

## **Supplemental Data**

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

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#### **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.

### **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

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](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**

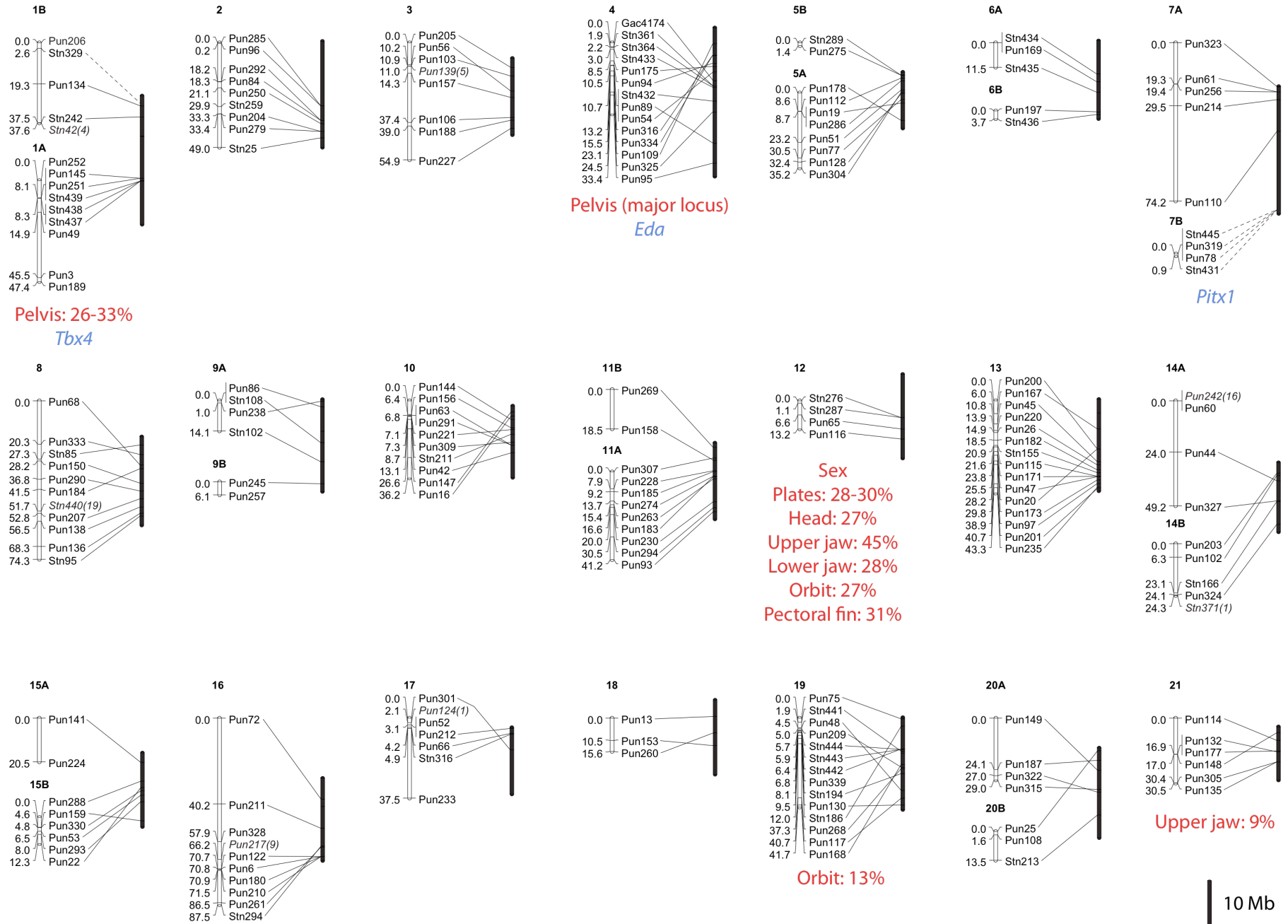
Skeletal measurements were performed using digital calipers under a dissecting microscope. Measurements included: standard length (from tip of upper lip to posterior edge of caudal peduncle), pelvic girdle length (from anterior tip of anterior process to posterior tip of posterior process), pelvic spine 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

(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.

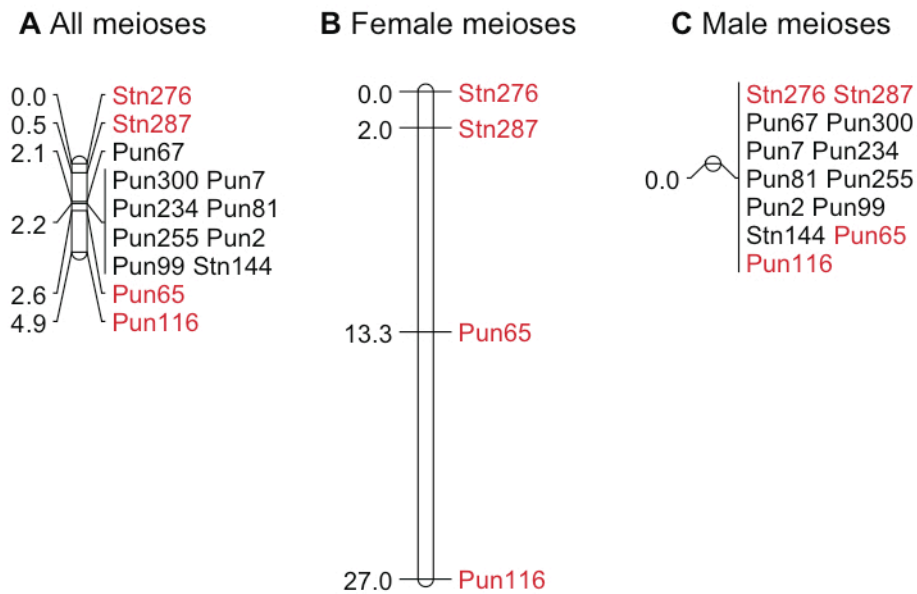
### **QTL analysis**

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

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.



**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 microsatellite 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.



**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.



**Table S1. Genomic locations of microsatellite markers used in this study.** Ninespine stickleback genomic fragments 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.

<b>Marker</b>	<b>Chromosome</b>	<b>Position (bp)</b>
<b>LG1A</b>		
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	
<b>LG1B</b>		
Pun206	many	
Stn329	I	NA
Pun134	I	2151610
Stn242	I	4631691
Stn42	IV	6107610
<b>LG2</b>		
Pun285	II	14246054
Pun96	II	14245722
Pun292	II	17425185
Pun84	II	17425185
Pun250	II	18087385
Stn259	II	NA
Pun204	II	19809441

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

**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	<i>Pitx1</i> BAC
Pun319	VII	<i>Pitx1</i> intron
Pun78	many	
Stn431	VII	<i>Pitx1</i> 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

Stn102	IX	13727189
<b>LG9B</b>		
Pun245	IX	18513334
Pun257	no hits	
<b>LG10</b>		
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
<b>LG11A</b>		
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
<b>LG11B</b>		
Pun269	XI	1043073
Pun158	XI	3751964
<b>LG12</b>		
Stn276	XII	9516858
Stn287	XII	9516581
Pun67*	XII	8475636
Pun300*	XII	13240670
Pun7 *	XII	8475019
Pun234*	XII	15612922
Pun81*	sc54	139500
Pun255*	XII	4778536

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	

**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

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				Significant difference
					N1A1	N1A2	N2A1	N2A2	
Complete versus absent pelvis	4	Pun316	82.16	NA					
Ascending branch height, left side									
<i>All fish</i>	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***
<i>Fish with pelvis</i>	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									
<i>All fish</i>	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									
<i>All fish</i>	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***
<i>Fish with pelvis</i>	1A	Pun252†	10.09	33.2	0.357 ± 0.142	-0.368 ± 0.089			A1 vs. A2****
Pelvic girdle length, right side									
<i>All fish</i>	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									
<i>All fish</i>	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									
<i>All fish</i>	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 ( $\pm$  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 ( $\pm$  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

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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								
<i>All fish</i>	4	Pun316	29.62	67.9	0.001 ± 0.118	0.012 ± 0.107	0.716 ± 0.071***	-0.689 ± 0.060
<i>Fish with pelvis</i>	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								
<i>All fish</i>	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								
<i>All fish</i>	4	Pun94	53.26	87.0	-0.152 ± 0.240	0.195 ± 0.281	1.890 ± 0.088***	-1.828 ± 0.100
<i>Fish with pelvis</i>	1A	Pun252†	10.09	33.2			0.357 ± 0.142****	-0.368 ± 0.089
Pelvic girdle length, right side								
<i>All fish</i>	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								
<i>All fish</i>	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								
<i>All fish</i>	4	Pun316	50.74	85.7	0.084 ± 0.153	-0.041 ± 0.115	0.941 ± 0.061***	-0.906 ± 0.038

Phenotypic means ( $\pm$  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 ( $\pm$  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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