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# The genetic architecture of skeletal convergence and sex determination in ninespine sticklebacks

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## SUMMARY

The history of life offers plentiful examples of convergent evolution, the independent derivation of similar phenotypes in distinct lineages [1]. Convergent phenotypes among closely related lineages (frequently termed "parallel" evolution) are often assumed to result from changes in similar genes or developmental pathways [2], but the genetic origins of convergence remains poorly understood. Ninespine (*Pungitius pungitius*) and threespine (*Gasterosteus aculeatus*) stickleback fish provide many examples of convergent evolution of adaptive phenotypes, both within and between genera. The genetic architecture of several important traits is now known for threespine sticklebacks [3-10]; thus, ninespine sticklebacks thus provide a unique opportunity to critically test whether similar or different chromosome regions control similar phenotypes in these lineages. We have generated the first genome-wide linkage map for the ninespine stickleback and used quantitative trait locus (QTL) mapping to identify chromosome regions controlling several skeletal traits and sex determination. In ninespine sticklebacks, these traits mapped to chromosome regions not previously known to control the corresponding traits in threespine sticklebacks. Therefore, convergent morphological evolution in these related, but independent, vertebrate lineages may have different genetic origins. Comparative genetics in sticklebacks provides an exciting opportunity to study the mechanisms controlling similar phenotypic changes in different groups of animals.

## RESULTS AND DISCUSSION

### Genome-wide linkage map

The last several years have witnessed substantial progress in characterizing the genetic basis of adaptive diversity in natural populations and species. In threespine sticklebacks, development of

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new genetic and molecular tools has made it possible to identify major loci controlling repeated evolution of armor plate, pelvic, opercular, and skin color changes in populations that colonized new lakes and streams generated by widespread deglaciation beginning about 20,000 years ago [3-6, 9, 10]. An emerging theme of genetics studies in threespine sticklebacks is that the same genes or chromosome regions underlie similar phenotypes in multiple natural populations, including major effects of *Pitx1* [4, 6, 11, 12] and *Ectodysplasin (Eda)* [5, 6, 8] in the evolution of derived pelvic and armor phenotypes, respectively, throughout the range of this species. Development of comparable genetic resources for ninespine sticklebacks makes it possible for us to critically compare the genetic basis of convergent evolution in a fish group that has also evolved a number of similar interesting morphological and physiological changes (Fig. 1), but last shared a common ancestor with threespine sticklebacks well over 13 million years ago [13].

To generate a genome-wide linkage map for quantitative trait locus (QTL) studies, we produced a *Pungitius pungitius* genomic library, screened it with a probe for microsatellite repeats, sequenced individual clones, and designed PCR primers that could amplify individual microsatellite repeat regions from ninespine stickleback genomic DNA samples. We typed 212 microsatellite markers (169 derived from ninespine sticklebacks and 43 from threespine sticklebacks) on 120 F1 progeny from a cross between Canadian and Alaskan ninespine sticklebacks, both lacking pelvic structures (Fig. 1C, E). The female parent came from Fox Holes Lakes (Northwest Territories, Canada), which is monomorphic for total absence of the pelvis [14]. The male parent came from an unnamed creek on Pt. MacKenzie (Matanuska-Susitna Borough, south-central Alaska), where ninespine sticklebacks are polymorphic for pelvic phenotypes (average pelvic score for the Pt. MacKenzie population is 1.96 on a scale [15] that

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ranges from 0 (bilateral absence of pelvic structures) to 8 (all 4 pelvic elements present on both sides)). The combined ninespine and threespine markers defined 30 genetic linkage groups (LGs), comprising 190 markers (151 from ninespine sticklebacks, 39 from threespine sticklebacks) and spanning a total genetic distance of 957.8 cM (Figure S1). Since cytological studies show that *Pungitius* has 21 chromosomes [16-18], we expect some current linkage groups to coalesce with others as additional markers are added to the map.

To compare LGs in ninepsine and threespine sticklebacks, we examined map locations of the 39 markers that could be amplified from genomic DNA in both species. We also used BLAST searches to compare the unique sequences from the 151 newly isolated and mapped ninespine stickleback markers with an initial genome assembly for the threespine stickleback (http://www.ensembl.org/Gasterosteus_aculeatus/index.html). In all, 88.7% (134/151) of ninespine stickleback marker sequences could be mapped to unique threespine stickleback chromosome scaffolds, 2.0% (3/151) mapped to unassembled scaffolds, and the remaining 9.3% (14/151) of sequences either produced no significant BLAST results or mapped to multiple genomic scaffolds (Table S1). At least 50% of markers in each ninespine linkage group were associated with a single threespine chromosome (87.9% of markers overall, mean of 85.2% of markers per linkage group). These results suggest that synteny has been well conserved between the two genera, both of which have 21 cytologically visible chromosomes [16]. For ease of comparison of results between species, we numbered linkage groups in the ninespine genetic map to match the syntenic linkage group in the threespine map.

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**Comparative mapping of pelvic reduction**

A dramatic example of convergent evolution between populations and genera of sticklebacks is the reduction or loss of the pelvic (hind fin) skeleton (Fig. 1). The pelvis is present in all marine and most freshwater populations of threespine and ninespine sticklebacks, but it has been lost repeatedly in several freshwater populations, likely as an adaptation to local predators and water chemistry [4, 14, 15, 19-24]. Previous studies of threespine sticklebacks have identified one QTL of major effect on LG7 that controls more than 50% of the variation in pelvic size in crosses from diverse geographic locations, including British Columbia, Alaska, Iceland, and Scotland [4, 6, 12]. Mapping, sequencing, and expression studies suggest that this major QTL corresponds to the *Pitx1* locus [4, 12, 25], a homeodomain transcription factor that is expressed in developing hindlimbs but not forelimbs of vertebrates [26-28]. Previous complementation and in situ studies show that Fox Holes Lakes ninespine sticklebacks have recessive genetic changes that also reduce *Pitx1* expression in the pelvis [29].

Presence or absence of a pelvic skeleton segregates in a 1:1 Mendelian ratio in our ninespine stickleback cross (Fig. 2). Of the 120 progeny analyzed, 59 had complete pelvic skeletons (bilateral presence of an anterior process, posterior process, ascending branch, and spine [30]), 8 had partial skeletons (6 of these had fewer than half of the normal structures), and 53 lacked all pelvic structures. The binary, qualitative trait of presence versus absence of the pelvic complex (partial phenotypes excluded) mapped to LG4 with a peak LOD score of 82.16 (Fig. 2D, Tables S2 and S4). Detailed analysis of marker genotypes shows that the striking dimorphism in this cross originates from the Alaskan male parent – inheritance of one Alaskan parental LG4 haplotype is usually associated with a complete pelvis in the F1 progeny, while inheritance of the

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other Alaskan haplotype is usually associated with the absence of pelvic structures (Table S4). Thus, the Alaskan male parent of the cross, which comes from a population that is polymorphic for pelvic phenotypes, was heterozygous for a dominant allele for pelvic reduction. The phenotypic effect of the LG4 region in the current cross is as large as that reported previously for the *Pitx1* (LG7) region in threespine sticklebacks [4, 6, 12], but maps to a completely different linkage group. This QTL on LG4 is unlinked to a marker in an intron of the ninespine stickleback *Pitx1* gene (Pun319), and to two markers on threespine stickleback BAC clones containing the *Pitx1* gene (Stn430, Stn431; Fig. 2E and Fig. S1). The region of the threespine stickleback genome that corresponds to the pelvic reduction region in the ninespine cross contains several genes with known roles in limb and fin development, including members of the *Fgf*, *Msx*, and *Wnt* families. We are currently investigating the potential roles of these candidate genes in pelvic reduction in the Point MacKenzie population.

Ninespine sticklebacks from the Pt. MacKenzie, Alaska, population show a key morphological difference compared to most other pelvic reduced populations. Most extant and fossil threespine stickleback populations [31], mice with knockouts in the *Pitx1* gene [32], and Florida manatees with vestigial pelvic structures [29] show greater pelvic reduction on the right than left side. In contrast, the Pt. MacKenzie ninespine sticklebacks tend to show greater pelvic reduction on the left than right side. Our linkage studies provide the first genetic evidence that populations with different types of directional asymmetry have changes in different major genes controlling pelvic reduction. Approximately 10% of threespine stickleback populations with extensive pelvic reduction show greater reduction on the left than right side [31]. It will be interesting to see if

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pelvic reduction in these populations maps to the same region detected in Pt. MacKenzie ninespine sticklebacks.

Although the major QTL for pelvic reduction in our ninespine cross is clearly distinct from the *Pitx1* locus, the position of the QTL on LG4 is in a region similar to a pelvic modifier QTL that controls less than 6% of the variation in pelvic spine length and pelvic girdle length in a cross between marine (complete pelvis) and pelvic-reduced threespine sticklebacks [4] (Table 1). It is possible that similar genes contribute to pelvic reduction in both threespine and ninespine sticklebacks, but the magnitude of their phenotypic effects differs dramatically between genera. The large impact of the LG4 region in the ninespine fish likely depends in part on a sensitized genetic background in the Pt. MacKenzie by Fox Holes Lakes cross, where all F1 progeny also inherited pelvic reduction alleles from the Fox Holes Lakes parent [29]. The LG4 region in the current cross has a larger phenotypic effect than *Eda*, *Pitx1*, or *Kit ligand* (*Kitlg*) genes in threespine sticklebacks, each of which has been successfully isolated by mapping or positional cloning studies [4, 8, 10]. Ninespine sticklebacks should thus provide a very useful system for identifying additional loci controlling major evolutionary phenotypes in natural populations.

A single region on ninespine LG4 largely controls the presence-versus-absence pelvic phenotype, yet other chromosome regions control quantitative variation in pelvic size in those progeny that do have a pelvis. For example, we identified a region on LG1A that controls up to 33.2% of the variation in left and right pelvic structures, with a more pronounced effect on the left than the right side (Fig. 2, Fig. S1, Tables S2 and S4). The LG1A QTL in the ninespine cross

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overlaps the broad location of a QTL interval that controls approximately 6% of the variance in pelvic girdle length in a threespine stickleback cross [4].

Notably, variation at the major and modifier pelvic loci reveals cryptic genetic variation (CGV) in the wild Pt. MacKenzie population. CGV is thought to be an important and pervasive, yet underappreciated, factor in the response of organisms to mutation, selection, and disease [33]. Both parents of our cross had similar pelvisless phenotypes, yet half of their progeny developed complete pelvises on the hybrid genetic background. Most of the fish in the wild Pt. MacKenzie population also exhibit extreme pelvic reduction, so much of the variation in the major and modifier pelvic loci may remain hidden except under extreme environmental conditions, or in response to genetic perturbations such as hybridization with other genetic backgrounds, as is the case on our cross (reviewed in [33]). This study provides a dramatic example of the phenotypic diversity that can result when admixture occurs between different outbred genetic backgrounds.

**A novel chromosome region controls lateral armor in ninespine sticklebacks**

Other skeletal traits mapped to different regions of the genome in ninespine sticklebacks relative to threespine sticklebacks (Table 1, Fig. S1, Tables S2-S5). In threespine sticklebacks, lateral plate variation maps to the *Eda* locus on LG4 [5, 6, 8]. We mapped two markers in and around the *Eda* locus in our ninespine cross, including Stn364 (located in an intron of the *Eda* gene itself), and the closely linked Stn361 marker (located 16 kb away, just 5' of the *Eda* locus). Both markers mapped to LG4 in the ninespine stickleback cross, in a chromosome region that did not have significant effects on plate phenotypes. Instead, lateral plate number variation in the ninespine stickleback cross (Fig. 1F) mapped to LG12, the same chromosome region that

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determines sex (see below). This linkage group accounted for nearly one-third (30.1% left-side and 28.4% right side) of the variance in plate number. Notably, unlike the major pelvic locus on LG4, segregation of different alleles on LG12 from both the Alaskan and Northwest Territories parents had significant effects on plate phenotypes (Tables S3 and S5). The armor QTL on LG12 is also distinct from all known chromosome regions that have smaller quantitative effects on armor phenotypes in threespine sticklebacks with reduced numbers of plates (Table 1).

**The sex determination locus differs between stickleback genera**

Several different mechanisms underlie sex determination among teleost fishes, and under normal conditions sex can be determined by genetic and/or environmental cues [34, 35]. Sex determination in threespine sticklebacks behaves as a simple Mendelian trait that maps to LG19 [7]. Sex determination in ninespine sticklebacks also behaves as a Mendelian trait, but it maps to LG12, in a completely different region of the genome relative to markers closely linked to the sex-determining region in threespine sticklebacks (Stn186, Stn194) [7] (Table 1, Fig. S1, Tables S3 and S5).

The sex determination region of LG19 in threespine sticklebacks shows striking differences in recombination rates in male versus female meiosis [7]. Similarly, LG12 in the ninespine stickleback cross covers approximately 13 cM, due largely to a lack of recombination in male meioses (Figs. S1 and S2). In contrast, when female meioses were analyzed independently of male meioses, the genetic distances between markers are greater and LG12 covers 27 cM (Fig. S2B). Hence, although different chromosomes are involved in sex determination in the two

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genera, the linkage group bearing the sex determination region in ninespine sticklebacks has some of the same recombination characteristics as the threespine stickleback Y chromosome.

The genomic positions of the major sex determination loci are different in threespine and ninespine sticklebacks, yet it is possible that the same molecular mechanisms determine this fundamental trait in both genera. For example, both genera may have inherited the same sex determination mechanism from a common ancestor, but the gene(s) underlying this mechanism may be located on different chromosomes due to different evolutionary translocations, as has occurred in salmonids [36]. Identification of the genes controlling sex determination on LG12 of ninespine sticklebacks and chromosome 19 of threespine sticklebacks will permit a direct test of this hypothesis.

**Multiple phenotypic traits cluster on the sex chromosome**

Several other phenotypic traits mapped to LG12 in ninespine sticklebacks, including jaw length, head length, orbit (eye) diameter, and pectoral fin length (Fig. S1, Tables S3 and S5). With the exception of pectoral fin length, the phenotypic means for each of these traits were larger in male than female fish. Sexual dimorphism in head size and other skeletal traits has previously been demonstrated for wild populations of *Pungitius* [37, 38], and for wild and lab-bred *Gasterosteus* [39-44]. Clustering of phenotypic traits on the sex chromosome could be due either to pleiotropic effects of a single sex-determining locus, or to multiple loci controlling sexually dimorphic traits that are physically and genetically linked on the sex chromosomes. When we repeated our analysis of sex-linked traits using residuals with the average effect of sex removed, we no longer detected significant QTL on LG12, suggesting that most LG12 QTL are detecting male-female

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differences rather than effects of alternative chromosomes within males or females. However, we did detect significant differences between the two female haplotypes on LG12 for lateral plate phenotypes using the transformed data ($p<0.05$, ANOVA with Tukey's Multiple Comparison Test), consistent with our QTL mapping of this trait described above.

Linkage between the primary sex determination locus and genes with differential effects in males and females is thought to be a key feature that drives evolution of sex chromosomes, including the accumulation of inversions and sequence divergence that suppresses recombination between the sex determining locus and neighboring genes [45]. Furthermore, the linkage between sex determination and LG12 in ninespine sticklebacks and at least one other stickleback species (*Gasterosteus wheatlandi*, the black-spotted stickleback) suggests that this chromosome "might have an abundance of genes with differential fitness effects in males and females and thus be predisposed to becoming a sex chromosome" [18]. The distinct linkage groups that control sex determination in threespine and ninespine sticklebacks will provide an excellent system to compare mechanisms of both sex determination and sex chromosome evolution in closely related lineages.

**Genetics of convergent evolution**

Several genetic studies have demonstrated that the same genes likely underlie similar changes among different animal lineages. For example, in *Drosophila*, *Ultrabithorax* and *Ovo/shavenbaby* control similar changes among different species in leg and abdominal trichome patterns, respectively, and repeated changes at the *yellow* locus control similar wing pigmentation in different species [46-49]. Among vertebrates, evolution of similar pigmentation

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phenotypes resulting from changes in the *Melanocortin 1 receptor* (*Mc1r*) in mammals, birds, and reptiles (reviewed in [50]); in *Oculocutaneous albinism 2* (*Oca2*) in multiple populations of cavefish [51]; and in *Kitlg* in both sticklebacks and humans [10] demonstrate that independent changes in the same gene can generate broadly similar phenotypes in multiple lineages. In contrast, other examples of convergent morphological evolution appear to depend on different genetic mechanisms. For instance, complementation crosses suggest that regressive eye loss in blind Mexican cavefish has occurred by different mechanisms in different cave populations [52, 53]. While variant alleles of *Mc1r* control pigmentation phenotypes in the beach mouse (*Peromyscus polionotus)* and rock pocket mouse (*Chaetodipus intermedius*), exceptions to this genetic trend are known for each species [54-56]. Likewise, different genes in different species of *Drosophila* control similar changes in abdominal pigmentation [57].

Because recent genetic studies in threespine sticklebacks show that similar chromosome regions control similar phenotypes in many different populations [4-6, 8, 10, 29], we recognized at the inception of this study that genetic mapping in ninespine sticklebacks might largely identify the same chromosome regions. However, for every trait we examined, we found that the major loci controlling skeletal traits and sex determination in ninespine sticklebacks mapped to different regions than the major loci controlling the corresponding traits in threespine sticklebacks. The convergent evolution of lateral plate and skin color changes in different threespine stickleback popultions has often taken place by repeated selection of ancient variants of the *Eda* and *Kitlg* genes, respectively [8, 10]. These variants are present at low levels in migratory marine populations, and were presumably introduced into new locations when marine ancestors colonized new lakes and streams. Perhaps recent evolution from standing variation within a

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single species of stickleback is more likely to involve the same genes in different populations, whereas convergent evolution between more distantly related genera may be more likely to arise from independent mutations. The current study suggests that ninespine sticklebacks provide an outstanding system to find additional genes responsible for morphological diversity in natural populations of vertebrates, and to compare the detailed genetic basis of convergent evolutionary change in long-separated lineages.

## SUPPLEMENTAL DATA

Supplemental data include Supplemental Experimental Procedures, two figures, and five tables.

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## FIGURE CAPTIONS

**Figure 1. Convergent skeletal evolution in ninespine and threespine sticklebacks.** Reduction and loss of the pelvic (hind) fin has evolved in multiple populations of both ninespine and threespine sticklebacks. (**A,B**) Ninespine (A) and threespine (B) sticklebacks with complete pelvic skeletal structures (arrow) from Airolo Lake, Alaska; and Little Campbell River, British Columbia. (**C,E**) Ninespine sticklebacks missing all pelvic structures (arrowhead) from Point MacKenzie, Alaska; and Fox Holes Lakes, Northwest Territories. These two populations were used in the mapping cross. (**D**) A similar pelvisless phenotype occurs in the benthic threespine sticklebacks of Paxton Lake, British Columbia. (**F**) Enlargement of boxed area in E showing detail of caudal portion of bony armor (arrowheads), which varies in numbers of plates among fish from different populations and in our laboratory cross. All specimens were cleared by digestion in trypsin and stained in alizarin red S to visualize ossified skeletal structures. Photographs are not to scale.

**Figure 2. Pelvic reduction maps to LG4, not to *Pitx1*, in a ninespine stickleback cross.** (**A**) Morphology of the ninespine stickleback pelvis and ectocoracoid in ventral (top) and lateral (bottom) views. A complete pelvis shows bilateral presence of the anterior process (AP), posterior process (PP), ascending branch (AB), and pelvic spine (PS). Anterior to the pelvis is the ectocoracoid bone (EC) of the pectoral girdle. (**B,C**) The 120 progeny showed a 1:1 ratio of (**B**) complete to (**C**) reduced pelvic phenotypes. Anterior is to the left in both images. (**D**) A QTL on LG4 controlled presence versus absence of the pelvis. Only informative markers (polymorphic in the Alaskan male parent) are shown. The plateau of the LOD peak is due to low

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recombination between LG4 haplotypes in the Alaskan parent of the cross. (**E**) The linkage group containing *Pitx1* did not have a significant effect on pelvic phenotype. (**F**) Restricted MQM analysis detected an additional QTL interval influencing left ascending process height (L asc pr; red) and pelvic girdle length (L pel; blue), and this interval includes the *Tbx4* gene, a transcription factor involved in hindlimb development [27]. Pelvic girdle length was measured from the anterior tip of AP to the posterior tip of PP. Dashed lines: LOD significance threshold (95% genome-wide level of $\geq 4.5$ in **D** [58] and $\geq 4.3$ in **F;** not shown in **E** to limit LOD scale preserve visibility of plot). Diagrams in **A** modified after [30].

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**Table 1. Comparison of QTL for skeletal traits and sex determination in ninespine and threespine sticklebacks (similar mapping results highlighted in bold italics).**

| Trait | Ninespine LG | Threespine LG | References |
|---|---|---|---|
| Pelvis (complete vs. reduced) | 4 | 7 | [4, 6, 12] |
| Ascending branch height | 1, 4 | 7, 10 | [4] |
| Pelvic girdle length | ***1***, ***4*** | ***1***, 2, ***4***, 7 | [4] |
| Pelvic spine length | ***4*** | 2, ***4***, 7, 8 | [3, 4] |
| Lateral plate number | 12 | 4, 7, 10, 26* | [5, 6, 8] |
| Sex determination | 12 | 19 | [7] |

* Chromosome 21 in threespine stickleback genome assembly.

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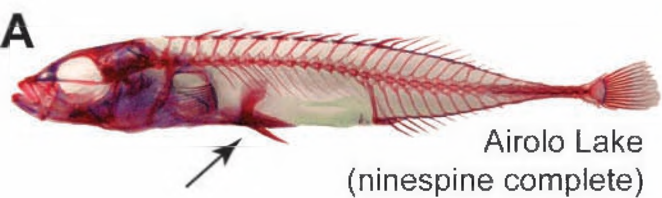
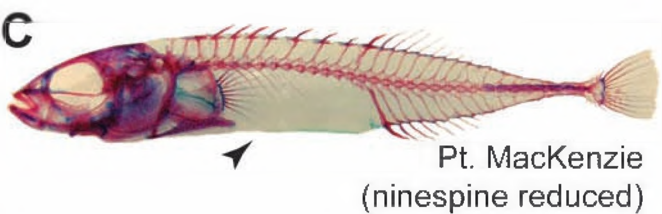
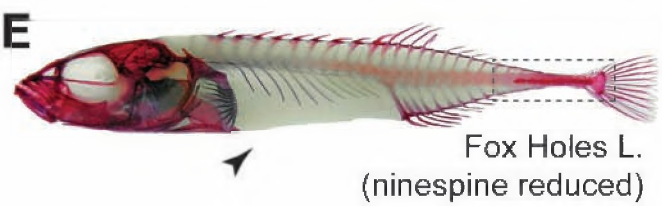
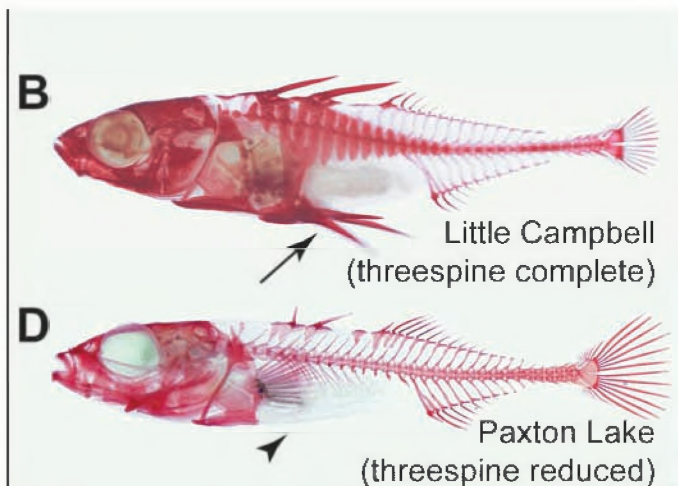
Figure 1

Figure 1



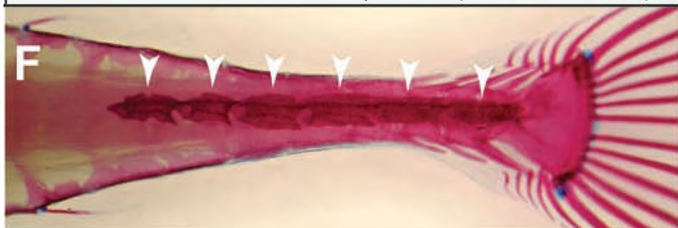
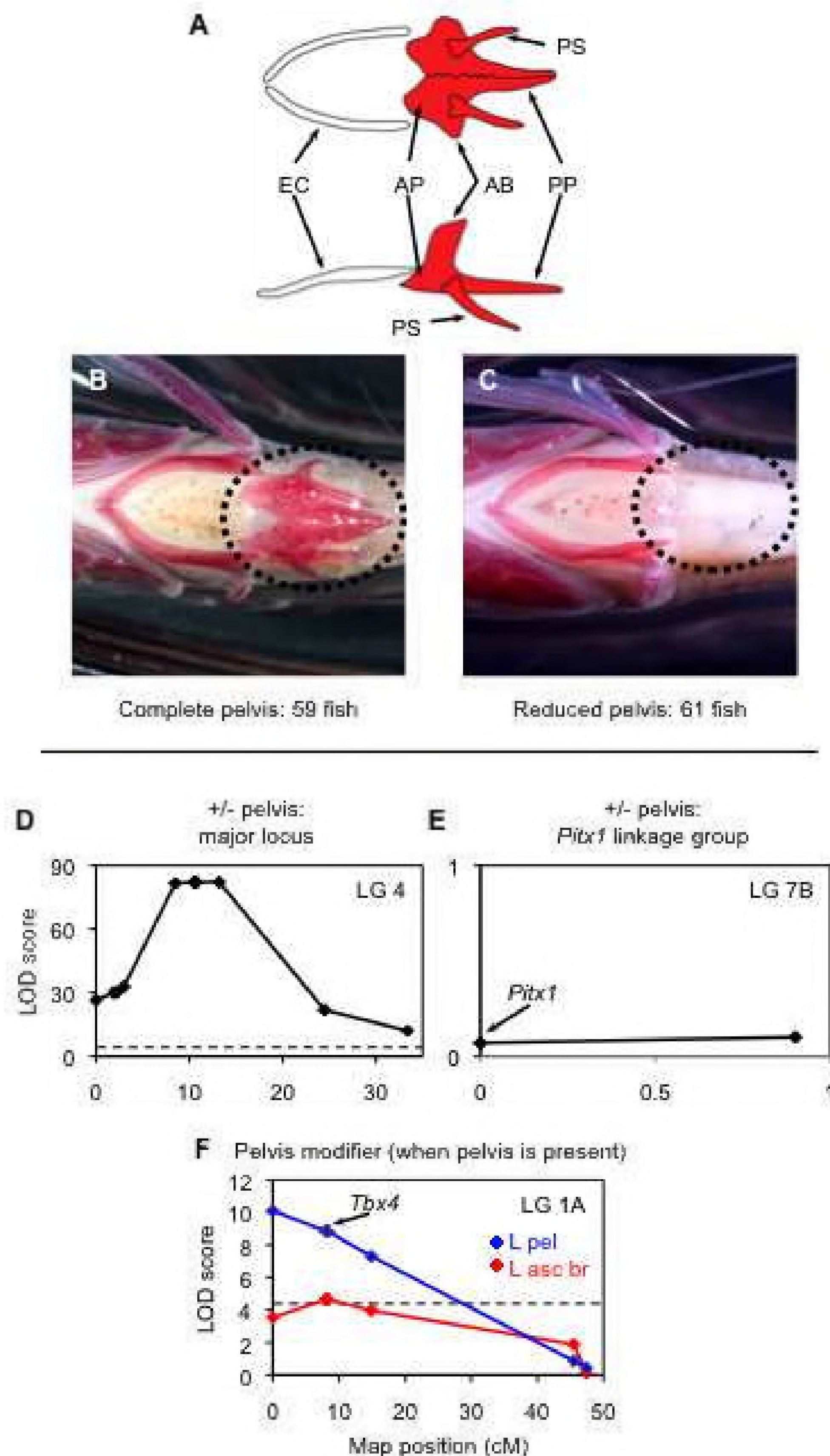



Figure 2
Click here to download high resolution image



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## Supplemental Data

# The Genetic Architecture of Skeletal Convergence and Sex Determination in Ninespine Sticklebacks

Michael D. Shapiro, Brian R. Summers, Sarita Balabhadra, Jaclyn T. Aldenhoven, Ashley L. Miller, Christopher B. Cunningham, Michael A. Bell, David M. Kingsley

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Mapping cross and husbandry

A female ninespine stickleback from Fox Holes Lakes, Northwest Territories, was crossed to a male ninespine stickleback from an unnamed creek at Pt. MacKenzie, south-central Alaska. Both fish lacked all pelvic structures. The female used in this cross was the same specimen also used in an intergeneric hybrid cross with a pelvisless Paxton Lake benthic threespine stickleback [1]. One hundred twenty progeny from the Fox Holes Lakes ninespine by Pt. MacKenzie ninespine cross were raised to at least 28.5 mm standard length (SL) in 29-gal aquaria with 16 h light 8 h dark light cycle. All fish were anesthetized, preserved in 100% ethanol, and tissue samples were removed from the liver, gut, and right pectoral fin for DNA analysis. Specimens were then fixed in 10% neutral buffered formalin, stained with alizarin red to visualize the skeleton as described elsewhere [2], and preserved in 70% ethanol for phenotypic analysis.

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**Microsatellite markers and genotyping**

High-molecular weight DNA from a single ninespine stickleback from Pine Lake, northeastern Alberta, was cut with RsaI or HincII and size-selected for fragments of 1 to 1.5 kb. Fragments were cloned into pBluescriptSK(+) and screened for microsatellite repeats as described previously [2]. Positive clones were sequenced on an ABI 377 DNA analyzer (Applied Biosystems, Foster City, CA) and fragments containing microsatellites were used to design mapping primers using Primer3 software [3]. In addition, a large set of microsatellite markers previously developed for mapping experiments in threespine sticklebacks [2, 4-7] was also tested for PCR amplification from genomic DNA of the two parents of the ninespine mapping cross to identify additional markers for mapping. PCR and genotyping were performed as described by Peichel et al. [2] using an ABI 3730xl DNA analyzer. Additional markers were designed around microsatellites from sequenced threespine stickleback BACs containing the coding regions of *Tbx4* (Stn437-Stn439) and *Pitx1* (Stn430-Stn431), and from an intron of the *Pitx1* gene in the ninespine stickleback (Pun319). New microsatellite marker data were submitted to GenBank dbSTS, accession numbers GF089519-GF089702.

**Map construction**

A genetic linkage map was constructed using genotype data from 212 polymorphic microsatellite markers. Segregation of microsatellite alleles was analyzed using JoinMap3.0 software [8] with parameters described by Peichel et al. [2]. Markers were assembled into 30 linkage groups at a LOD threshold of 4.0. Linkage groups shown were derived from the second round of analysis and include 151 ninespine markers and 39 threespine markers (190 total markers). The remaining 18 ninespine and 4 threespine markers were incorporated in the less stringent third round of

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analysis and are listed in Table S1 (212 total markers). A graphical map was generated using MapChart software [9] (Figure S1).

**Comparison of ninespine and threespine stickleback linkage maps**

Linkage groups in the ninespine map were examined for broad correspondence with chromosomes in the version 1.0 release of the threespine stickleback genome sequence assembly (http://www.ensembl.org/Gasterosteus_aculeatus/index.html). We performed a BLAST search through the Ensembl web interface (http://www.ensembl.org/Multi/blastview) to estimate the corresponding positions in the threespine genome of the ninespine genomic fragments used to generate microsatellite markers. BLAST hits were considered significant at a threshold of $E < 10^{-5}$ using the BLASTN search tool with no optimization of search sensitivity [10]. For ease of comparison, linkage groups in the new *Pungitius* linkage map are designated using the number of the threespine linkage group containing the most orthologous markers. In some cases, *Pungitius* linkage groups received "A" or "B" designations because 2 linkage groups shared homology with the same threespine stickleback chromosome, but did not show sufficient linkage in our cross to be joined during map construction.

**Phenotyping**

Skeletal measurements were performed using digital calipers under a dissecting microscope. Measurements included: standard length (from tip of upper lip to posterior edge of caudal peduncle), pelvic girdle length (from anterior tip of anterior process to posterior tip of posterior process), pelvic spine length (from proximal-most part of base to distal tip), length of ascending branch of pelvis (from midpoint of pelvic spine articulation to dorsal tip of branch), head length

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(from anterior tip of upper lip to posterior of operculum), upper jaw length (from lateral corner of the mouth to midline of upper lip; a proxy for mediolateral length of the premaxilla and maxilla), lower jaw length (from ventral angle of lower jaw formed by the articulation of the angular/articular with the quadrate, to midline of lower lip; a proxy for mandibular length), orbit (eye) diameter (measured along the longitudinal body axis of the fish), and pectoral fin length (from the dorsal base of the fin to the most distal point). Each measurement was taken three separate times and averaged to reduce errors, and the same person measured individual traits in all fish. We made separate measurements of left and right sides of pelvic structures and lateral plates to assay for genomic regions that might play a role in bilateral asymmetry; other structures were measured on the left side only. Phenotypic sex was determined by dissection and gonadal morphology in 89 of the 120 progeny (74.2% of the cross). Fish with ambiguous or highly immature gonads were not scored. The following traits were also measured but did not produce significant QTL: snout length, interorbital distance, body depth, length and width of caudal peduncle, length of anal fin base, anal spine length, length of pectoral fin base, length of dorsal fin base, length of most posterior dorsal spine, and number of dorsal spines.

**QTL analysis**

Phenotypic and genotypic data were analyzed using the interval and restricted MQM mapping functions of MapQTL4.0 [ref. 11] using the following parameters: mapping step size of 5.0, maximum of 200 iterations, a functional tolerance value of $1.0e^{-8}$, and automatic cofactor selection for restricted MQM. Regression analysis was performed on the linear measurements to remove the effects of size (standard length) and sex; the adjusted measurements (residuals) were then used in the QTL analysis. Armor plate counts were analyzed as raw data as plate counts do

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not co-vary with standard length (tested by regression of plate phenotypes on standard length, slope not significantly different from 0, $p > 0.05$ for both right and left plate phenotypes). To detect additional QTL for pelvic phenotypes in fish with a complete pelvis, we ran a separate analysis with absent pelvis phenotypes treated as missing data. LOD scores of $\geq 4.5$ were considered significant based on conservative genome-wide criteria [12] and were confirmed by genome-wide permutation test in MapQTL4.0 [ref. 11]. For significant QTL markers with 4 alleles, we used one-way ANOVA with Tukey's multiple comparison test to examine differences in phenotypic means for each allele using Prism 4 software (GraphPad Software, La Jolla, CA). Residuals for pelvic traits with a large number of zero measurements ("all fish" category) and upper jaw length were analyzed using Kruskal-Wallis and Dunn's multiple comparison tests due to non-normal distribution of phenotypic values. For markers with 2 alleles, we used an unpaired, two-tailed t-test with Welch's correction for unequal variances. We discarded potential QTL that had 5 or fewer members in one or more genotypic classes.

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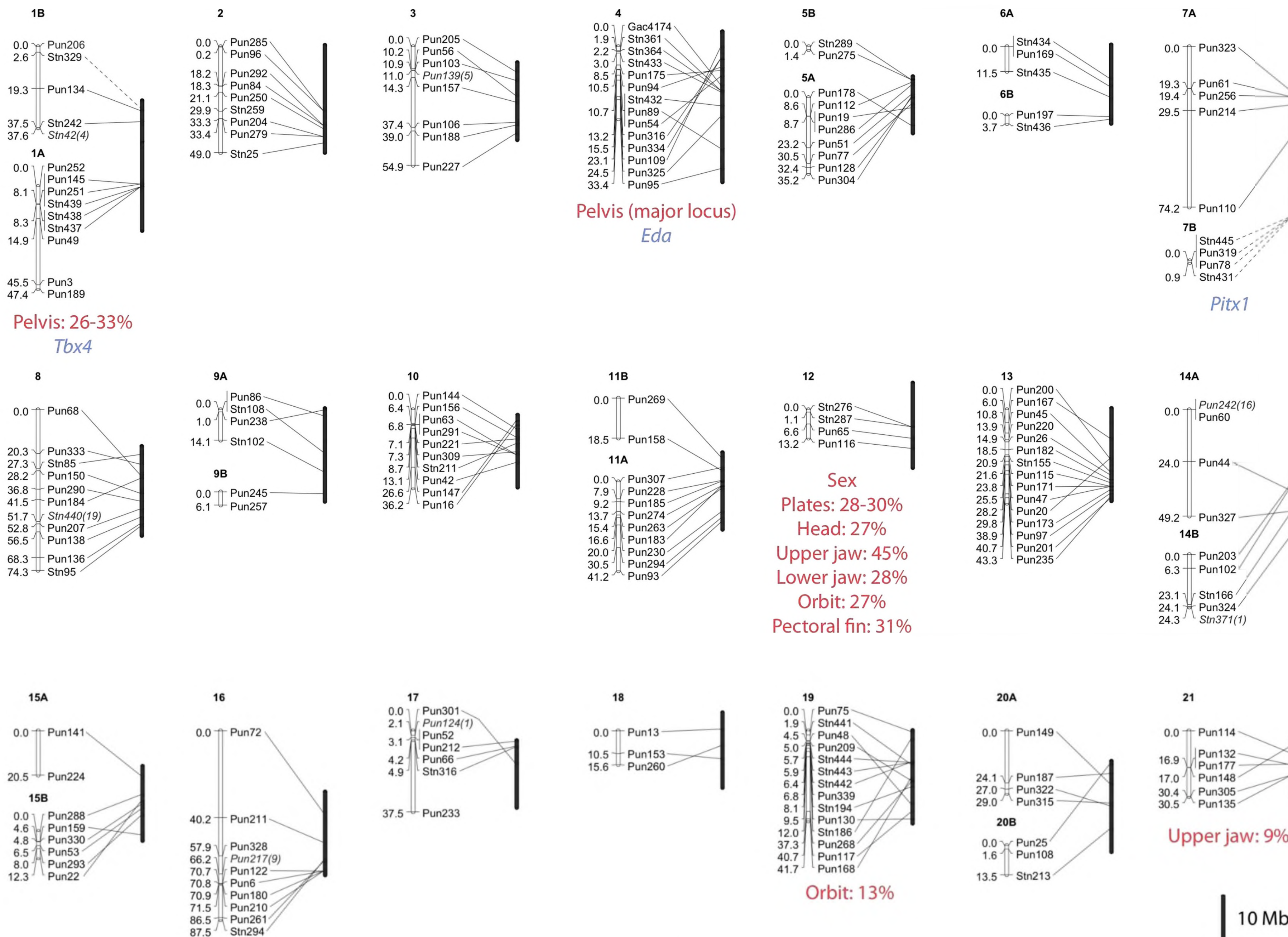

1B
0.0 Pun206
2.6 Stn329
19.3 Pun134
37.5 Stn242
37.6 Stn42(4)
1A
0.0 Pun252
Pun145
8.1 Pun251
Stn439
Stn438
8.3 Stn437
14.9 Pun49
45.5 Pun3
47.4 Pun189
Pelvis: 26-33%
Tbx4
2
0.0 Pun285
0.2 Pun96
18.2 Pun292
18.3 Pun84
21.1 Pun250
29.9 Stn259
33.3 Pun204
33.4 Pun279
49.0 Stn25
3
0.0 Pun205
10.2 Pun56
10.9 Pun103
11.0 Pun139(5)
14.3 Pun157
37.4 Pun106
39.0 Pun188
54.9 Pun227
4
0.0 Gac4174
1.9 Stn361
2.2 Stn364
3.0 Stn433
8.5 Pun175
10.5 Pun94
Stn432
10.7 Pun89
Pun54
13.2 Pun316
15.5 Pun334
23.1 Pun109
24.5 Pun325
33.4 Pun95
Pelvis (major locus)
Eda
5B
0.0 Stn289
1.4 Pun275
5A
0.0 Pun178
8.6 Pun112
Pun19
8.7 Pun286
23.2 Pun51
30.5 Pun77
32.4 Pun128
35.2 Pun304
6A
0.0 Stn434
Pun169
11.5 Stn435
6B
0.0 Pun197
3.7 Stn436
7A
0.0 Pun323
19.3 Pun61
19.4 Pun256
29.5 Pun214
74.2 Pun110
7B
Stn445
0.0 Pun319
Pun78
0.9 Stn431
Pitx1
8
0.0 Pun68
20.3 Pun333
27.3 Stn85
28.2 Pun150
36.8 Pun290
41.5 Pun184
51.7 Stn440(19)
52.8 Pun207
56.5 Pun138
68.3 Pun136
74.3 Stn95
9A
Pun86
0.0 Stn108
1.0 Pun238
14.1 Stn102
9B
0.0 Pun245
6.1 Pun257
10
0.0 Pun144
6.4 Pun156
6.8 Pun63
Pun291
7.1 Pun221
7.3 Pun309
8.7 Stn211
13.1 Pun42
26.6 Pun147
36.2 Pun16
11B
0.0 Pun269
18.5 Pun158
11A
0.0 Pun307
7.9 Pun228
9.2 Pun185
13.7 Pun274
15.4 Pun263
16.6 Pun183
20.0 Pun230
30.5 Pun294
41.2 Pun93
12
0.0 Stn276
1.1 Stn287
6.6 Pun65
13.2 Pun116
Sex
Plates: 28-30%
Head: 27%
Upper jaw: 45%
Lower jaw: 28%
Orbit: 27%
Pectoral fin: 31%
13
0.0 Pun200
6.0 Pun167
10.8 Pun45
13.9 Pun220
14.9 Pun26
18.5 Pun182
20.9 Stn155
21.6 Pun115
23.8 Pun171
25.5 Pun47
28.2 Pun20
29.8 Pun173
38.9 Pun97
40.7 Pun201
43.3 Pun235
14A
Pun242(16)
0.0 Pun60
24.0 Pun44
49.2 Pun327
14B
0.0 Pun203
6.3 Pun102
23.1 Stn166
24.1 Pun324
24.3 Stn371(1)
15A
0.0 Pun141
20.5 Pun224
15B
0.0 Pun288
4.6 Pun159
4.8 Pun330
6.5 Pun53
8.0 Pun293
12.3 Pun22
16
0.0 Pun72
40.2 Pun211
57.9 Pun328
66.2 Pun217(9)
70.7 Pun122
70.8 Pun6
70.9 Pun180
71.5 Pun210
86.5 Pun261
87.5 Stn294
17
0.0 Pun301
2.1 Pun124(1)
3.1 Pun52
Pun212
4.2 Pun66
4.9 Stn316
37.5 Pun233
18
0.0 Pun13
10.5 Pun153
15.6 Pun260
19
0.0 Pun75
1.9 Stn441
4.5 Pun48
5.0 Pun209
5.7 Stn444
5.9 Stn443
6.4 Stn442
6.8 Pun339
8.1 Stn194
9.5 Pun130
12.0 Stn186
37.3 Pun268
40.7 Pun117
41.7 Pun168
Orbit: 13%
20A
0.0 Pun149
24.1 Pun187
27.0 Pun322
29.0 Pun315
20B
0.0 Pun25
1.6 Pun108
13.5 Stn213
21
0.0 Pun114
16.9 Pun132
Pun177
17.0 Pun148
30.4 Pun305
30.5 Pun135
Upper jaw: 9%
10 Mb

**Figure S1. Genome-wide microsatellite linkage map for the ninespine stickleback.** Linkage groups are numbered according to orthologous linkage groups of the threespine stickleback; genetic distances (at left of each group) are listed in centimorgans. Solid lines are drawn from genetic locations of micrsatellite markers to the approximate physical locations of marker sequences on threespine stickleback chromosome sequence assemblies (black vertical bars) based on significant BLAST hits. Dashed lines indicate approximate locations based on previous genetic studies of threespine sticklebacks [5, 7]. Significant QTL are listed in red text under linkage groups with percent of the phenotypic variance explained (expressed as a percentage); see Tables S2-S5 for details. The locations of two genes important in vertebrate hindlimb development (*Pitx1* and *Tbx4*) and one important in lateral plate development in threespine sticklebacks (*Eda*) are shown in blue text, as is the location of *Eda*, a key determinant of lateral plate variation in threespine sticklebacks. Markers in italics shared sequence homology or a previous genetic mapping result with a different threespine stickleback linkage group, noted in parentheses. For example, Stn42 was mapped to LG1B in this study, but this marker shares sequence homology with chromosome 4 of the threespine stickleback, and was previously mapped to LG4 (see Table S1 for details). Thus, this marker appears as *Stn42(4)* on LG1B.

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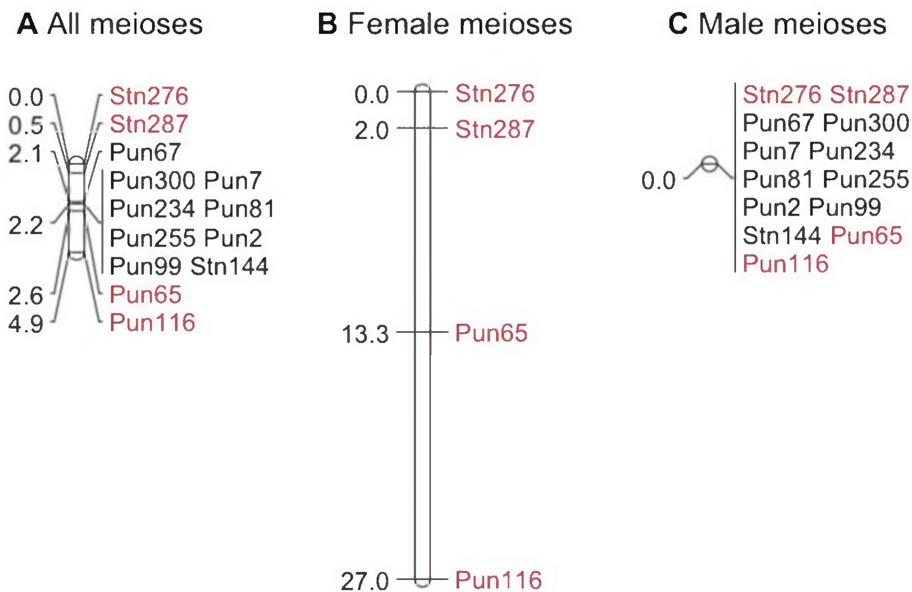


**Figure S2. Genetic linkage maps of the LG12 sex chromosome in the ninespine stickleback.** Markers in red were used to construct the linkage map and were used in QTL analyses. Markers in black were added in the third (less stringent) round of analysis in JoinMap (see Table S1). (**A**) Combined linkage map from male and female meioses. All markers were polymorphic in males, while only those highlighted in red were polymorphic (and thus mappable) in females. (**B**) Linkage map based only on recombination seen in the female parent. Exclusion of male meioses generated greater genetic distances between markers. (**C**) Linkage map based only on recombination seen in the male parent. No recombination was observed between markers. Genetic distances given in centimorgans.

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**Table S1. Genomic locations of microsatellite markers used in this study.** Ninespine stickleback genomic fragements containing microsatellites were BLASTed against the threespine stickleback genome assembly to estimate their chromosomal positions. The positions of threespine stickleback markers are also indicated, where available. Marker sequences that did not produce any significant hits (E-value > 1E-05) are listed as "no hits", while those that produced multiple nearly equivalent hits (E-value within a factor of 1E-02) are listed as "many". Some marker sequences shared high sequence identity with unmapped threespine sequence scaffolds. These BLAST hits are denoted with an "sc" prefix in the Chromosome column.

| Marker | Chromosome | Position (bp) |
|---|---|---|
| **LG1A** | | |
| Pun252 | no hits | |
| Pun145 | I | 18123372 |
| Pun251 | I | 18124421 |
| Stn439 | I | 18648811 |
| Stn438 | I | 18660849 |
| Stn437 | I | 18650677 |
| Pun49 | no hits | |
| Pun3 | sc393 | 14719 |
| Pun189 | no hits | |
| | | |
| **LG1B** | | |
| Pun206 | many | |
| Stn329 | I | NA |
| Pun134 | I | 2151610 |
| Stn242 | I | 4631691 |
| Stn42 | IV | 6107610 |
| | | |
| **LG2** | | |
| Pun285 | II | 14246054 |
| Pun96 | II | 14245722 |
| Pun292 | II | 17425185 |
| Pun84 | II | 17425185 |
| Pun250 | II | 18087385 |
| Stn259 | II | NA |
| Pun204 | II | 19809441 |

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| | | |
|---|---|---|
| Pun279 | II | 19809606 |
| Stn25 | II | 21161570 |
| **LG3** | | |
| Pun205 | III | 2013452 |
| Pun56 | III | 7248015 |
| Pun103 | III | 3918274 |
| Pun139 | V | 745819 |
| Pun157 | III | 8557342 |
| Pun106 | III | 12992253 |
| Pun188 | III | 13567224 |
| Pun227 | III | 15356028 |
| **LG4** | | |
| Gac4174 | IV | 11586126 |
| Stn361 | IV | 12790351 |
| Stn364 | IV | 12807468 |
| Stn433 | IV | 13143527 |
| Pun175 | IV | 8488036 |
| Pun94 | IV | 7820645 |
| Stn432 | IV | 16055450 |
| Pun89 | IV | 25298841 |
| Pun54 | IV | 9740859 |
| Pun316 | IV | 5979902 |
| Pun334 | IV | 5979484 |
| Pun109 | IV | 3461291 |
| Pun325 | IV | 18472454 |
| Pun95 | IV | 29653393 |
| **LG5A** | | |
| Pun178 | V | 10649677 |
| Pun112 | V | 1293256 |
| Pun19 | V | 6758830 |
| Pun286 | V | 1893130 |
| Pun51 | V | 3862434 |
| Pun77 | V | 5976177 |
| Pun128 | V | 4727346 |
| Pun304 | V | 4374934 |
| **LG5B** | | |
| Stn289 | V | 588714 |
| Pun275 | V | 745819 |

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|---|---|---|
| **LG6A** | | |
| Stn434 | VI | 7286525 |
| Pun169 | VI | 8985662 |
| Stn435 | VI | 11292179 |
| **LG6B** | | |
| Pun197 | VI | 15572597 |
| Stn436 | VI | 16171983 |
| **LG7A** | | |
| Pun98* | VII | 245926 |
| Pun323 | VII | 83643 |
| Pun61 | VII | 1183661 |
| Pun256 | VII | 1183661 |
| Pun214 | VII | 2893656 |
| Pun299* | VII | 6469786 |
| Pun110 | VII | 9813897 |
| Stn71* | VII | 7478503 |
| **LG7B** | | |
| Stn81* | VII | 26449238 |
| Stn445 | VII | *Pitx1* BAC |
| Pun319 | VII | *Pitx1* intron |
| Pun78 | many | |
| Stn431 | VII | *Pitx1* BAC |
| **LG8** | | |
| Pun68 | VIII | 6224460 |
| Pun333 | VIII | 3868882 |
| Stn85 | VIII | 1770045 |
| Pun150 | VIII | 10185898 |
| Pun290 | VIII | 11887975 |
| Pun184 | VIII | 7016002 |
| Stn440 | XIX | 7780014 |
| Pun207 | VIII | 13561575 |
| Pun138 | VIII | 15373500 |
| Pun136 | VIII | 16757299 |
| Stn95 | VIII | 17370467 |
| **LG9A** | | |
| Pun86 | IX | 1596473 |
| Stn108 | IX | 9534952 |
| Pun238 | IX | 418496 |



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|---|---|---|
| Stn102 | IX | 13727189 |
| **LG9B** | | |
| Pun245 | IX | 18513334 |
| Pun257 | no hits | |
| **LG10** | | |
| Pun144 | X | 3038567 |
| Pun156 | X | 4760712 |
| Pun63 | X | 8582358 |
| Pun291 | X | 8581800 |
| Pun221 | X | 5213183 |
| Pun309 | X | 8039801 |
| Stn211 | X | 6169732 |
| Pun42 | X | 10254491 |
| Pun312* | X | 2760918 |
| Pun147 | X | 1264102 |
| Pun16 | X | 2193902 |
| **LG11A** | | |
| Pun307 | XI | 3314586 |
| Pun228 | XI | 6089953 |
| Pun185 | XI | 6264140 |
| Pun274 | XI | 7270896 |
| Pun263 | XI | 7893784 |
| Pun183 | XI | 7418791 |
| Pun230 | XI | 12896222 |
| Pun294 | XI | 14190954 |
| Pun93 | XI | 15088893 |
| **LG11B** | | |
| Pun269 | XI | 1043073 |
| Pun158 | XI | 3751964 |
| **LG12** | | |
| Stn276 | XII | 9516858 |
| Stn287 | XII | 9516581 |
| Pun67* | XII | 8475636 |
| Pun300* | XII | 13240670 |
| Pun7 * | XII | 8475019 |
| Pun234* | XII | 15612922 |
| Pun81* | sc54 | 139500 |
| Pun255* | XII | 4778536 |

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|---|---|---|
| Pun2 * | XII | 12276617 |
| Pun99* | XII | 5576441 |
| Stn144 * | XII | 11036972 |
| Pun65 | XII | 11979558 |
| Pun116 | XII | 14193816 |
| **LG13** | | |
| Pun192* | XIII | 8108921 |
| Pun18 * | XIII | 6212413 |
| Pun163* | XIII | 6213467 |
| Pun200 | XIII | 10906831 |
| Pun167 | XIII | 6515101 |
| Pun45 | XIII | 13652251 |
| Pun220 | no hits | |
| Pun26 | XIII | 14365608 |
| Pun182 | XIII | 15491846 |
| Stn155 | XIII | 16102101 |
| Pun115 | XIII | 16909448 |
| Pun171 | XIII | 16909448 |
| Pun47 | XIII | 17018388 |
| Pun20 | XIII | 10906831 |
| Pun173 | XIII | 17675134 |
| Pun97 | XIII | 18472513 |
| Pun201 | XIII | 19629395 |
| Pun235 | XIII | 19339536 |
| Pun254* | sc200 | 37331 |
| **LG14A** | | |
| Pun242 | XVI | 6217516 |
| Pun60 | no hits | |
| Pun44 | XIV | 3969287 |
| Pun327 | XIV | 8379643 |
| **LG14B** | | |
| Pun203 | XIV | 2264289 |
| Pun102 | XIV | 2848067 |
| Stn166 | XIV | 8491339 |
| Pun324 | XIV | 13535451 |
| Stn371 | I | NA |
| **LG15A** | | |
| Pun141 | XV | 2092699 |
| Pun224 | no hits | |

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| **LG15B** | | |
|---|---|---|
| Pun288 | XV | 6209371 |
| Pun159 | XV | 14913510 |
| Pun330 | XV | 8275179 |
| Pun53 | XV | 9265667 |
| Pun293 | XV | 10691970 |
| Pun22 | XV | 7560619 |
| **LG16** | | |
| Pun72 | XVI | 4596098 |
| Stn315* | XVI | 4544301 |
| Pun211 | XVI | 11037756 |
| Pun328 | sc69 | 101867 |
| Pun217 | IX | 7039524 |
| Pun122 | XVI | 17240548 |
| Pun6 | XVI | 17210652 |
| Pun180 | XVI | 17218156 |
| Pun210 | XVI | 17218844 |
| Pun261 | XVI | 15144293 |
| Stn294 | XVI | 14888733 |
| **LG17** | | |
| Pun301 | XVII | 4853449 |
| Pun193* | XVII | 1759093 |
| Pun124 | I | 8817318 |
| Pun52 | no hits | |
| Pun212 | XVII | 192048 |
| Pun66 | XVII | 1287801 |
| Stn316 | XVII | 1513741 |
| Pun233 | no hits | |
| Pun196* | sc89 | 417125 |
| **LG18** | | |
| Pun13 | XVIII | 3558622 |
| Pun153 | no hits | |
| Pun260 | XVIII | 7198653 |
| **LG19** | | |
| Pun75 | XIX | 315554 |
| Stn441 | XIX | 7407774 |
| Pun48 | XIX | 16007354 |
| Pun209 | XIX | 11247610 |

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|---|---|---|
| Stn444 | XIX | 7047694 |
| Stn443 | XIX | 7019934 |
| Stn442 | XIX | 6937054 |
| Pun339 | no hits | |
| Stn194 | XIX | 12275340 |
| Pun130 | XIX | 19230879 |
| Stn186 | XIX | 1942745 |
| Pun268 | XIX | 10890440 |
| Pun117 | XIX | 17734849 |
| Pun168 | XIX | 10324618 |
| **LG20A** | | |
| Pun162* | XX | 7386639 |
| Pun149 | no hits | |
| Pun187 | XX | 2708397 |
| Pun322 | XX | 9712659 |
| Pun315 | XX | 9147709 |
| **LG20B** | | |
| Pun25 | XX | 607686 |
| Pun108 | sc229 | 64878 |
| Stn213 | XX | 14682616 |
| **LG21** | | |
| Pun114 | XXI | 3030175 |
| Pun132 | XXI | 5487078 |
| Pun177 | XXI | 5298431 |
| Pun148 | XXI | 1320705 |
| Pun305 | XXI | 7717593 |
| Pun135 | XXI | 7717593 |

* Marker added in the third round of analysis in JoinMap [8], but not used in the (second round) linkage map in Figure S1 or for QTL analysis.

** Sequence containing microsatellite did not produce significant BLAST hit; E-value is for reverse read off of same clone.

**Table S2. Summary of QTL and phenotypic means for pelvic traits.**

| Trait | LG | Marker | LOD | PVE (%) | Genotype N1A1 | N1A2 | N2A1 | N2A2 | Significant difference |
|---|---|---|---|---|---|---|---|---|---|
| Complete versus absent pelvis | 4 | Pun316 | 82.16 | NA | | | | | |
| Ascending branch height, left side | | | | | | | | | |
| *All fish* | 4 | Pun316 | 29.62 | 67.9 | 0.583 ± 0.106 | -0.581 ± 0.127 | 0.807 ± 0.093 | -0.761 ± 0.050 | A1 vs. A2*** |
| *Fish with pelvis* | 1A | Pun145† | 4.65 | 26.0 | -0.248 ± 0.070 | 0.167 ± 0.096 | -0.044 ± 0.108 | 0.152 ± 0.066 | A1 vs. A2** |
| Ascending branch height, right side | | | | | | | | | |
| *All fish* | 4 | Pun316 | 32.68 | 71.5 | 0.785 ± 0.127 | -0.765 ± 0.134 | 0.962 ± 0.098 | -0.917 ± 0.072 | A1 vs. A2*** |
| Pelvic girdle length, left side | | | | | | | | | |
| *All fish* | 4 | Pun94 | 53.26 | 87.0 | 1.77 ± 0.118 | -1.85 ± 0.128 | 2.02 ± 0.129 | -1.79 ± 0.162 | A1 vs. A2*** |
| *Fish with pelvis* | 1A | Pun252† | 10.09 | 33.2 | 0.357 ± 0.142 | -0.368 ± 0.089 | | | A1 vs. A2**** |
| Pelvic girdle length, right side | | | | | | | | | |
| *All fish* | 4 | Pun316 | 52.18 | 86.5 | 1.96 ± 0.153 | -1.82 ± 0.195 | 2.05 ± 0.125 | -2.03 ± 0.130 | A1 vs. A2*** |
| Pelvic spine length, left side | | | | | | | | | |
| *All fish* | 4 | Pun316 | 43.20 | 80.7 | 1.03 ± 0.094 | -0.856 ± 0.115 | 0.890 ± 0.064 | -0.949 ± 0.077 | A1 vs. A2*** |
| Pelvic spine length, right side | | | | | | | | | |
| *All fish* | 4 | Pun316 | 50.74 | 85.7 | 1.05 ± 0.093 | -0.887 ± 0.071 | 0.862 ± 0.078 | -0.919 ± 0.043 | A1 vs. A2*** |

Phenotypic means (± standard error) are listed for each genotype at the marker with the peak LOD score for each trait. All phenotypic means are expressed as residuals of a regression on standard length. Phenotypic means for each allele were also analyzed, and significant mean phenotypic

differences between alleles from the same parent are noted in the “Significant difference” column: **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. See Table S4 for allelic means for traits listed.

† Detected using restricted multiple QTL mapping with LG4 marker as co-factor.

Abbreviations: LG, linkage group; PVE, percent variance explained; N1, N2: Northwest Territories (female parent) alleles; A1, A2: Alaskan (male parent) alleles.

**Table S3. Summary of QTL and phenotypic means for sex-linked traits.**

| Trait | LG | Marker | LOD | PVE (%) | Genotype | | | | Significant difference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | N1A1 | N1A2 | N2A1 | N2A2 | |
| Sex determination | 12 | Pun65 | 45.64 | NA | | | | | |
| Lateral plates, left side | 12 | Stn276 | 9.17 | 30.1 | 5.833 ± 0.155 | 7.231 ± 0.320 | 6.758 ± 0.185 | 8.318 ± 0.380 | N1 vs. N2*, A1 vs. A2*** |
| Lateral plates, right side | 12 | Stn287 | 8.55 | 28.4 | 5.739 ± 0.157 | 6.870 ± 0.379 | 6.743 ± 0.206 | 8.200 ± 0.374 | N1 vs. N2*** A1 vs. A2** |
| Head length | 12 | Pun65 | 8.22 | 27.1 | -0.209 ± 0.060 | 0.120 ± 0.089 | -0.168 ± 0.076 | 0.384 ± 0.055 | A1 vs. A2*** |
| Upper jaw length | 12 | Pun116 | 15.43 | 44.8 | -0.116 ± 0.022 | 0.156 ± 0.023 | -0.088 ± 0.024 | 0.087 ± 0.026 | A1 vs. A2*** |
| | 21 | Pun114† | 4.69 | 9.2 | -0.067 ± 0.036 | 0.017 ± 0.030 | -0.027 ± 0.029 | 0.073 ± 0.029 | A1 vs. A2* |
| Lower jaw length | 12 | Stn276 | 8.64 | 28.3 | -0.045 ± 0.027 | 0.604 ± 0.023 | -0.078 ± 0.025 | 0.103 ± 0.040 | A1 vs. A2*** |
| Orbit diameter | 12 | Pun116 | 8.06 | 27.4 | -0.083 ± 0.019 | 0.051 ± 0.024 | -0.039 ± 0.023 | 0.118 ± 0.024 | A1 vs. A2*** |
| | 19 | Stn186† | 5.02 | 13.3 | 0.088 ± 0.033 | 0.033 ± 0.025 | -0.026 ± 0.032 | -0.053 ± 0.025 | N1 vs. N2** |
| Pectoral fin length | 12 | Pun116 | 9.55 | 31.3 | 0.211 ± 0.069 | -0.394 ± 0.076 | 0.282 ± 0.076 | -0.224 ± 0.095 | A1 vs. A2*** |

Phenotypic means (± standard error) are listed for each genotype at the marker with the peak LOD score for each trait. All phenotypic means except mean lateral plates counts are expressed as residuals of a regression on standard length. Phenotypic means for each allele were also analyzed, and significant mean phenotypic differences between alleles from the same parent are noted in the "Significant difference" column: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Abbreviations follow Table S2. See Table S5 for allelic means for traits listed.

† Detected using restricted multiple QTL mapping with LG12 marker as co-factor.

**Table S4. Summary of pelvic QTL and phenotypic means for each allele.**

| Trait | LG | Marker | LOD | PVE (%) | Alleles | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | N1 | N2 | A1 | A2 |
| Complete versus absent pelvis | 4 | Pun316 | 82.16 | NA | | | | |
| Ascending branch height, left side | | | | | | | | |
| *All fish* | 4 | Pun316 | 29.62 | 67.9 | 0.001 ± 0.118 | 0.012 ± 0.107 | 0.716 ± 0.071*** | -0.689 ± 0.060 |
| *Fish with pelvis* | 1A | Pun145† | 4.65 | 26.0 | -0.074 ± 0.067 | 0.082 ± 0.059 | -0.175 ± 0.061** | 0.158 ± 0.055 |
| Ascending branch height, right side | | | | | | | | |
| *All fish* | 4 | Pun316 | 32.68 | 71.5 | 0.010 ± 0.145 | 0.010 ± 0.128 | 0.890 ± 0.078*** | -0.856 ± 0.069 |
| Pelvic girdle length, left side | | | | | | | | |
| *All fish* | 4 | Pun94 | 53.26 | 87.0 | -0.152 ± 0.240 | 0.195 ± 0.281 | 1.890 ± 0.088*** | -1.828 ± 0.100 |
| *Fish with pelvis* | 1A | Pun252† | 10.09 | 33.2 | | | 0.357 ± 0.142**** | -0.368 ± 0.089 |
| Pelvic girdle length, right side | | | | | | | | |
| *All fish* | 4 | Pun316 | 52.18 | 86.5 | 0.073 ± 0.302 | -0.017 ± 0.260 | 2.017 ± 0.096*** | -1.945 ± 0.110 |
| Pelvic spine length, left side | | | | | | | | |
| *All fish* | 4 | Pun316 | 43.20 | 80.7 | 0.087 ± 0.156 | -0.042 ± 0.121 | 0.947 ± 0.054*** | -0.912 ± 0.065 |
| Pelvic spine length, right side | | | | | | | | |
| *All fish* | 4 | Pun316 | 50.74 | 85.7 | 0.084 ± 0.153 | -0.041 ± 0.115 | 0.941 ± 0.061*** | -0.906 ± 0.038 |

Phenotypic means (± standard error) listed for each allele at marker with the peak LOD score for each trait. All phenotypic means are expressed as residuals of a regression on standard length. Significant mean phenotypic differences from alternative allele from same parent are noted with asterisks: *, $p<0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

† Detected using restricted multiple QTL mapping with LG4 marker as co-factor.

Abbreviations: LG, linkage group; PVE, percent variance explained; N1, N2: Northwest Territories (female parent) alleles; A1, A2: Alaskan (male parent) alleles.

**Table S5. Summary of sex-linked QTL and phenotypic means for each allele.**

| Trait | LG | Marker | LOD | PVE (%) | Alleles | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | N1 | N2 | A1 | A2 |
| Sex determination | 12 | Pun65 | 45.64 | NA | | | | |
| Lateral plates, left side | 12 | Stn276 | 9.17 | 30.1 | 6.560 ± 0.206* | 7.382 ± 0.213 | 6.368 ± 0.139*** | 7.729 ± 0.256 |
| Lateral plates, right side | 12 | Stn287 | 8.55 | 28.4 | 6.304 ± 0.220*** | 7.350 ± 0.216 | 6.345 ± 0.153** | 7.563 ± 0.281 |
| Head length | 12 | Pun65 | 8.22 | 27.1 | -0.066 ± 0.056 | 0.080 ± 0.060 | -0.188 ± 0.048*** | 0.257 ± 0.054 |
| Upper jaw length | 12 | Pun116 | 15.43 | 44.8 | 0.011 ± 0.023 | -0.013 ± 0.021 | -0.100 ± 0.017*** | 0.124 ± 0.018 |
| | 21 | Pun114† | 4.69 | 9.2 | -0.022 ± 0.024 | 0.020 ± 0.021 | -0.044 ± 0.023* | 0.045 ± 0.021 |
| Lower jaw length | 12 | Stn276 | 8.64 | 28.3 | 0.010 ± 0.018 | -0.006 ± 0.023 | -0.064 ± 0.019*** | 0.080 ± 0.019 |
| Orbit diameter | 12 | Pun116 | 8.06 | 27.4 | -0.021 ± 0.017 | 0.029 ± 0.020 | -0.061 ± 0.015*** | 0.082 ± 0.018 |
| | 19 | Stn186† | 5.02 | 13.3 | 0.065 ± 0.022** | -0.042 ± 0.020 | 0.047 ± 0.026 | -0.013 ± 0.019 |
| Pectoral fin length | 12 | Pun116 | 9.55 | 31.3 | -0.061 ± 0.064 | 0.061 ± 0.068 | 0.245 ± 0.051*** | -0.314 ± 0.061 |

Phenotypic means (± standard error) listed for each allele at marker with the peak LOD score for each trait. All phenotypic means except mean lateral plates counts are expressed as residuals of a regression on standard length. Notations and abbreviations follow Table S4.

† Detected using restricted multiple QTL mapping with LG12 marker as co-factor.

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