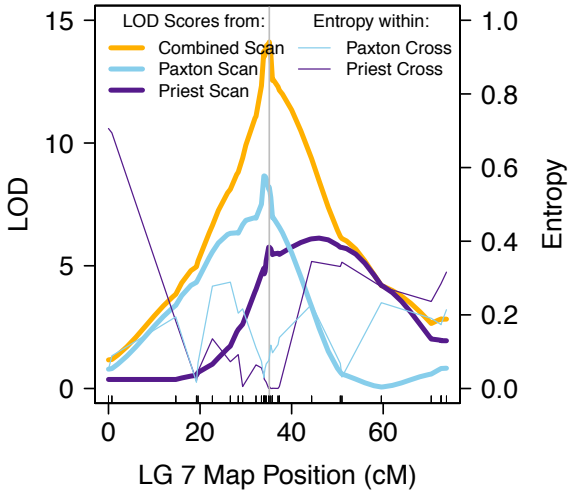
Meristics

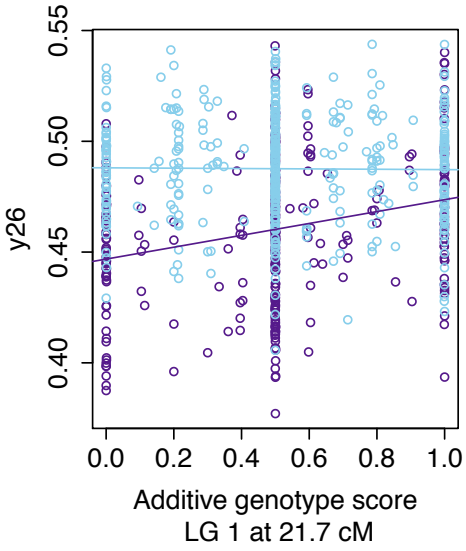
- lateral plate count
- 1st dorsal spine presence/absence
- 2nd dorsal spine presence/absence
- long gill raker count (on the first gill arch)
- short gill raker count (on the first gill arch)

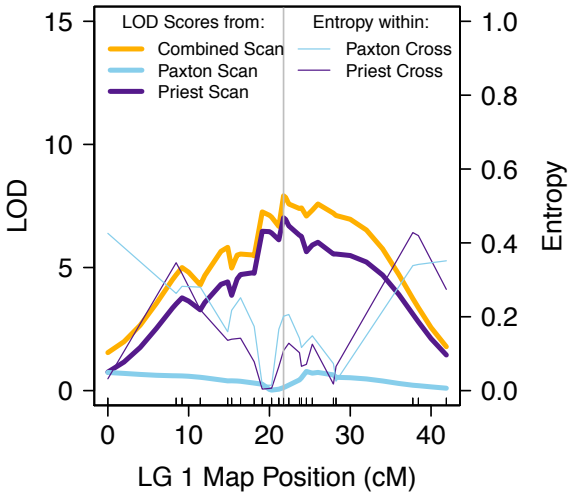
Figure C.2 Examples of mapping candidate QTL

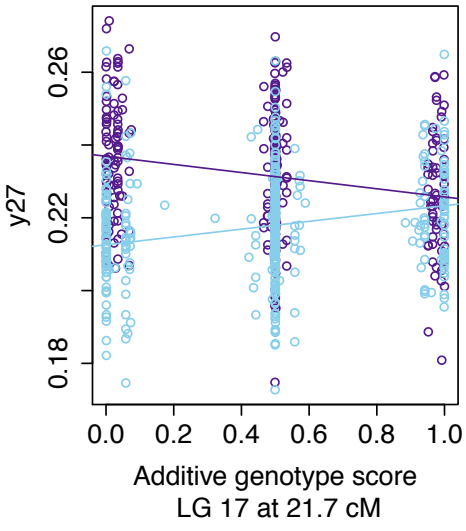
(Starts on next page) The three depicted candidate QTL were determined to have the following effects: top row - parallel effects; middle row - effect in only a single lake; bottom row - opposite effects. The left column (also depicted in Figure 4.2) shows examples of phenotype by genotype relationships at candidate QTL in F_2 hybrids from the Paxton Lake cross (light blue) and the Priest Lake cross (purple). Phenotypes are shown on the *y*-axes. The *x*-axes show the additive genotype score at the candidate QTL with 0 indicating the limnetic genotype, 1 the benthic genotype and 0.5 the heterozygote (values in between indicate uncertain genotypes, with score reflecting genotype probability). Lines represent the fitted values of linear models fitted to the phenotype and genotype data for each lake separately (light blue: Paxton Lake cross, purple: Priest Lake cross), using family identity and sex as covariates. Phenotypic measurements shown here are corrected for family identity. For the same three QTL (one QTL per row), the plots in the right column show the LOD profiles (left *y*-axis and bold lines) from the three distinct QTL scans across the entire linkage group on which the QTL was detected (*x*-axis). They also show the Entropy scores (an index of missing genotype information) for each lake's cross across the entire linkage group (right *y*-axis and un-bolded lines). The tick marks along the *x*-axis represent the positions of SNP markers on the linkage group. The vertical grey line represents the position of the peak marker in the combined scan (gold).


long gill raker count
18
20
22
24
0.0
0.2
0.4
0.6
0.8
1.0
Additive genotype score
LG 7 at 35.1 cM


LOD Scores from:
Combined Scan
Paxton Scan
Priest Scan
Entropy within:
Paxton Cross
Priest Cross
LOD
Entropy
0
5
10
15
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
LG 7 Map Position (cM)


y26
0.40
0.45
0.50
0.55
0.0
0.2
0.4
0.6
0.8
1.0
Additive genotype score
LG 1 at 21.7 cM


LOD Scores from:
Combined Scan
Paxton Scan
Priest Scan
Entropy within:
Paxton Cross
Priest Cross
LOD
Entropy
0
5
10
15
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
LG 1 Map Position (cM)


y27
0.18
0.22
0.26
0.0
0.2
0.4
0.6
0.8
1.0
Additive genotype score
LG 17 at 21.7 cM

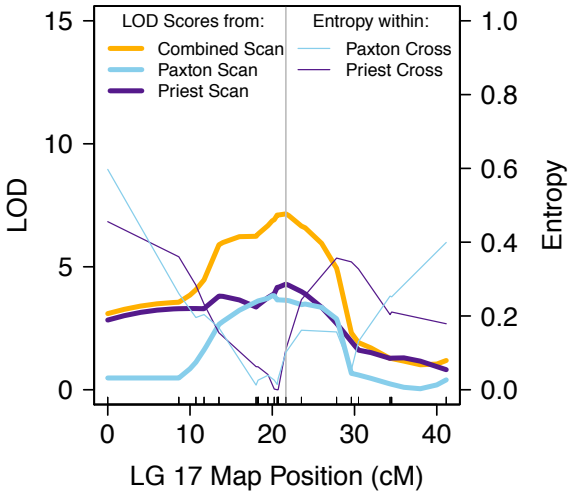

LOD Scores from:
Combined Scan
Paxton Scan
Priest Scan
Entropy within:
Paxton Cross
Priest Cross
LOD
Entropy
0
5
10
15
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
LG 17 Map Position (cM)

Figure C.3 Map of candidate QTL

(Starts on next page) Map of 58 'candidate QTL' (i.e. QTL with an effect in one or both lakes). Linkage groups on which QTL were detected are shown. For each, the positions of SNP markers are depicted by tick marks on the left. Colored bars span the 1.5 LOD confidence intervals of candidate QTL. Black dots within bars represent the peak marker position. The phenotype affected by each candidate QTL is printed to the left its bar. Colors of bars represent the 'QTL Effect' category, as follows: parallel effects – blue; effect in only one lake – grey; opposite effects – red. Tan colored bars represent the candidate QTL for which more than one QTL effect category fit the data nearly equally well.

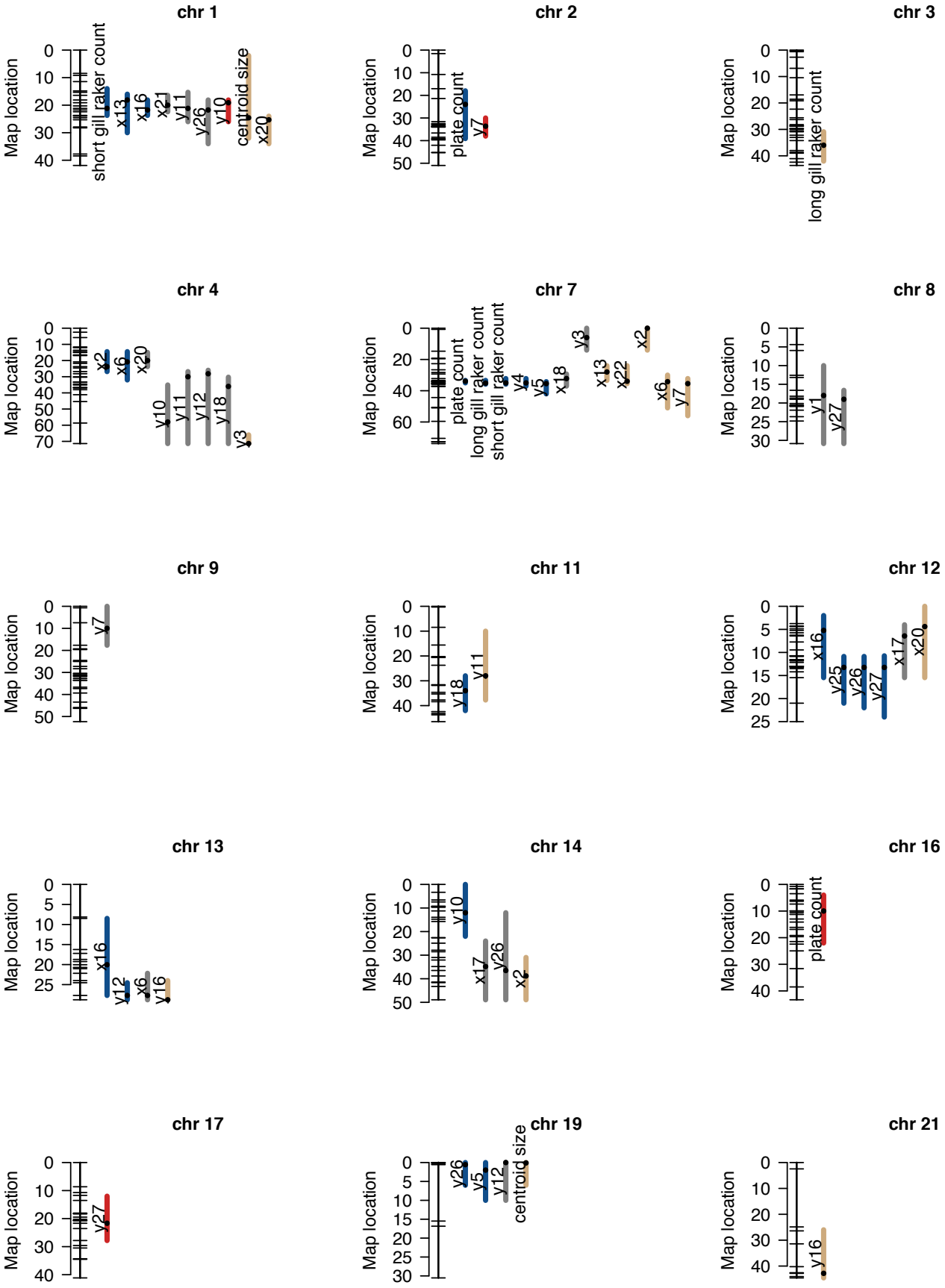

chr 1
Map location
short gill raker count
x13
x16
x21
y11
y26
y10
centroid size
x20
chr 2
plate count
y7
chr 3
long gill raker count
chr 4
x2
x6
x20
y10
y11
y12
y18
y3
chr 7
long gill raker count
short gill raker count
y3
x2
y4
y5
x18
x13
x22
x6
y7
chr 8
y1
y27
chr 9
y7
chr 11
y18
y11
chr 12
x16
y25
y26
y27
x17
x20
chr 13
x16
y12
x6
y16
chr 14
y10
x17
y26
x2
chr 16
plate count
chr 17
y27
chr 19
y26
y5
y12
centroid size
chr 21
y16

Figure C.4 Proportions of QTL effect categories per chromosome

The average proportion of candidate QTL per chromosome (n=13) with parallel effects (blue), an effect in only a single lake (grey) and opposite effects (red) is shown with standard error bars. The 43 candidate QTL considered are shown in Figure C.3 in blue, grey and red.

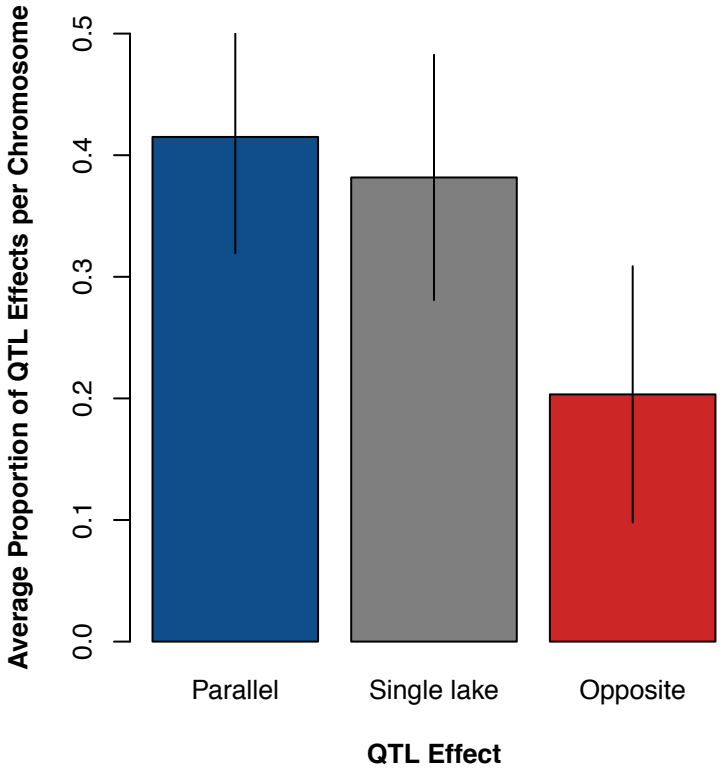


Table C.1 Trait divergence categories

Trait divergence was considered 'parallel' when either the best model of the species effect was 'same effect', or when the best model of species effect was 'different effect' but the direction of divergence was 'same'. Trait divergence was considered only in a 'single lake' when the best model of the species effect was either 'effect only in Paxton' or 'effect only in Priest'. Trait divergence was considered 'opposite' when the best model of species effect was 'different effect' and the direction of divergence was 'opposite'. The second best model of species effect and the delta AICc between it and the best model is also shown. When the delta AICc was less than two and the 2nd best model called for a different trait divergence category than the best model did, we dropped the trait from further study, though all detected QTL are shown in Tables C.3 – C.5.

Trait	'Trait divergence' based on AICc model selection	Direction of divergence	Best model of species effect	2nd best model of species effect	Delta AICc
1st dorsal spine	Single lake	opposite	effect in Paxton only	different effect	2.14
2nd dorsal spine	Neither lake	same	no effect	effect in Priest only	0.95
gill raker count	Parallel	same	same effect	different effect	1.24
plate count	Parallel	same	different effect	same effect	35.62
x1	Neither lake	opposite	no effect	effect in Priest only	0.17
y1	Parallel	same	same effect	different effect	1.72
x2	Parallel	same	different effect	same effect	5.78
y2	Parallel	same	same effect	different effect	1.28
x3	Opposite	opposite	different effect	effect in Paxton only	3.97
y3	Parallel	same	different effect	same effect	1.14
x4	Single lake	opposite	effect in Priest only	different effect	2.12
y4	Parallel	same	different effect	same effect	31.59
x5	Single lake	opposite	effect in Priest only	different effect	0.02
y5	Parallel	same	different effect	same effect	2.77
x6	Parallel	same	same effect	different effect	2.20
y6	Opposite	opposite	different effect	effect in Priest only	5.36
x7	Opposite	opposite	different effect	effect in Priest only	1.47
y7	Parallel	same	same effect	different effect	1.90
x8	Single lake	opposite	effect in Paxton only	different effect	0.32
y8	Parallel	same	different effect	same effect	0.53
x9	Single lake	same	effect in Priest only	different effect	1.36
y9	Single lake	same	effect in Paxton only	different effect	0.26
x10	Single lake	same	effect in Priest only	different effect	2.18
y10	Parallel	same	same effect	different effect	1.01
x11	Single lake	same	effect in Paxton only	different effect	1.69
y11	Parallel	same	same effect	different effect	1.83
x12	Opposite	opposite	different effect	effect in Priest only	19.50
y12	Parallel	same	same effect	different effect	1.03
x13	Parallel	same	different effect	same effect	2.97
y13	Parallel	same	same effect	different effect	1.72
x15	Parallel	same	same effect	effect in Priest only	1.70
y15	Parallel	same	same effect	different effect	1.01
x16	Parallel	same	different effect	same effect	0.78
y16	Parallel	same	same effect	different effect	0.67
x17	Parallel	same	different effect	same effect	3.19
y17	Parallel	same	different effect	same effect	0.37
x18	Parallel	same	different effect	same effect	0.84
y18	Parallel	same	different effect	same effect	0.43
x19	Opposite	opposite	different effect	effect in Priest only	1.00
y19	Single lake	same	effect in Paxton only	different effect	2.13
x20	Parallel	same	different effect	effect in Priest only	3.10

Trait	'Trait divergence' based on AICc model selection	Direction of divergence	Best model of species effect	2nd best model of species effect	Delta AICc
y20	Parallel	same	different effect	same effect	17.17
x21	Parallel	same	same effect	different effect	2.09
y21	Neither lake	opposite	no effect	effect in Priest only	0.37
x22	Parallel	same	same effect	different effect	2.17
y22	Single lake	same	effect in Paxton only	different effect	0.27
x23	Opposite	opposite	different effect	effect in Paxton only	4.95
y23	Parallel	same	different effect	effect in Priest only	0.29
x24	Single lake	same	effect in Priest only	different effect	1.32
y24	Opposite	opposite	different effect	effect in Paxton only	17.01
x25	Single lake	same	effect in Priest only	different effect	2.19
y25	Parallel	same	different effect	effect in Paxton only	4.15
x26	Parallel	same	different effect	effect in Priest only	1.63
y26	Parallel	same	different effect	same effect	3.30
x27	Parallel	same	same effect	different effect	2.12
y27	Parallel	same	same effect	different effect	2.15
centroid	Parallel	same	different effect	effect in Paxton only	25.59

Table C.2 Identities, map positions, and physical locations of SNPs

(Starts on next page) Identities, map positions, and physical locations of the 430 single nucleotide polymorphism (SNP) markers used in linkage and QTL analysis. The linkage group (LG) and map position in centimorgans (cM) are provided for each SNP. Each marker name is a combination of the chromosome number (before the colon) and the physical position in base pairs (after the colon) of the SNP in the initial stickleback genome assembly (Broad S1, Feb. 2006). Markers identified from unassembled regions of the genome are indicated with 'chrUN'. In such cases, the position in base pairs is based on the composite chrUN in the UCSC genome browser. Marker information can be obtained from the Single Nucleotide Polymorphism Database (dbSNP, available at http://www.ncbi.nlm.nih.gov/projects/SNP/), which is hosted by the National Center for Biotechnology Information (NCBI) of the U.S. National Institutes of Health. Data for specific markers may be found by searches of the dbSNP using the submitted SNP ID numbers (ss#). Two SNPs are still awaiting ss# assignment.

Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)	Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)
1	0	chrI:27642534	418642015	2	45.24	chrII:919438	244222781
1	8.45	chrUn:18660323	418642624	2	45.324	chrUn:23384875	418642627
1	9.18	chrI:22716347	418642010	2	50.981	chrII:533883	120258418
1	11.439	chrI:3310077	244222768	3	0	chrUn:30223426	418642641
1	14.833	chrI:19946499	418642005	3	0.219	chrUn:27149198	418642632
1	15.322	chrI:2718044	418641984	3	0.593	chrUn:27040022	418642631
1	16.421	chrI:4219350	244222770	3	2.685	chrUn:30323959	418642642
1	18.111	chrI:3494580	120258412	3	6.884	chrIII:16463929	244222796
1	19.109	chrI:14261764	418641998	3	10.455	chrIII:16251071	120258431
1	20.267	chrI:15145305	418642000	3	16.956	chrIII:15793968	418642089
1	21.162	chrI:4171190	244222769	3	18.59	chrIII:15185662	418642088
1	21.745	chrI:17306554	418642003	3	19.173	chrIII:15157782	418642087
1	22.395	chrI:7545826	418641993	3	22.347	chrIII:14892994	244222794
1	23.715	chrI:20584613	418642006	3	25.596	chrIII:13397314	418642078
1	23.959	chrI:22899825	418642011	3	26.289	chrIII:13520975	252841102
1	24.572	chrI:22361077	120258417	3	28.19	chrIII:14393183	418642084
1	25.305	chrI:3538018	418641987	3	28.59	chrIII:14048561	252841058
1	27.917	chrI:26879230	244222777	3	28.741	chrIII:13911180	418642080
1	28.238	chrI:25560380	418642013	3	28.761	chrIII:11836494	418642072
1	37.745	chrI:1550	418641979	3	29.684	chrIII:13699701	418642079
1	38.432	chrUn:37631434	244223001	3	29.727	chrIII:12930427	418642076
1	41.893	chrI:913033	120258411	3	29.906	chrIII:14135608	418642081
2	0	chrII:22443700	244222787	3	30.523	chrIII:14456990	252841063
2	1.55	chrII:22644752	418642054	3	30.9	chrIII:14248039	418642083
2	10.77	chrII:21231538	244222786	3	32.208	chrIII:11302839	418642071
2	17.049	chrII:21013052	418642052	3	33.049	chrIII:2376699	418642065
2	21.421	chrII:19985741	244222785	3	34.228	chrIII:1968625	418642063
2	31.618	chrII:5914538	418642030	3	37.992	chrIII:1198125	120258428
2	32.478	chrII:10092618	418642034	3	38.662	chrIII:639237	418642059
2	32.693	chrII:8305286	418642033	3	38.988	chrIII:1651721	252841079
2	33.053	chrII:6475468	244222782	3	41.069	chrIII:269753	418642057
2	33.629	chrII:17453243	418642042	3	42.403	chrIII:105665	418642055
2	33.653	chrII:5935944	252841148	3	43.645	chrIII:186390	418642056
2	33.707	chrII:12292176	120258425	4	0	chrUn:27478064	244222993
2	33.978	chrII:14611516	244222784	4	2.47	chrUn:27589750	418642633
2	34.026	chrII:17312835	418642041	4	5.799	chrUn:27402745	252841068
2	36.632	chrII:4530808	120258423	4	11.6	chrIV:32592491	418642150
2	38.498	chrII:19324477	418642044	4	12.021	chrIV:32487875	244222812
2	39.102	chrII:3931852	418642025	4	13.638	chrIV:32387818	120258447
2	39.262	chrII:4157699	252841112	4	14.49	chrIV:32277841	418642146
2	39.701	chrII:3516452	120258422	4	15.02	chrIV:32236655	418642145
2	42.057	chrII:3384330	120258421	4	16.56	chrIV:32092919	252841132

Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)	Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)
4	16.927	chrIV:32005807	120258445	5	46.025	chrV:7791830	252841093
4	20.838	chrIV:31740478	244222809	5	50.516	chrUn:10540032	418642614
4	21.77	chrIV:31350187	418642140	5	53.473	chrUn:10213240	418642613
4	23.776	chrIV:29763654	120258443	5	53.596	chrUn:11980918	252841136
4	24.599	chrIV:31611147	252841084	5	56.444	chrUn:12390868	120258569
4	26.831	chrIV:30568387	252841083	6	0	chrVI:487411	418642183
4	28.149	chrIV:5165268	418642111	6	3.991	chrVI:6312798	418642187
4	30.064	chrIV:21232476	418642127	6	5.262	chrVI:1440771	244222823
4	30.311	chrIV:21605258	252841082	6	5.672	chrVI:10415741	418641920
4	33.352	chrIV:15721538	244222806	6	6.903	chrVI:11954719	418642192
4	33.352	chrIV:15737291	244222807	6	7.644	chrVI:13220597	252841044
4	34.536	chrIV:15530121	244222805	6	7.721	chrVI:11873663	120258454
4	35.12	chrIV:15052901	244222804	6	7.97	chrVI:12427477	418642193
4	36.67	chrIV:10997988	244222801	6	12.157	chrVI:3116218	244222825
4	36.782	chrIV:9220132	418642120	6	12.259	chrVI:16870159	244222834
4	36.984	chrIV:8545605	418642119	6	13.529	chrVI:218630	244222820
4	38.029	chrIV:11367975	120258435	6	15.74	chrVI:14571427	418642200
4	41.136	chrIV:4065598	244222799	6	20.547	chrVI:15413799	418642203
4	45.377	chrIV:3334208	418642103	6	23.176	chrVI:14976508	418642201
4	58.662	chrIV:2045971	418642099	6	24.097	chrVI:15654034	418642204
4	71.359	chrIV:219384	418642093	6	24.282	chrVI:15692312	418642205
5	0	chrUn:25831365	418642629	7	0	chrVII:27918897	418642257
5	2.542	chrUn:25946639	244222990	7	0.743	chrUn:29400087	418642638
5	8.438	chrV:11316476	252841077	7	14.707	chrVII:26769148	418642251
5	9.302	chrV:11368893	418642177	7	19.214	chrVII:26538823	244222842
5	13.732	chrV:11509827	418642178	7	19.55	chrVII:26448674	252841125
5	17.208	chrV:11642284	418642179	7	22.7	chrVII:26227403	120258461
5	19.847	chrV:11722274	418642180	7	26.614	chrVII:25662266	120258460
5	20.126	chrV:10649179	252841089	7	28.341	chrVII:25193081	418642246
5	23.802	chrV:10674055	418642173	7	29.302	chrVII:24988330	
5	30.785	chrV:10028353	418642167	7	32.219	chrVII:24217606	418642245
5	31.771	chrV:9884672	418642164	7	33.407	chrVII:19857837	418642237
5	31.771	chrV:9911653	418642165	7	33.931	chrVII:16848769	418642232
5	32.969	chrV:9768052	252841108	7	34.008	chrVII:24203557	120258459
5	34.423	chrV:9157076	244222818	7	34.209	chrVII:23703797	418642243
5	40.776	chrV:8327818	244222816	7	34.447	chrVII:22798737	418642240
5	42.038	chrV:1238066	120258448	7	34.985	chrVII:21302029	418642238
5	43.017	chrV:1727383	418642153	7	35.124	chrVII:20883742	252841067
5	43.695	chrV:2528528	244222814	7	35.45	chrVII:18353106	244222839
5	44.689	chrUn:11085407	418642615	7	35.809	chrVII:13452516	244222836
5	45.499	chrV:5064057	418642160	7	35.815	chrVII:5552972	252841066
5	45.501	chrV:4819972	418642158	7	37.029	chrVII:5936068	120258457

Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)	Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)
7	37.322	chrVII:4310181	418642225	9	32.728	chrIX:13852312	418642311
7	44.369	chrVII:2559099	418642220	9	33.542	chrIX:803523	252841065
7	50.636	chrVII:1569236	418642218	9	33.594	chrIX:16779825	244222869
7	50.989	chrVII:1481322	418642217	9	36.679	chrIX:2360337	244222859
7	59.593	chrVII:835236	252841091	9	37.317	chrIX:2310926	418642299
7	70.492	chrUn:29087782	244222996	9	39.339	chrIX:2089567	244222858
7	72.645	chrVII:537136	252841113	9	43.455	chrIX:1273244	244222857
7	72.679	chrVII:393417	418642213	9	45.903	chrIX:1417909	418642292
7	73.819	chrUn:28671327	244222995	9	46.308	chrIX:1571056	418642294
8	0	chrVIII:19282658	418642286	9	52.345	chrIX:639609	244222856
8	4.379	chrVIII:868226	418642258	10	0	chrX:1275840	418642326
8	5.961	chrVIII:18760705	244222855	10	4.412	chrX:14831394	418642358
8	12.586	chrVIII:2505620	418642263	10	4.526	chrX:14456479	252841100
8	13.173	chrVIII:1929053	244222843	10	4.527	chrX:14549101	252841122
8	16.606	chrVIII:2257915	418642261	10	6.198	chrX:14265366	120258486
8	18.186	chrVIII:3765115	418642265	10	7.06	chrUn:14127611	418642619
8	18.689	chrVIII:3627706	244222844	10	8.977	chrUn:14043112	418642618
8	19.011	chrVIII:3987295	120258464	10	9.768	chrUn:24511995	418642628
8	20.132	chrVIII:6680213	418642268	10	10.14	chrUn:29017220	418642637
8	20.538	chrVIII:8858242	418642273	10	10.338	chrX:13132917	418642352
8	20.747	chrVIII:14278829	418642277	10	16.368	chrX:10080391	418642338
8	20.771	chrVIII:12472630	252841158	10	16.858	chrX:11139448	252841128
8	20.929	chrVIII:13412707	244222846	10	17.302	chrX:4696470	418642330
8	21.923	chrVIII:15261158	418642279	10	19.902	chrX:8703061	120258485
8	23.667	chrVIII:13577518	252841097	10	20.486	chrX:7113953	120258483
8	24.825	chrVIII:14472465	244222848	10	22.444	chrX:11252137	244222875
8	30.855	chrVIII:16843576	418642285	10	24.019	chrX:12844036	418642350
9	0	chrIX:19781202	244222870	10	28.446	chrX:12507632	244222877
9	0.675	chrIX:20090929	244222871	11	0	chrXI:16701186	244222888
9	7.45	chrIX:19745222	418642321	11	0.287	chrXI:16655205	120258495
9	17.662	chrIX:18494397	418642317	11	8.458	chrXI:15154801	418642382
9	19.295	chrIX:18826248	418642319	11	15.6	chrUn:32523521	418642646
9	19.628	chrIX:19322448	418642320	11	20.238	chrXI:14631875	418642379
9	24.547	chrIX:5109672	244222860	11	20.482	chrXI:14691162	418642380
9	25.033	chrIX:4882924	120258472	11	20.626	chrXI:14830913	244222885
9	27.249	chrIX:5403530	120258474	11	23.738	chrXI:15005173	244222886
9	28.314	chrIX:5568375	244222863	11	31.588	chrXI:12097498	418642375
9	30.384	chrIX:12933483	244222865	11	31.877	chrXI:10976029	244222883
9	30.606	chrIX:7146708	418642304	11	34.655	chrXI:9039275	252841094
9	31.139	chrIX:15670033	244222868	11	35.303	chrXI:7355052	418642370
9	31.408	chrIX:7893416	418642306	11	37.738	chrXI:12746496	244222884
9	31.862	chrIX:13553866	252841127	11	38.425	chrXI:3120961	244222880

Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)	Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)
11	42.418	chrXI:1017481	120258488	13	21.03	chrXIII:2632698	244222901
11	43.126	chrXI:1449684	120258489	13	22.175	chrXIII:2523163	120258505
11	43.715	chrXI:1266618	418642362	13	23.993	chrXIII:1909687	244222900
11	46.473	chrXI:234849	120258487	13	24.529	chrXIII:1698554	418642421
12	0	chrXII:17758877	244222897	13	27.703	chrXIII:1001571	120258503
12	3.744	chrXII:16628544	418642412	13	28.789	chrXIII:2105469	418642423
12	4.281	chrXII:2242677	418642394	14	0	chrXIV:14049917	252841090
12	4.39	chrUn:26305459	244222991	14	3.337	chrUn:21213332	120258571
12	4.88	chrXII:16877465	418642413	14	6.577	chrXIV:11054767	120258517
12	5.223	chrXII:3026329	418642398	14	7.427	chrXIV:9742642	418642458
12	5.738	chrXII:18221941	244222899	14	9.222	chrXIV:6992838	418642456
12	6.258	chrXII:4123972	418642400	14	9.409	chrXIV:15137805	418642462
12	6.422	chrUn:30606854	244222997	14	9.506	chrXIV:6641188	418642455
12	7.731	chrUn:38378170	120258576	14	9.686	chrXIV:7313827	418642457
12	9.452	chrXII:3810254	418642399	14	11.233	chrXIV:15033103	418642461
12	10.731	chrXII:6012527	418642404	14	13.935	chrXIV:3414352	120258514
12	10.731	chrXII:5828898	418642403	14	14.748	chrXIV:3598443	418642452
12	10.863	chrXII:5521301	418642402	14	15.715	chrXIV:3534175	120258515
12	11.573	chrXII:6399147	252841133	14	22.579	chrXIV:2084777	418642446
12	11.61	chrXII:6924609	418642405	14	22.648	chrXIV:1798136	418642443
12	11.639	chrXII:6745006	244222892	14	24.911	chrXIV:1713227	120258513
12	11.825	chrXII:6913126	120258500	14	27.904	chrXIV:1641269	418642442
12	12.164	chrXII:7504339	418642406	14	28.549	chrXIV:1442872	120258512
12	13.016	chrXII:16454328	418642411	14	30.976	chrXIV:1383447	244222908
12	13.016	chrXII:1589655	120258497	14	32.066	chrXIV:1311694	418642441
12	13.016	chrXII:2157795	418642393	14	34.828	chrXIV:1087388	418642439
12	13.016	chrXII:15046849	418642410	14	36.498	chrXIV:800076	418642438
12	13.24	chrXII:11472159	418642407	14	38.817	chrXIV:721170	244222907
12	13.498	chrXII:13045611	244222894	14	41.408	chrXIV:451065	120258511
12	14.199	chrXII:14223760	244222895	14	41.654	chrXIV:348659	418642435
12	15.462	chrXII:1483544	244222889	14	43.257	chrUn:35285565	418642649
12	20.999	chrXII:880748	418642389	14	48.931	chrUn:36334731	244223000
12	25.007	chrXII:548804	252841119	15	0	chrXV:13047331	418642481
13	0	chrXIII:18470329	252841124	15	0.602	chrXV:12281774	418642480
13	8.132	chrXIII:17392141	120258510	15	3.131	chrXV:6446874	418642477
13	8.48	chrXIII:17249562	418642432	15	6.668	chrXV:2507809	244222914
13	16.245	chrXIII:8085851	418642430	15	7.328	chrXV:3703641	418642475
13	17.207	chrXIII:4401535	418642425	15	9.649	chrXV:2169610	244222912
13	18.717	chrXIII:4868788	418642428	15	12.33	chrXV:1902350	244222911
13	19.246	chrXIII:4621027	418642426	15	13.521	chrXV:1800560	418642468
13	20.037	chrXIII:2969182	418642424	15	19.973	chrXV:414608	120258519
13	20.712	chrXIII:3109522	120258506	15	20.789	chrXV:505537	418642465

Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)	Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)
15	26.144	chrXV:979445	418642466	17	27.817	chrXVII:12022612	120258536
15	28.551	chrXV:215800	418642464	17	29.614	chrXVII:1264852	418642508
15	29.834	chrXV:11818	418642463	17	30.48	chrXVII:12528572	252841151
16	0	chrXVI:2764206	120258523	17	34.32	chrXVII:769372	244222939
16	0.74	chrXVI:2650854	244222922	17	34.515	chrXVII:645029	418642506
16	1.67	chrXVI:2392758	244222921	17	41.148	chrXVII:14127979	418642528
16	1.674	chrXVI:2483136	252841051	18	0	chrXVIII:15478444	120258549
16	3.48	chrXVI:3206769	244222923	18	19.259	chrXVIII:13773116	418642545
16	5.889	chrXVI:13588796	244222930	18	23.155	chrXVIII:13753579	244222958
16	6.329	chrXVI:14093156	244222931	18	24.456	chrXVIII:13193140	244222957
16	7.378	chrXVI:14963879	244222933	18	24.798	chrXVIII:12818939	120258545
16	9.956	chrXVI:12996432	244222929	18	26.138	chrXVIII:12273872	252841150
16	9.978	chrXVI:5562355	244222924	18	26.312	chrXVIII:11896010	244222954
16	10.609	chrXVI:6415385	418642487	18	27.539	chrXVIII:11765327	120258543
16	11.281	chrXVI:9428786	244222926	18	27.765	chrXVIII:11702469	418642543
16	12.994	chrXVI:13148331	418642492	18	27.765	chrXVIII:11641450	244222953
16	14.134	chrXVI:14283264	244222932	18	28.292	chrXVIII:11504306	418642542
16	16.126	chrXVI:15039503	418642494	18	29.831	chrXVIII:13352631	120258546
16	16.894	chrXVI:16058672	252841101	18	31.323	chrXVIII:5765162	120258540
16	19.173	chrXVI:17471373	418642502	18	31.327	chrXVIII:4836241	120258539
16	19.623	chrXVI:18106789	120258529	18	34.367	chrXVIII:3137228	
16	21.464	chrXVI:17895677	244222938	18	41.287	chrXVIII:1211531	418642530
16	22.417	chrXVI:17405918	418642501	19	0	chrXIX:8190806	120258554
16	24.961	chrXVI:17236926	244222936	19	0.054	chrXIX:14650559	418641975
16	31.626	chrXVI:16673569	120258528	19	0.099	chrXIX:18045399	120258558
16	38.499	chrUn:37016121	418642651	19	0.102	CH213.119K16:14070	418641977
16	43.334	chrUn:26389255	244222992	19	0.102	CH213.21C23:188808	418641953
17	0	chrXVII:1733515	418642509	19	0.434	chrXIX:3737235	418641965
17	8.642	chrXVII:12666712	418642526	19	0.554	chrXIX:18043409	252841059
17	10.714	chrXVII:2664810	244222940	19	15.488	chrXIX:1546489	418641958
17	11.707	chrXVII:2626658	418642511	19	16.847	chrXIX:1472847	120258551
17	13.517	chrXVII:2872553	418642512	19	30.53	chrXIX:897343	418641956
17	18.03	chrXVII:3906379	244222942	20	0	chrXX:12622695	244222966
17	18.272	chrXVII:10329401	418642524	20	0	chrXX:12810044	252841048
17	18.272	chrXVII:9697366	244222947	20	0.278	chrXX:14562943	418642569
17	19.473	chrXVII:3843835	120258534	20	0.588	chrXX:14462157	244222968
17	20.238	chrXVII:4909843	244222944	20	0.724	chrXX:14859034	418642571
17	20.598	chrUn:2474754	418642603	20	1.646	chrXX:5734841	418642558
17	20.713	chrUn:2776586	120258568	20	3.948	chrXX:15996390	418642573
17	20.713	chrUn:2632376	252841074	20	8.045	chrXX:16253512	252841060
17	21.65	chrXVII:2999556	418642513	20	16.409	chrXX:2080510	418642553
17	23.546	chrXVII:9881295	418642523	20	22.695	chrUn:30545876	120258573

Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)	Linkage Group	Map Position (cM)	Marker name (chromosome: position)	NCBI submitted SNP ID numbers (ss#)
21	0	chrUn:31339987	244222998				
21	2.453	chrUn:28158103	418642634				
21	24.879	chrXXI:11060209	120258566				
21	26.406	chrXXI:10969152	244222981				
21	31.416	chrUn:23042966	418642626				
21	40.24	chrXXI:9820534	418642589				
21	42.589	chrXXI:7002178	244222977				
21	42.816	chrXXI:5737465	244222973				
21	44.076	chrXXI:3082227	418642585				
21	44.583	chrUn:6720054	244222987				

Table C.3 Paxton Lake QTL scan results

The QTL scan results for all QTL detected in our Paxton Lake scan are shown. Together, the '1.5 LOD C.I. low (cM)' and '1.5 LOD C.I. high (cM)' columns indicate the range of the 1.5 LOD confidence interval of the genomic location of the QTL. The 'LOD' column indicates the LOD score at the QTL's peak marker (the maker at which genotypes showed the strongest association with phenotypes). The 'p-value' column indicates the genome-wide significance of the peak marker's LOD score for the associated trait.

Trait	Linkage group	Peak Marker Position (cM)	1.5 LOD C.I. low (cM)	1.5 LOD C.I. high (cM)	LOD	p-value	Candidate QTL?
plate count	7	33.93	33.41	34.99	16.65	<1.00E-04	no
long gill raker count	3	36	30.9	42	6.43	1.70E-03	no
long gill raker count	7	34.01	32.22	35.81	8.66	<1.00E-04	no
short gill raker count	1	21.16	16	23.72	6.05	3.50E-03	no
short gill raker count	7	34.99	32.22	35.81	5.54	7.60E-03	no
1st dorsal spine	2	33.63	22	39.26	8.11	<1.00E-04	no
2nd dorsal spine	20	1.65	0	22.7	4.39	3.68E-02	no
x1	1	20	16.42	21.75	5.05	1.86E-02	no
y1	8	18.19	10	30	5.12	1.58E-02	no
x2	7	0	0	14	6.38	1.70E-03	yes
x3	1	21.16	16.42	23.72	9.14	<1.00E-04	no
x3	5	52	26	56.44	5.65	6.60E-03	no
x3	12	13.5	12.16	24	10.23	<1.00E-04	no
y3	7	6	0	14	11.12	<1.00E-04	no
x4	3	10	4	16	5.68	5.20E-03	no
x4	7	34.21	32.22	35.81	7.61	4.00E-04	no
x4	12	20	7.73	25.01	5.09	1.50E-02	no
y4	7	34.99	32	37.03	5.27	1.08E-02	no
y5	19	2	0	10	5.11	1.55E-02	yes
x6	7	35.45	30	54	5.61	5.50E-03	no
y6	7	35.45	14.71	40	4.47	4.79E-02	no
y6	13	18.72	0	24.53	5.1	1.80E-02	no
y6	19	0.1	0	4	11.3	<1.00E-04	no
x10	7	40	34.45	50	5.54	7.80E-03	no
y10	4	58	35.12	71.36	6.47	1.10E-03	yes
y10	14	11.23	0	22	7.32	2.00E-04	no
x11	1	21.16	16.42	23.72	5.94	3.90E-03	no
y11	1	21.75	16.42	23.72	6.82	9.00E-04	no
y11	4	30	26.83	71.36	8.11	3.00E-04	no
x12	1	19.11	16	23.72	4.71	2.95E-02	no
y12	4	28.15	26	71.36	4.62	3.74E-02	yes
y12	19	0.1	0	8	5.13	1.59E-02	no
x13	1	19.11	16.42	27.92	7.15	3.00E-04	no
x13	7	35.12	24	40	4.58	4.24E-02	no
x16	1	19.11	16.42	27.92	4.76	2.85E-02	no
x16	13	20.04	8.48	27.7	4.44	4.93E-02	yes
x18	7	32.22	26.61	33.93	4.84	2.52E-02	no
y18	4	36	30.31	71.36	4.53	4.11E-02	yes
x19	10	8	6.2	24	4.96	2.00E-02	no
y19	8	26	19.01	30.86	6.83	1.00E-03	no
y19	12	12.16	10	25.01	4.84	2.73E-02	no
x20	1	25.31	24	34	4.36	5.30E-02	yes

Trait	Linkage group	Peak Marker Position (cM)	1.5 LOD C.I. low (cM)	1.5 LOD C.I. high (cM)	LOD	p-value	Candidate QTL?
x21	1	20	16.42	22.4	4.9	2.32E-02	yes
x22	7	33.93	24	35.45	4.62	4.06E-02	yes
x23	1	19.11	16.42	27.92	8.51	<1.00E-04	no
x24	1	19.11	16	26	5.62	6.40E-03	no
x25	1	20	16	26	4.53	4.33E-02	no
x25	16	12.99	0	24	4.48	4.74E-02	no
y25	12	18	12.16	25.01	7.67	<1.00E-04	no
x26	1	19.11	16	26	5.13	1.47E-02	no
y26	19	0.1	0	6	4.85	2.63E-02	no
centroid	19	0.1	0	6	4.67	3.42E-02	yes

Table C.4 Priest Lake QTL scan results

The QTL scan results for all QTL detected in our Priest Lake scan are shown. Together, the '1.5 LOD C.I. low (cM)' and '1.5 LOD C.I. high (cM)' columns indicate the range of the 1.5 LOD confidence interval of the genomic location of the QTL. The 'LOD' column indicates the LOD score at the QTL's peak marker (the maker at which genotypes showed the strongest association with phenotypes). The 'p-value' column indicates the genome-wide significance of the peak marker's LOD score for the associated trait.

Trait	Linkage group	Peak Marker Position (cM)	1.5 LOD C.I. low (cM)	1.5 LOD C.I. high (cM)	LOD	p-value	Candidate QTL?
plate count	2	26	18	44	4.41	2.34E-02	no
plate count	7	35.45	30	42	8.58	<1.00E-04	no
plate count	16	14	6.33	22	5.5	2.70E-03	no
long gill raker count	7	46	32.22	58	6.13	1.70E-03	no
short gill raker count	1	14.83	6	32	4.67	1.69E-02	no
x1	2	24	18	33.63	9.52	<1.00E-04	no
x2	14	38.82	30.98	48.93	4.04	4.88E-02	yes
x3	14	38.82	28.55	48.93	4.38	2.47E-02	no
y3	4	71.36	66	71.36	4.67	1.51E-02	no
y5	7	40	34.21	56	6.16	1.40E-03	no
x6	4	20.84	14.49	32	3.99	5.35E-02	yes
x6	13	27.7	22.18	28.79	4.06	4.64E-02	yes
x7	3	6	0	14	5.32	4.80E-03	no
y7	7	35.45	30	50.64	5.58	3.50E-03	no
y7	9	10	0	17.66	4.32	2.97E-02	yes
y10	1	23.96	4	27.92	5.8	1.80E-03	no
y11	11	30	8.46	37.74	4.86	8.30E-03	no
x15	3	2.69	0	12	4.2	3.39E-02	no
x16	1	21.75	18.11	34	5.4	3.20E-03	no
x16	12	13.5	2	15.46	5.68	1.70E-03	no
y16	21	42.82	26	44.58	4.19	4.17E-02	yes
x17	12	6.42	4	13.24	6.31	7.00E-04	no
x17	14	34.83	24	48.93	4.1	4.36E-02	yes
y18	11	34.66	28	40	4.31	3.04E-02	no
y19	1	22	16	37.75	5.87	9.00E-04	no
y19	12	12.16	0	24	4.3	3.20E-02	no
y19	14	34.83	0	41.41	4.53	2.15E-02	no
x20	12	4.39	0	15.46	4.56	1.86E-02	yes
x23	3	4	0	12	4.69	1.54E-02	no
y23	21	44	20	44.58	5.35	4.70E-03	no
y25	12	11.57	9.45	15.46	4.09	4.82E-02	no
y26	1	21.75	18.11	30	7.02	<1.00E-04	no
y26	12	15.46	3.74	25.01	4.7	1.42E-02	no
y26	14	36.5	12	48.93	4.13	3.94E-02	yes
y27	12	4.39	0	15.46	4.47	2.19E-02	no
y27	17	21.65	0	27.82	4.29	2.99E-02	no
centroid	1	24.57	22.4	32	6.74	3.00E-04	no

Table C.5 'Combined' QTL scan results

The QTL scan results for all QTL detected in our 'combined scan' (i.e. Paxton and Priest Lakes, and including a genotype by lake interaction covariate) are shown. Together, the '1.5 LOD C.I. low (cM)' and '1.5 LOD C.I. high (cM)' columns indicate the range of the 1.5 LOD confidence interval of the genomic location of the QTL. The 'LOD' column indicates the LOD score at the QTL's peak marker (the maker at which genotypes showed the strongest association with phenotypes). The 'p-value' column indicates the genome-wide significance of the peak marker's LOD score for the associated trait.

Trait	Linkage group	Peak Marker Position (cM)	1.5 LOD C.I. low (cM)	1.5 LOD C.I. high (cM)	LOD	p-value	Candidate QTL?
plate count	2	24	18	39.1	5.59	5.27E-02	yes
plate count	7	33.93	33.41	34.99	24.81	<1.00E-04	yes
plate count	16	9.98	4	22	5.66	4.68E-02	yes
long gill raker count	3	36	30.9	42	7.29	2.30E-03	yes
long gill raker count	7	35.12	33.41	35.81	14.12	<1.00E-04	yes
short gill raker count	1	21.16	14	23.72	9.43	2.00E-04	yes
short gill raker count	7	34.99	32.22	35.81	6.67	9.40E-03	yes
1st dorsal spine	2	33.63	24	39.26	10.39	<1.00E-04	no
x1	2	26	18	33.05	10.61	<1.00E-04	no
y1	8	18	10	30.86	5.97	3.45E-02	yes
x2	4	23.78	14.49	26.83	5.97	5.01E-02	yes
x3	1	21.16	16.42	22.4	8.16	1.40E-03	no
x3	5	50.52	30	56.44	6.4	1.57E-02	no
x3	12	18	8	25.01	8.57	6.00E-04	no
y3	4	71.36	66	71.36	5.8	4.04E-02	yes
y3	7	6	0	14	10.65	<1.00E-04	yes
x4	7	33.93	26.61	35.81	8.4	1.10E-03	no
y4	7	34.99	32.22	37.03	5.81	4.95E-02	yes
y5	7	35.45	34.21	42	9.77	<1.00E-04	yes
x6	7	34.21	30	50.99	6.18	1.92E-02	yes
y6	7	37.32	26.61	44	6.86	6.80E-03	no
y6	13	12	0	23.99	7.32	2.80E-03	no
y6	19	0	0	4	11.42	<1.00E-04	no
x7	3	6	0.22	12	7.61	1.70E-03	no
y7	2	33.63	30	38	6.05	2.52E-02	yes
y7	7	35.45	32.22	56	6.41	1.36E-02	yes
x9	3	4	0	10	5.89	3.10E-02	no
x10	2	36.63	28	42	5.71	4.66E-02	no
y10	1	19.11	18.11	26	8.36	5.00E-04	yes
y10	14	12	0	22	10.43	<1.00E-04	yes
x11	1	21.16	16.42	23.72	5.96	3.77E-02	no
y11	1	21.16	15.32	26	5.86	5.35E-02	yes
y11	4	30	26.83	71.36	6.31	2.86E-02	yes
y11	11	28	10	37.74	6.7	1.58E-02	yes
x12	19	0.55	0	6	6.12	4.57E-02	no
y12	13	27.7	24.53	28.79	6	4.21E-02	yes
y12	19	0	0	10	6.85	1.28E-02	yes
x13	1	18.11	16	30	6.51	2.34E-02	yes
x13	7	28	24	33.41	6.87	1.37E-02	yes
x16	1	21.75	18.11	23.72	9.67	<1.00E-04	yes
x16	12	5.22	2	15.46	6.92	5.60E-03	yes
y16	13	28.79	24	28.79	6.18	2.02E-02	yes

Trait	Linkage group	Peak Marker Position (cM)	1.5 LOD C.I. low (cM)	1.5 LOD C.I. high (cM)	LOD	p-value	Candidate QTL?
x17	12	6.42	4	15.46	6.87	9.50E-03	yes
x18	7	32.22	29.3	37.03	6.51	1.12E-02	yes
y18	11	34	28	42	6.44	1.24E-02	yes
y19	1	21.75	14	41.89	6.25	2.17E-02	no
y19	4	34.54	30.31	38	6.7	1.02E-02	no
y19	8	26	19.01	30.86	6.97	6.50E-03	no
y19	12	12.16	10.86	15.46	9.25	1.00E-04	no
y19	14	11.23	0	36	6.01	3.22E-02	no
y19	19	0	0	6	6.19	2.46E-02	no
x20	4	20	15.02	23.78	6.42	1.28E-02	yes
y22	1	18	15.32	20.27	6.71	9.20E-03	no
x23	1	21.16	16.42	23.72	6.75	1.67E-02	no
x23	3	6	0	10.46	8.19	2.00E-03	no
y25	12	13.24	10.86	21	11.06	<1.00E-04	yes
y26	1	21.75	18.11	34	7.91	9.00E-04	yes
y26	12	13.24	10.86	22	9.37	<1.00E-04	yes
y26	19	0.55	0	6	6.83	9.00E-03	yes
y27	8	19.01	16.61	30.86	5.54	5.42E-02	yes
y27	12	13.24	10.73	24	7.51	2.90E-03	yes
y27	17	21.65	12	27.82	7.15	4.40E-03	yes
centroid	1	24.57	2	32	9.64	3.00E-04	yes

Table C.6 QTL effects of candidate QTL

(Starts on next page) QTL effect was considered 'parallel' when either the best model of the QTL effect was 'same effect', or when the best model of QTL effect was 'different effect' but the direction of additive effects were 'same'. QTL effect was considered only in a 'single lake' when the best model of the QTL effect was either 'effect in Paxton only' or 'effect in Priest only'. QTL effect was considered 'opposite' when the best model of QTL effect was 'different effect' and the direction of additive effects were 'opposite'. The second best model of QTL effect and the delta AICc between it and the best model is also shown. When the delta AICc was less than two and the 2nd best model called for a different QTL effect category than the best model did, we dropped the QTL from any analysis in which QTL effect category was a variable. PVE for each QTL in each lake was determined using 'single QTL, single lake linear models'. The 'Priest Entropy' and 'Paxton Entropy' columns show the entropy values (an index of genotype information content, where lower values indicate greater information content), in each lake's cross at the QTL's peak marker.

Trait	Scan QTL was detected in	Linkage group	Peak Marker Position (cM)	Direction of additive effects	'QTL Effect' based on AICc model selection	Best model	2nd best model	Delta AICc	PVE in Priest	PVE in Paxton	Priest entropy	Paxton entropy
plate count	combined	2	24	same	Parallel	same effect	different effect	2.21	4.9	1.32	0.28	0.27
plate count	combined	7	33.93	same	Parallel	different effect	same effect	0.94	9.1	12.09	0.04	0.03
plate count	combined	16	9.98	opposite	Opposite	different effect	effect in Priest only	2.03	6.06	0.73	0.06	0.17
long gill raker count	combined	7	35.12	same	Parallel	same effect	different effect	2.26	6.51	6.3	0.01	0.08
long gill raker count	combined	3	36	same	Single lake	effect in Paxton only	different effect	1.12	1.08	5	0.14	0.14
short gill raker count	combined	1	21.16	same	Parallel	same effect	different effect	2.72	4.13	4.32	0.07	0.17
short gill raker count	combined	7	34.99	same	Parallel	same effect	different effect	0.68	1.67	3.97	0	0.08
y1	combined	8	18	same	Single lake	effect in Paxton only	same effect	4.14	1.58	4.18	0.09	0.12
x2	combined	4	23.78	same	Parallel	same effect	different effect	2.99	1.48	1.13	0.08	0.21
x2	Paxton	7	0	same	Single lake	effect in Paxton only	same effect	0.58	0.07	2.39	0.71	0.05
x2	Priest	14	38.82	opposite	Single lake	effect in Priest only	different effect	0.39	1.92	0.37	0.08	0.16
y3	combined	4	71.36	opposite	Opposite	different effect	effect in Priest only	1.38	5.29	0.9	0.06	0.88
y3	combined	7	6	opposite	Single lake	effect in Paxton only	different effect	2.49	0.13	8.86	0.5	0.13
y4	combined	7	34.99	same	Parallel	same effect	different effect	2	1.56	4.08	0	0.08
y5	combined	7	35.45	same	Parallel	same effect	different effect	2.42	4.34	2.51	0	0.12
y5	Paxton	19	2	same	Parallel	same effect	different effect	0.42	0.21	3.63	0.19	0.05
x6	combined	7	34.21	opposite	Opposite	different effect	effect in Paxton only	0.92	0.68	3.4	0.02	0.05
x6	Priest	4	20.84	same	Parallel	same effect	different effect	1.81	3.09	0.7	0.05	0.2
x6	Priest	13	27.7	same	Single lake	effect in Priest only	same effect	7.08	3.15	0.08	0.1	0.2
y7	combined	7	35.45	same	Single lake	effect in Priest only	different effect	0.94	5.9	0.52	0	0.12
y7	combined	2	33.63	opposite	Opposite	different effect	effect in Paxton only	5.61	2.41	3.14	0.03	0.04
y7	Priest	9	10	same	Single lake	effect in Priest only	same effect	3.44	4.61	0.59	0.35	0.34
y10	combined	1	19.11	opposite	Opposite	different effect	effect in Priest only	9.03	2.77	1.63	0	0.01
y10	combined	14	12	same	Parallel	same effect	different effect	0.31	1.23	4.22	0.06	0.2
y10	Paxton	4	58	opposite	Single lake	effect in Paxton only	different effect	2.57	0.09	3.76	0.03	0.74
y11	combined	11	28	same	Single lake	effect in Priest only	different effect	0.57	2.45	0.37	0.13	0.12
y11	combined	1	21.16	opposite	Single lake	effect in Paxton only	different effect	5.1	0.35	3.49	0.07	0.17
y11	combined	4	30	same	Single lake	effect in Paxton only	different effect	2.4	0.38	4.13	0.01	0.24
y12	combined	19	0	same	Single lake	effect in Priest only	different effect	13.99	2.03	3.11	0.22	0.03
y12	combined	13	27.7	same	Parallel	same effect	different effect	3.01	1.96	1.58	0.1	0.2
y12	Paxton	4	28.15	same	Single lake	effect in Paxton only	different effect	2.77	0.55	2.81	0.06	0.19
x13	combined	7	28	opposite	Parallel	same effect	different effect	0.23	2.3	1.91	0.09	0.22
x13	combined	1	18.11	same	Parallel	same effect	different effect	0.31	0.57	3.71	0.08	0.17
x16	combined	1	21.75	same	Parallel	same effect	different effect	2.97	6.19	3.32	0.11	0.2

Trait	Scan QTL was detected in	Linkage group	Peak Marker Position (cM)	Direction of additive effects	'QTL Effect' based on AICc model selection	Best model	2nd best model	Delta AICc	PVE in Priest	PVE in Paxton	Priest entropy	Paxton entropy
x16	combined	12	5.22	same	Parallel	different effect	same effect	0.87	5.45	1.36	0.06	0.16
x16	Paxton	13	20.04	same	Parallel	different effect	same effect	1.02	0.98	3.39	0.05	0.07
y16	combined	13	28.79	opposite	Opposite	different effect	same effect	0.83	3.55	2.08	0.16	0.26
y16	Priest	21	42.82	same	Parallel	same effect	effect in Priest only	0.34	4.22	0.54	0.01	0.87
x17	combined	12	6.42	opposite	Single lake	effect in Priest only	different effect	3.69	6.84	0.01	0.16	0.22
x17	Priest	14	34.83	same	Single lake	effect in Priest only	different effect	2.58	4.52	0.29	0	0.22
x18	combined	7	32.22	opposite	Single lake	effect in Paxton only	different effect	4.02	2.06	3.84	0.06	0.11
y18	combined	11	34	same	Parallel	same effect	different effect	1.45	2.71	1.27	0.03	0.03
y18	Paxton	4	36	opposite	Single lake	effect in Paxton only	different effect	9.02	0.16	2.85	0.02	0.04
x20	combined	4	20	opposite	Single lake	effect in Priest only	effect in Paxton only	3.16	3.58	2.15	0.04	0.19
x20	Paxton	1	25.31	same	Parallel	different effect	effect in Paxton only	1.62	1.18	3.32	0.13	0.15
x20	Priest	12	4.39	opposite	Opposite	different effect	effect in Priest only	1.21	4.34	0.75	0.05	0.17
x21	Paxton	1	20	same	Single lake	effect in Paxton only	same effect	2.57	0.25	2.51	0.01	0.01
x22	Paxton	7	33.93	same	Parallel	different effect	effect in Paxton only	0.06	0.73	3.94	0.04	0.03
y25	combined	12	13.24	same	Parallel	same effect	different effect	2.88	4.71	5.64	0.02	0.04
y26	combined	1	21.75	opposite	Single lake	effect in Priest only	different effect	2.66	4.71	0.07	0.11	0.2
y26	combined	12	13.24	same	Parallel	same effect	different effect	2.36	3.17	2.57	0.02	0.04
y26	combined	19	0.55	same	Parallel	different effect	effect in Priest only	5.04	1.18	2.79	0.19	0.04
y26	Priest	14	36.5	opposite	Single lake	effect in Priest only	different effect	6.62	2.83	0.05	0.07	0.18
y27	combined	12	13.24	same	Parallel	same effect	different effect	2.55	4.16	2.91	0.02	0.04
y27	combined	17	21.65	opposite	Opposite	different effect	effect in Priest only	10.7	4.81	2.95	0.11	0.1
y27	combined	8	19.01	same	Single lake	effect in Paxton only	effect in Priest only	1.4	2.76	2.53	0.01	0.1
centroid	combined	1	24.57	same	Single lake	effect in Priest only	different effect	1.14	6.4	0.91	0.07	0.13
centroid	Paxton	19	0.1	opposite	Single lake	effect in Priest only	same effect	1.28	1.24	2.96	0.21	0.02

Table C.7 Proportional similarity of QTL use underlying parallel traits

For each QTL, 'PVE in Priest' and 'PVE in Paxton' were determined using a 'multiple QTL linear model' containing genotypic effects of each QTL affecting the same trait (as well as family identity and sex as covariates). These models were run for each lake separately. If the QTL genotype (both additive and dominant components) did not show a significant effect when dropped from a 'single lake, single QTL linear model' then it was not entered in the multiple QTL model for that lake. In this case, the PVE column is left blank. In each lake, proportional contributions of QTL to traits were calculated by scaling the PVEs of all QTL affecting the same trait so that they summed to 1. The proportional similarity of a QTL was taken as the overlap in the proportional contributions of that QTL in the two lakes. The 'proportional similarity of QTL use' underlying any given trait is then the sum of the proportional similarities of all QTL affecting that trait.

Trait	QTL (LG # @ position (cM))	PVE in Priest	Proportional Contribution in Priest	PVE in Paxton	Proportional Contribution in Paxton	Proportional Similarity
plate count	16@10.0	4.79	0.31			0.00
plate count	2@24.0	3.39	0.22	1.28	0.10	0.10
plate count	7@33.9	7.32	0.47	12.04	0.90	0.47
long gill raker count	7@35.1	6.51	1.00	6.23	0.56	0.56
long gill raker count	3@36.0			4.93	0.44	0.00
short gill raker count	1@21.2	4.13	1.00	4.89	0.52	0.52
short gill raker count	7@35.0			4.54	0.48	0.00
y1	8@18.0	1.58	1.00	4.18	1.00	1.00
x2	14@38.8	1.70	0.57			0.00
x2	4@23.8	1.26	0.43	1.11	0.32	0.32
x2	7@0.0			2.38	0.68	0.00
y3	4@71.4	5.29	1.00			0.00
y3	7@6.0			8.86	1.00	0.00
y4	7@35.0			4.08	1.00	0.00
y5	7@35.5	4.34	1.00	2.44	0.41	0.41
y5	19@2.0			3.57	0.59	0.00
x6	13@27.7	3.11	0.50			0.00
x6	4@20.8	3.06	0.50			0.00
x6	7@34.2			3.40	1.00	0.00
y7	2@33.6	1.43	0.12	3.14	1.00	0.12
y7	7@35.5	5.98	0.49			0.00
y7	9@10.0	4.74	0.39			0.00
y10	1@19.1	2.61	0.71	0.73	0.11	0.11
y10	14@12.0	1.07	0.29	3.51	0.51	0.29
y10	4@58.0			2.68	0.39	0.00
y11	11@28.0	2.45	1.00			0.00
y11	1@21.2			2.60	0.44	0.00
y11	4@30.0			3.24	0.56	0.00
y12	13@27.7	1.54	0.49	1.35	0.19	0.19
y12	19@0.0	1.61	0.51	3.14	0.44	0.44
y12	4@28.1			2.69	0.37	0.00
x13	7@28.0	2.30	1.00	1.31	0.30	0.30
x13	1@18.1			3.11	0.70	0.00
x16	1@21.7	4.53	0.54	2.31	0.37	0.37
x16	12@5.2	3.79	0.46	1.16	0.18	0.18
x16	13@20.0			2.83	0.45	0.00
y16	13@28.8	3.03	0.45	2.08	1.00	0.45
y16	21@42.8	3.70	0.55			0.00
x17	12@6.4	6.00	0.62			0.00

Trait	QTL (LG # @ position (cM))	PVE in Priest	Proportional Contribution in Priest	PVE in Paxton	Proportional Contribution in Paxton	Proportional Similarity
x17	14@34.8	3.68	0.38			0.00
x18	7@32.2	2.06	1.00	3.84	1.00	1.00
y18	11@34.0	2.71	1.00	0.88	0.26	0.26
y18	4@36.0			2.47	0.74	0.00
x20	12@4.4	4.15	0.55			0.00
x20	4@20.0	3.40	0.45	1.61	0.37	0.37
x20	1@25.3			2.78	0.63	0.00
x21	1@20.0			2.51	1.00	0.00
x22	7@33.9			3.94	1.00	0.00
y25	12@13.2	4.71	1.00	5.64	1.00	1.00
y26	1@21.7	3.02	0.38			0.00
y26	12@13.2	2.07	0.26	3.00	0.48	0.26
y26	14@36.5	1.62	0.20			0.00
y26	19@0.6	1.21	0.15	3.22	0.52	0.15
y27	12@13.2	3.47	0.34	2.87	0.35	0.34
y27	17@21.7	4.36	0.43	3.17	0.38	0.38
y27	8@19.0	2.36	0.23	2.21	0.27	0.23
centroid size	1@24.6	6.40	1.00			0.00
centroid size	19@0.1			2.96	1.00	0.00