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Supporting Online Material for

Adaptive Evolution of Pelvic Reduction in Sticklebacks by Recurrent Deletion of a *Pitx1* Enhancer

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Supporting Online Material

Material and Methods

Fish Collection and Husbandry. Sticklebacks were collected in minnow traps from locations described in table S1. Laboratory crosses were generated, as described (S*1*).

BAC Screening. BAC libraries, generated from a pelvic-complete stickleback population, Salmon River, BC, Canada (CHORI-213; Children's Hospital Oakland Research Institute, Oakland, CA, USA); and from a pelvic-reduced population, Paxton Lake (benthic form), BC, Canada (CHORI-215) (S*2*) were screened on high-density filters with radioactively-labeled oligonucleotide probes as described (S3-*4*) using sequences near *Pitx1:* -43kb-OVa 5'-CTCCCTGGATGGCTGGGAAACTGA-3', -43kb-OVb 5'-GGTCTCTGGTCCCGGCCTCCCTGG-3', +38kb-OVa 5'-TTCCGTAGTGTAGTGGTTATCACG-3', +38kb-OVb 5'-TCGCGTGTTAGGCGAACGTGATAA-3',-51kb-Ova 5'-GAGGAGTGGAAACGAAAGAGTGAG-3',-51kb-OVb 5'-ATAACATCTCCAGCGGCTCACTCT-3'. Positive clones were secondarily screened with microsatellite markers Stn446, Stn454, Stn474 and end sequenced with T7 and SP6 primers on an ABI3730xl automatic sequencer (Applied Biosystems, Foster City, CA, USA). BAC Clones CH213-164F21, CH213-118G22, CH215-217P05 and CH215-196J14 (Genbank accession numbers: GU130433-4, GU130436-7) were sequenced to completion as described (S*2*).

Allele-Specific Expression. Allele-specific expression of *Pitx1* was analyzed using FRIL as the control parent. All FRIL individuals show robust expression of the pelvic spines and girdle. Wild-caught females from FRIL were crossed to males from a pelvic-complete marine LITC population and the pelvic-reduced freshwater PAXB population. The progeny were raised to stage 29/30 (S*5*), a stage when *Pitx1* is strongly expressed in both head and pelvic regions (S*6-S8*). Larvae were anesthetized in 0.0003% tricaine (Ethyl 3- aminobenzoate methanesulfonate, Sigma, St. Louis, MO, USA). Heads and pelvic regions were dissected and placed individually in tubes. Tissue samples were immediately frozen and stored at –80 ºC. RNA was isolated from head and pelvis samples with the RNeasy Micro Kit (Qiagen, Valencia, CA, USA). The remainder of each larva was used for DNA isolation. We analyzed twenty larvae from each cross type. Allele specific RT-PCR was carried out with gene-specific primers, the forward primer was labeled with a 6-FAM fluorophore (Forward: 5'-CCCCTTCTCTTCAAGCTGAG-3'. Reverse: 5'-AGCTGCTGGCTGGTGAAG-3'). We distinguished the alleles using a six base-pair deletion in a GCG repeat, resulting in a poly-alanine polymophism in the poorly conserved N-terminal region of the *Pitx1* protein. The PCR product is approximately 250bp from spliced RNA, depending on the length of the GCG repeat. Any residual DNA in the samples would result in an approximately 2.2kb product that was not visible in our assays. RT-PCR reactions were done with the OneStep RT-PCR (Qiagen). Each 50 µl reaction contained: 16 µl water, 10 µl 5X Qiagen PCR buffer, 10 µl Q solution, 2 µl dNTPs, 3 µl each primer, 2 µl OneStep RT-PCR enzyme mix, and 4 µl RNA from pelvis samples or 2 µl RNA from head samples plus an additional 2 µl of water. To ensure accurate quantification of peak intensity, 5 µl aliquots were

removed during the extension stage every 5 cycles between 25 and 40 cycles. Of each 5 µl aliquot, 1 µl was mixed with 10 µl HiDi formamide (Applied Biosystems) and 0.25 µl of GeneScan 500 LIZ (Applied Biosystems) and analyzed with an ABI3730xl and GeneMapper v3.5 software (Applied Biosystems).
Reaction products were detectable in pelvis samples by cycle 30, robust at cycle 35 and off-scale by cycle 40. Head samples reached saturation by cycle 35 and were analyzed at cycle 30. For each sample, allele ratios were computed as follows: the peak height (arbitrary units of peak intensity) of the experimental allele (LITC, PAXB) was divided by the height of the control allele (FRIL).

Association Mapping. Thirty-six fluorescent-labeled markers (Genbank GF100639- GF100673, GF101813, table S2) were designed with Primer3 as described (S*1, S9*), with an average spacing of 8kb across a 259kb Pitx1 region derived from BAC clones CH213-164F21 (5') and CH213-118G22 (3'). Two populations segregating pelvic phenotypes, (PAXB/PAXL and WALC/WALR) were screened for alleles associated with pelvic reduction. In each case, 48 pelvic-reduced individuals and 48 pelvic-complete individuals were genotyped (Paxton Lake: Stn431, Stn445-9, Stn452-4, Stn456, Stn458, Stn462, Stn464, Stn471-2, Stn474, Stn47-9, Stn482; Wallace Lake: Stn336, Stn445, Stn448, Stn450-1, Stn453-7, Stn459- Stn470, Stn472-Stn480). Microsatellites were amplified and genotyped on an ABI3730xl sequencer with GeneMapper v3.7 software as described (S*1*). Differences in microsatellite allele frequency distributions were expressed in log(p-values) between the pelvic-complete and pelvic-reduced fish with a modified χ^2 test on a coalesced 2 x 2 contingency table (T4) as implemented by CLUMP (S*10*). The candidate region was delimited by haplotypes strongly associated with pelvic reduction, as determined by PHASE v2.1 (S*11*).

Transgenic Assays. Transgenic sticklebacks were generated by microinjection (S*12*) with the following modifications to the injection mount: a glass plate measuring 20 x 15 cm was used as the injection platform instead of a microfabricated injection chamber. Freshly fertilized embryos at one-cell stage were placed, with the blastodisc facing the microinjection needle, in the indentations between teeth of a 6" plaster saw blade (HILTI, Tulsa, OK, USA: #00374342, 6 teeth per inch) with an artists' paintbrush.
Plasmids were co-injected with tol2 transposon mRNA as described (S*12*). We synthesized mature *tol*2 mRNA by in vitro transcription using the mMessage mMachine SP6 kit (Ambion, Austin, TX, USA). For enhancer assays using EGFP as the reporter, we used fertilized embryos from either MATA or LITC pelvic-complete populations. For the functional rescue assays, we used fertilized embryos from the BEPA pelvic-reduced population. All larvae were raised under standard aquarium conditions to Swarup St 29/30 (S*5*), when pelvic bud development is initiated, for phenotyping. Larvae were anesthetized in 0.0003% w/v tricaine (Ethyl 3-aminobenzoate methanesulfonate, Sigma). Microscopic observation for EGFP and mCherry expression was conducted with a MZFLIII fluorescent microscope (Leica Microsystems, Bannockburn, IL) with GFP2, GFP3 and DsRed filters. Some larvae were also grown to adulthood for skeletal visualization by Alizarin Red staining, as described (S*1*).

pTol2[drHsp70:eGFP] (pBHR) vector. The *drHsp70* promoter from pHSP70-4-EGFP (S*13-14*) was released by digestion with *AvaI* and cloned into the *AgeI* site of pBH-mcs-YFP. EGFP was PCR amplified from pHSP70-4-EGFP with primers 5'-AAAATCGTCGACGGTCGCCACCATGGTGAGCAAGG-3' and 5'-AAAATCGATCGATTAAGATACATTGATGAGTTTGG-3'. The EGFP was then cloned into pBH mcs YFP using digestion with *SalI* and *ClaI*, replacing YFP as the fluoroflore, yielding the pBHR vector. Potential enhancer sequences were then PCR amplified, *NotI* digested, and cloned into pBHR at the *PspOMI* site.

Transgenic rescue vector. Individual fragments matching *Pitx1* exons were PCR amplified with primers pairs 5'-AAAACTGTCGACATGGAGCTAAATCTTTCGAGCAAACAAAACGTCATTTCCGGCGCGGACGCAGGTGGGATGAATTCGGACC-3' (designated "primer 1") and 5'-CTCGGACGCGGAGCCGCCAGAGAAGGAGC-3'; 5'-AGCCGCCAGAGAAGGAGCGCGGCG-3' and 5'-CGGCGGTTCTTGAACCACACCCGCACTCGGGCCTCG-3'; 5'-GAGTGCGGGTGTGGTTCAAGAACCGCCG-3' and 5'-AAAACTGATCAGCTGTTGTACTGGCACGCG-3' (primer 5). For the PCR, we used Phusion Polymerase (Finnzymes, Woburn, MA) and the following cycling conditions: 98ºC for 5'30"; 98ºC for 30"; (58ºC for 20" -0.5ºC per cycle; 72ºC for 2') x 4 times; (98ºC for 30'; 58ºC for 20"; 72ºC for 2') x 29 times; 72ºC for 7'; 4ºC to hold. All fragments were amplified from pooled genomic DNA extracted from 4 individuals from Bear Paw Lake, AK. A second PCR amplification was performed to fuse the individual fragments into a *Pitx1* minigene with identical cycling conditions, but only with primers 1 and 5. The fused *Pitx1* minigene fragment was then cloned into pBHR using digestion with *SalI* and *ClaI*, replacing eGFP from the vector pBHR described above. With the exception of the signature sequence located in primer 1, the amplified region is identical in BEPA and SALR populations. Injected individuals were genotyped with primers 5'-CGGGCATTTACTTTATGTTGCT-3' (primer Geno-F) and 5'-GTCCGAATTCATCCCACCTG-3' (primer Geno-R) to confirm incorporation of the transgene, with the same overall PCR cycling conditions as above, but with only 30" for the elongation step.

***Pel* enhancers.** Fragments containing the *Pel*-2.5kb^{SALR} enhancer were cloned with a three-step process with primers 5' -AAACTGGCGGCCGCCAGTTATTAAGACGGTTATTAATGT-3' and 5'-AAGCACTAGTGTCAGGGCCGGACCATCTAACTC-3' (cut with *NotI* and *SpeI*); 5'-AACTGTCTAGACACACAGGAGATCTGAGGC-3' and 5'-AAAACTGAATTCTGACGCGGCGCTCCATCACCGAGCC-3' (cut with *EcoRI* and *XbaI*). Fragments amplified from BAC clone CH213-164F21, all with the PCR cycling conditions described above, were individually cloned into a pBluescript KS+ vector that has been modified with linkers 5'-TCGACCTCGAGGGGGGGCGGCCGCCCGGTACCCAGC-3' and 5'-GCTGGGTACCGGGCGGCCGCCCCCCCTCGAGGTCGA-3' to introduce an additional *NotI* site between *ApaI* and *KpnI*. Using the endogenous *NsiI* site within the insert itself, the released fragment bounded by *EcoRI* and *NsiI* was then cloned into the other construct linearized by *NsiI* and *NotI* site. This fused fragment was further released using *NotI* and cloned into the *PspOMI*

site in the pBHR vector for an enhancer assay. The *Pel*-Δ2.5kb^{PAXB} construct from PAXB (1868bp deletion) was directly amplified from genomic DNA with primers 5'-AAACTGGCGGCCGCCAGTTATTAAGACGGTTATTAATGT-3' and 5'-AAACTGCGGCCGCTGACGCGGCGCTCCATCACCGAGCC-3' with *NotI*-digested overhangs for the pBHR vector. The *Pel*-501bp^{SALR} insert was amplified from BAC clone CH213-164F21 as well, with primers 5'-AACTGTCTAGACACACAGGAGATCTGAGGC-3' and 5'-AAGCACTAGTCCTCAAATCTGCAGCGTT-3' (cut with *XbaI* and *SpeI*), and cloned into the aforementioned modified pBlueScript KS+ vector linearized with *SpeI*. The resulting plasmid was then re-cut with *SpeI*, now only re-opening on the original *SpeI* site. The concatenated insert was then released by *NotI* digest and cloned into pBHR at the *PspOMI* site.

Alignment against distantly related teleosts. Sequences from the stickleback *Pitx1* region (derived from an alignment of the 5' and the 3' SALR *Pitx1* BACs, (CH213-164F21 & CH213-118G22, aligned reference submitted as Genbank: GU130435) were aligned pairwise against tetraodon (TETRAODON 7, Apr 2003), fugu (FUGU 4.0, Apr 2006), medaka (assembly 200506, Jun 2005) and zebrafish sequences (Zv6, Mar 2006) using mLAGAN (S15) following repeat filtering with RepeatMasker (S*16*) with a custom stickleback-specific repeat library, or with default options for other teleost sequences. The alignments were then visualized using mVISTA (http://genome.lbl.gov/vista/index.shtml; S*15*)

Transcription factor binding sites. Predicted transcription factor binding sites in the *Pel*-501bp enhancer sequence (SALR) were identified using MatInspector (version 8.01, July 2009, Genomatix, Munich, Germany). Matrix Family Library Version 8.1 (June 2009) was used to identify elements belonging to the classes General Core Promoter Elements (parameters: 0.75 for the core score and matrix similarity setting "optimized") and Vertebrates (0.75/Optimized), with "family matches" option.

TwistFlex. Complete *Pitx1* SALR BAC sequence (see above) and individual chromosomes from the stickleback genome assembly were analyzed by TwistFlex (S*17*). The following parameters were used for all regions: window size=50bp; leap size=1bp; threshold value =13.7°; normalization value=10kb; discrete display value=5kb; maximal distance between adjacent peaks in a cluster=2kb; minimal number of peaks in a cluster=3.

SNP genotyping. Complete BAC sequences from SALR, PAXB and the initial stickleback genome assembly from a pelvic-reduced BEPA individual (Broad gasAcu1, Feb 2006), were pairwise aligned with mLAGAN (S*15*) following repeat filtering with RepeatMasker (S*16*) with a custom stickleback-specific repeat library. Single nucleotide polymorphisms (SNPs) were identified from these comparisons. In addition, a number of SNPs were ascertained by amplification and resequencing of portions of the *Pitx1* coding and intergenic regions from a worldwide panel of sticklebacks. 149 SNPs (table S3; Genbank: ss158145672-849) from the *Pitx1* region were genotyped on 399 individuals on the Illumina GoldenGate 1536 platform (Illumina, San Diego, CA, USA) according to manufacturer's protocols with the BeadStudio v3.0 (Illumina) software. The raw results were individually inspected with the BeadStudio v3.0 software to minimize clustering errors.

Signature of Selection. Since SNP ascertainment may bias towards overestimates of frequencies of common alleles and may cause spurious selective sweeps, only SNPs with at least 10% minor allele frequencies amongst the ancestral marine populations were included in our calculation for various signatures of selection. Population genetics statistics for $\theta\pi$ and Fay and Wu's H were calculated as described (S*18-20*). Fay and Wu's H calculation was based on a sliding window of 11 consecutive SNPs, similar to (S*19*). Therefore the resulting values should not be compared against those obtained from re-sequencing data, due to an inflation of segregating sites (S) from the use of concatenated SNP genotypes in the place of sequences.

Supporting figures

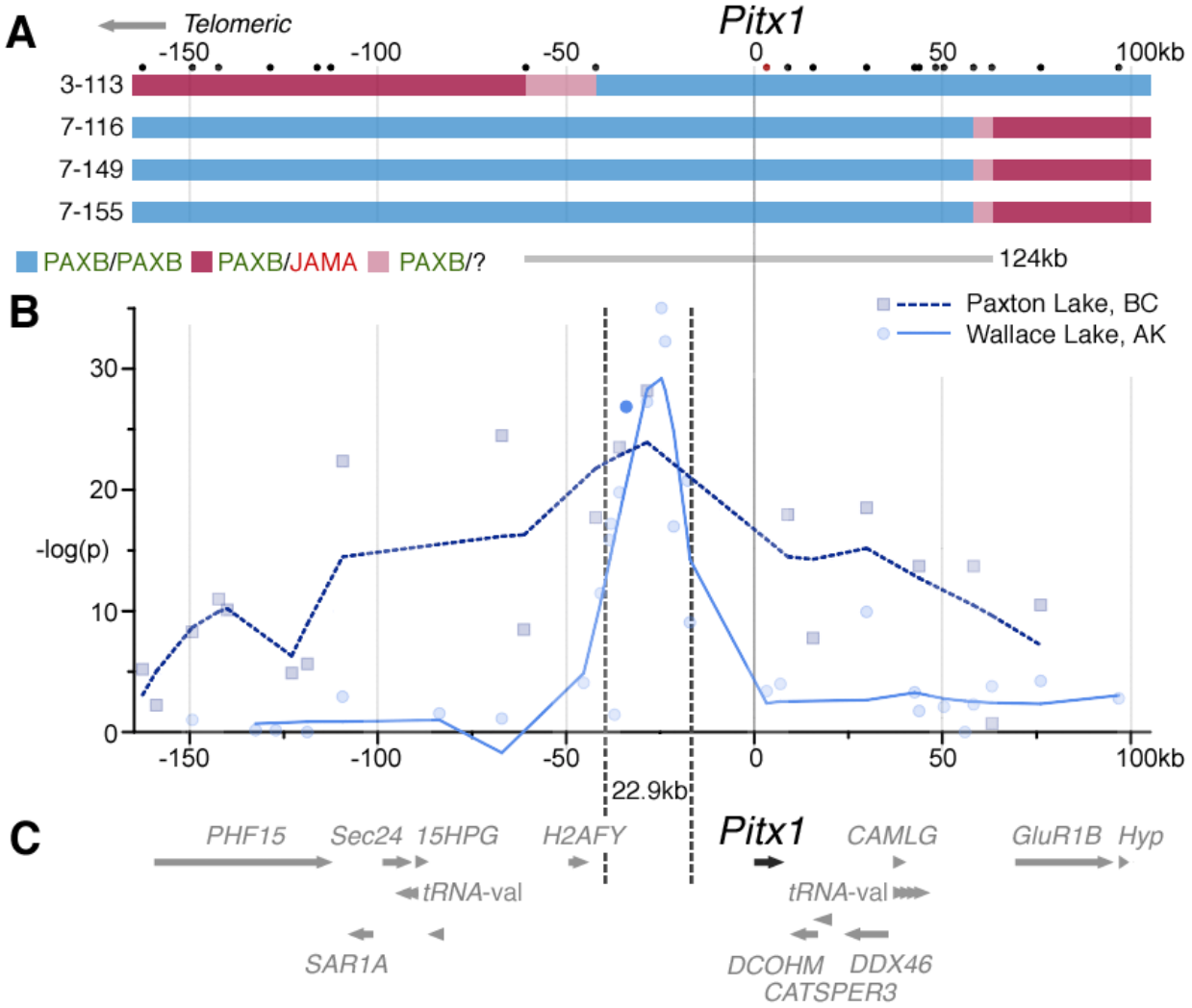


Fig. S1. Fine mapping of pelvic reduction to *Pitx1* upstream region. **(A)** Recombinant animals from a pelvic-complete (JAMA) by pelvic-reduced (PAXB) cross (S*1*, S*3*) define a minimal region that is always homozygous for PAXB alleles in F2 progeny completely missing a pelvis. Symbols: ●, microsatellite markers, red marker represents *Stn336*, peak marker for pelvic QTL (S*3*); blue bars, homozygous PAXB genotypes; red bars: PAXB/JAMA heterozygous genotypes; pink bars: transition zone between known genotypes; grey bar, candidate non-recombinant interval (124kb). **(B)** Association mapping of dimorphic pelvic phenotypes in Paxton and Wallace Lakes. Symbols (● &☐) indicate markers and phenotype-genotype association at different positions in 259kb interval surrounding *Pitx1*. Solid blue marker: *Stn463*. Log p-values on y-axis are based on a modified χ^2 test, T4 as implemented in CLUMP (S*10*). Dotted vertical lines delimit a strongly associated 23kb candidate region that maps to the 5' intergenic region of *Pitx1*, and was used for both enhancer studies and statistical analyses in Fig. 5C & S9F. **(C)** Names and locations of genes in region. Arrows indicate transcription orientation and abbreviations correspond to nomenclature of human orthologs where possible.

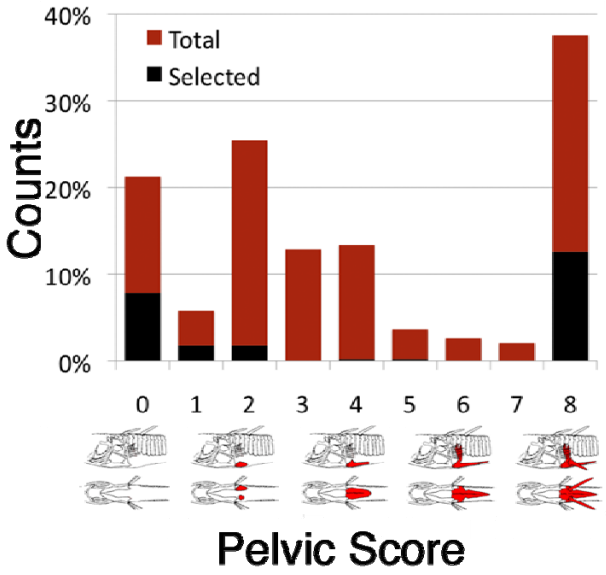
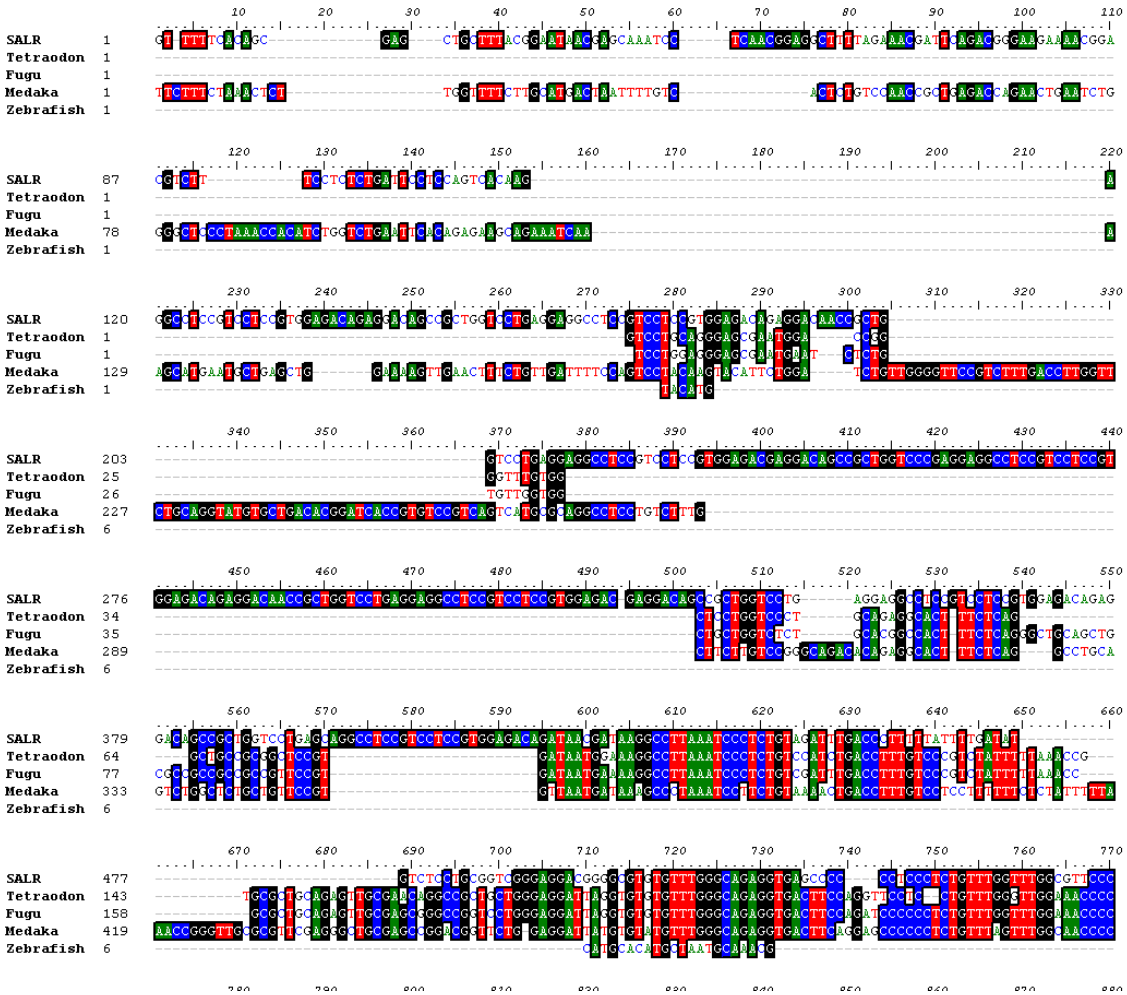


Fig. S2. Phenotypic distribution in Wallace Lake, AK. Pelvic scores were assessed according to (S*21*). Zero (0) is absent, pelvic size and complexity increases to the right, and 8 is complete. The 96 specimens were collected in Summer, 2005. Stickleback drawings are modified after (S*22*).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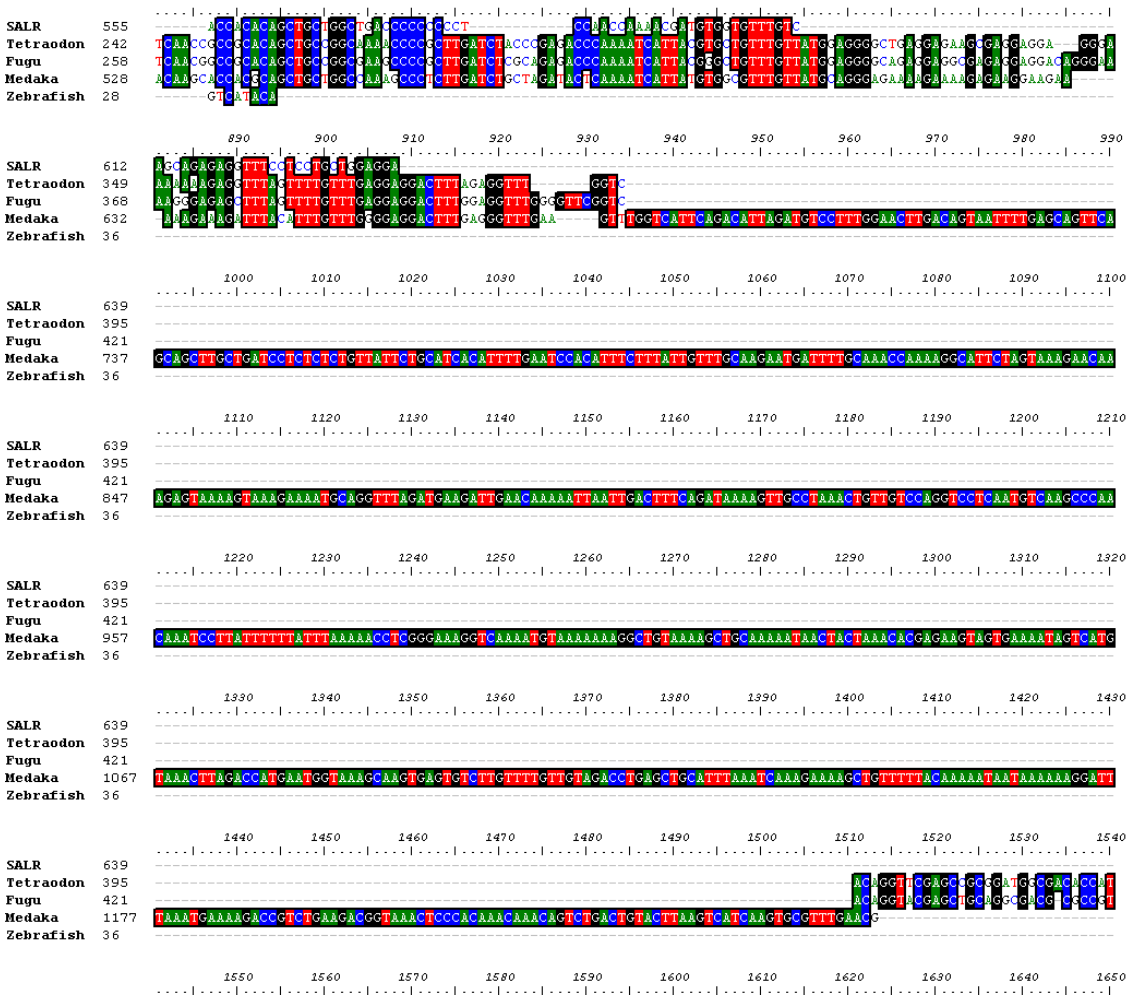
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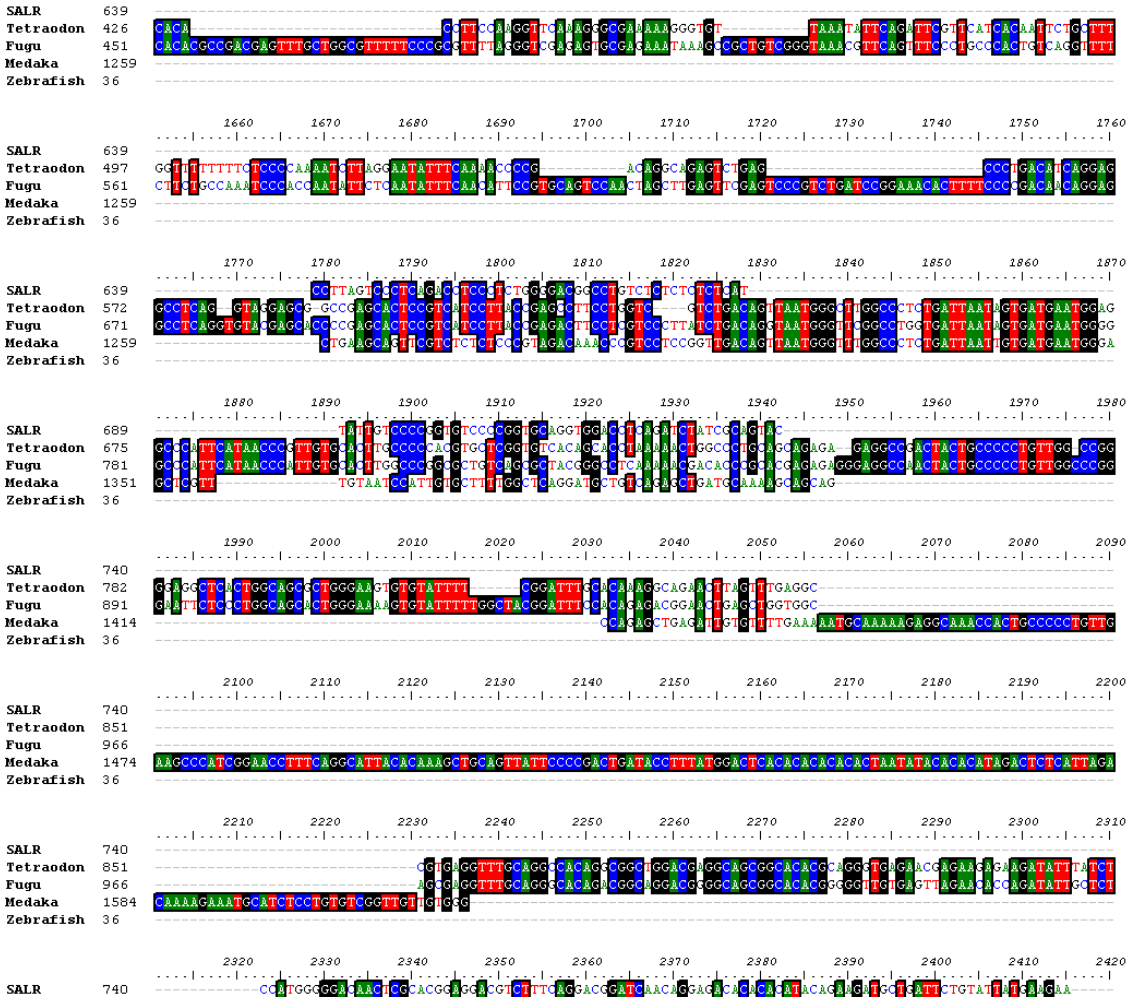
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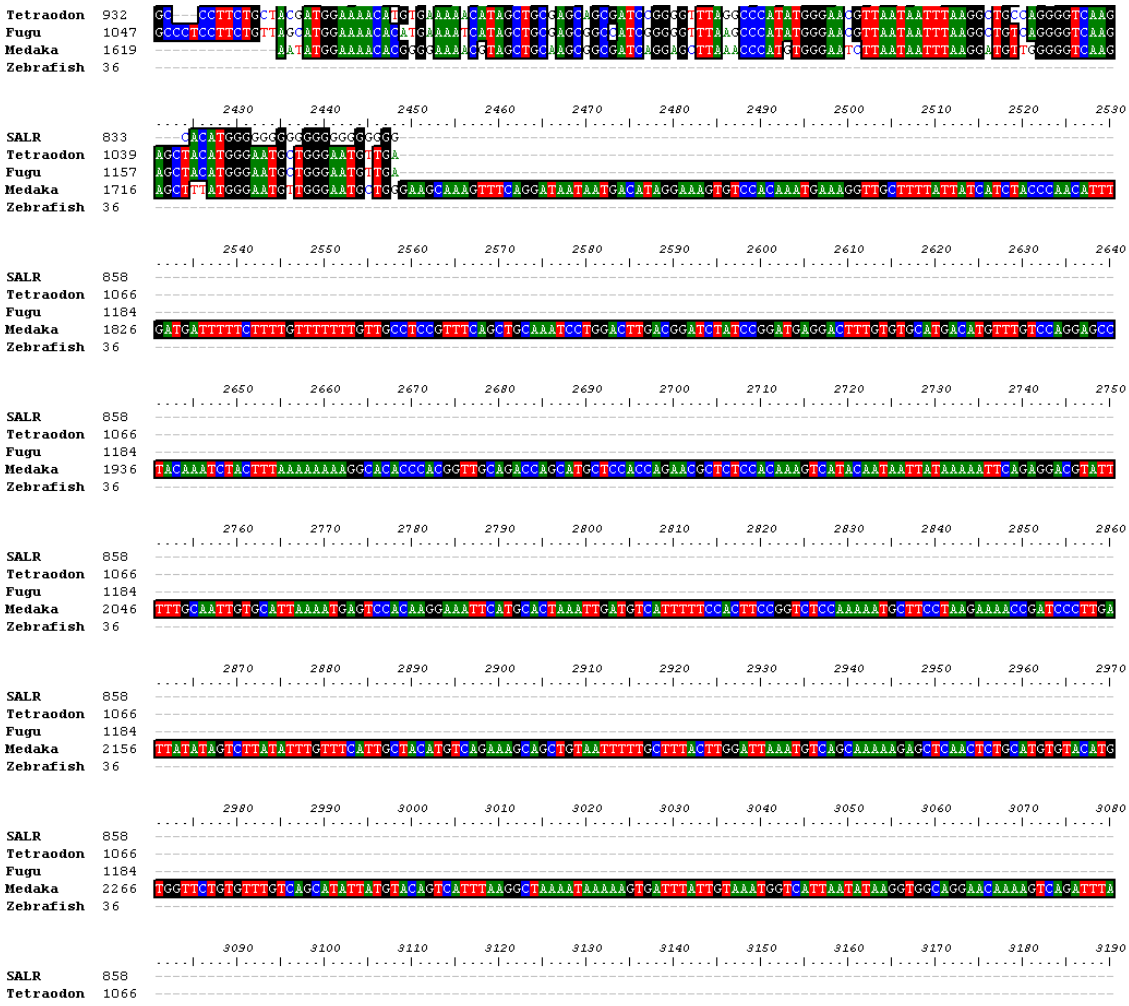
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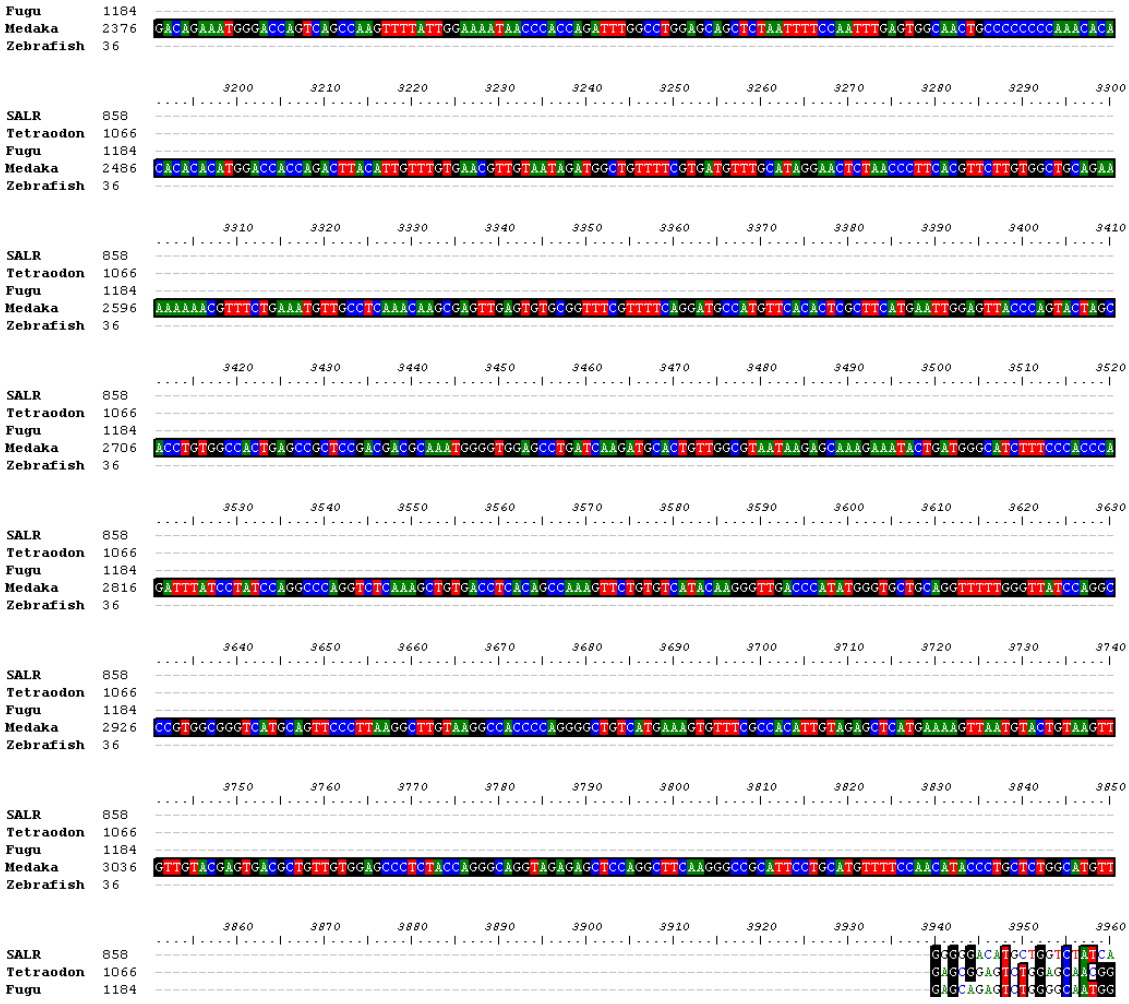
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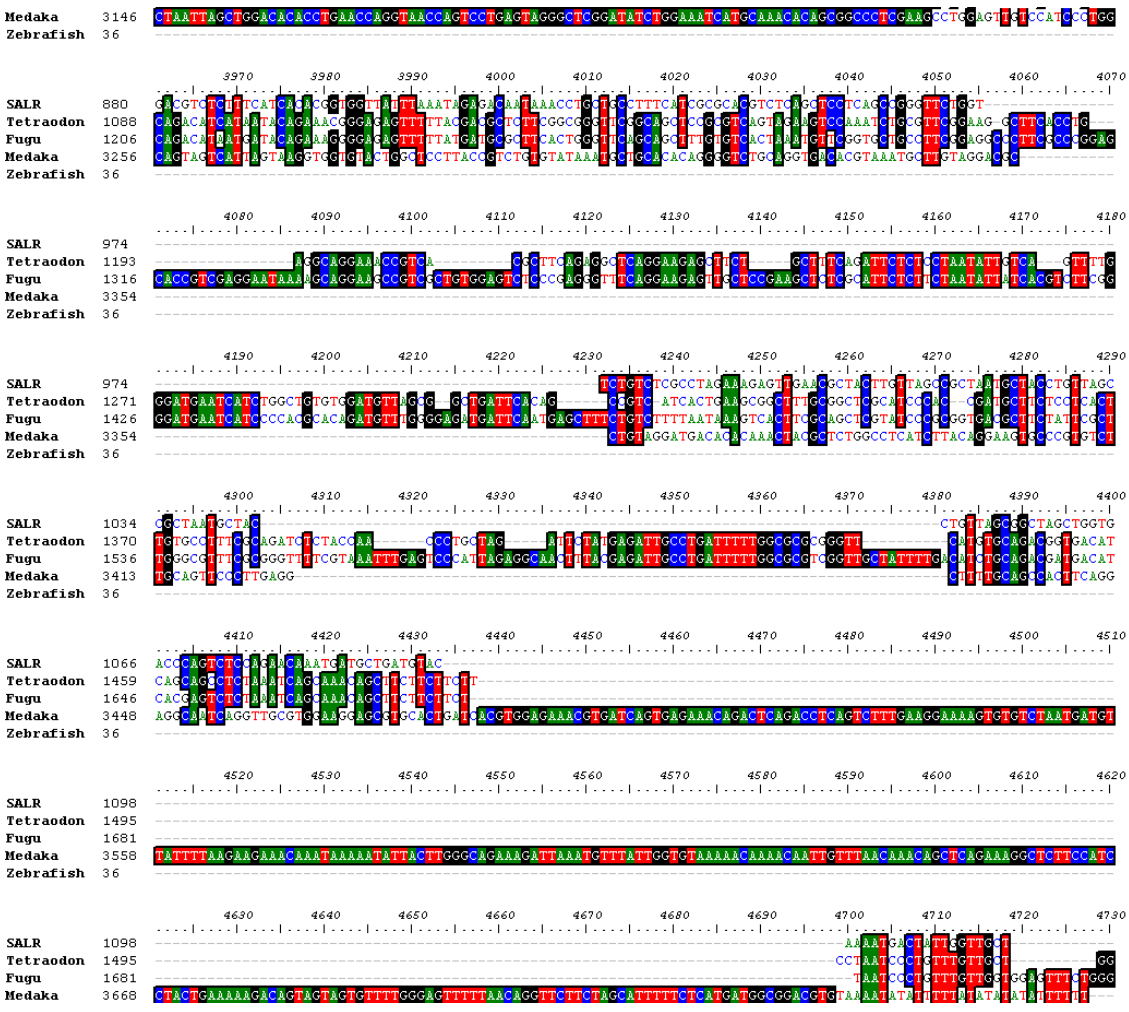
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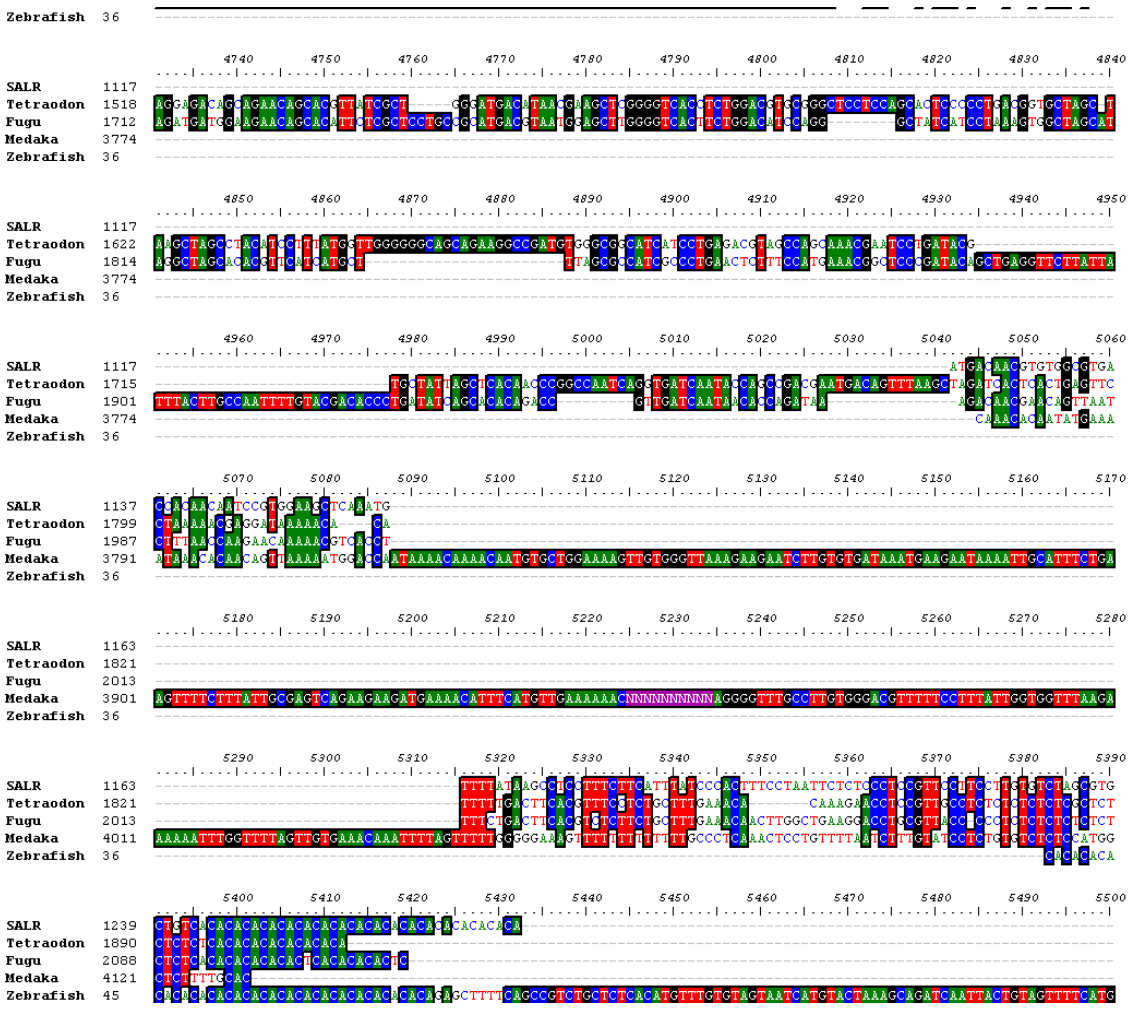
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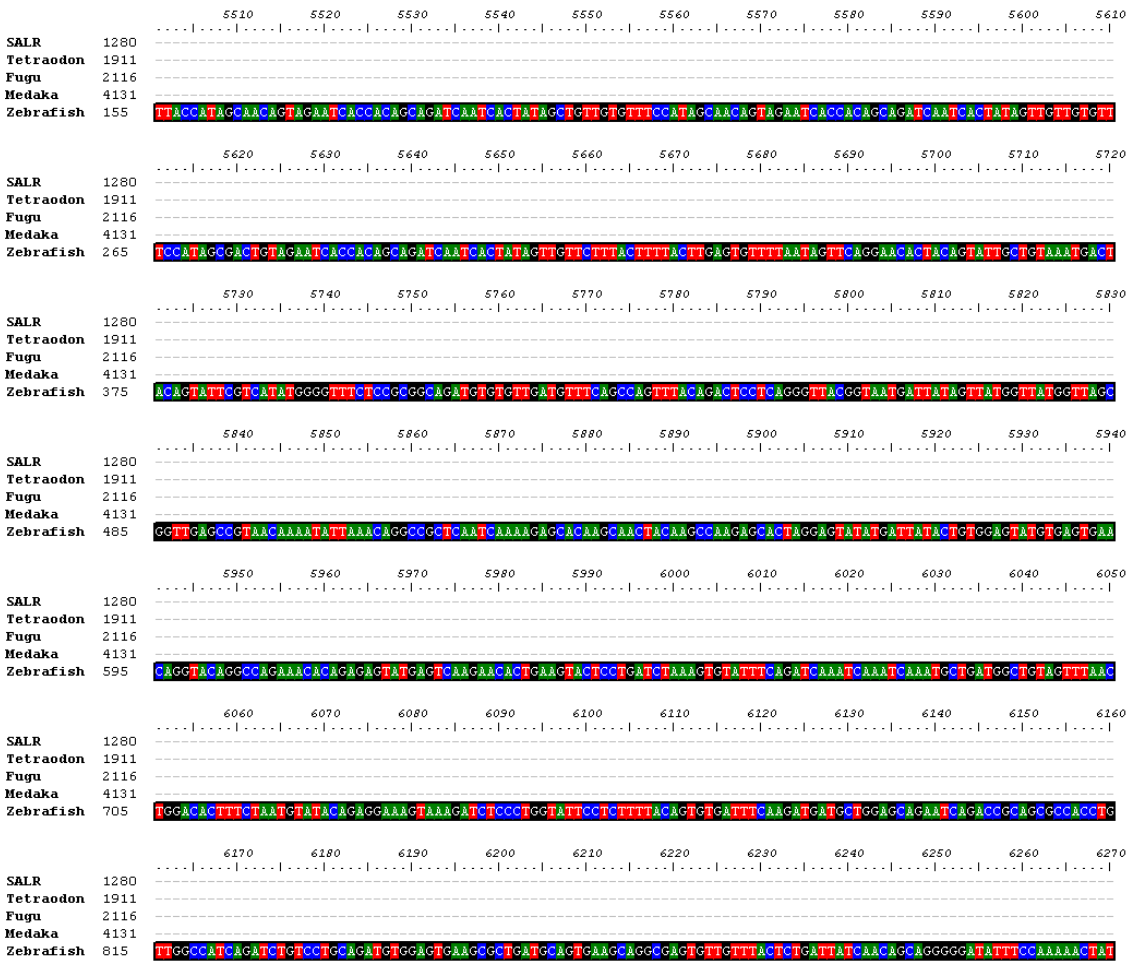
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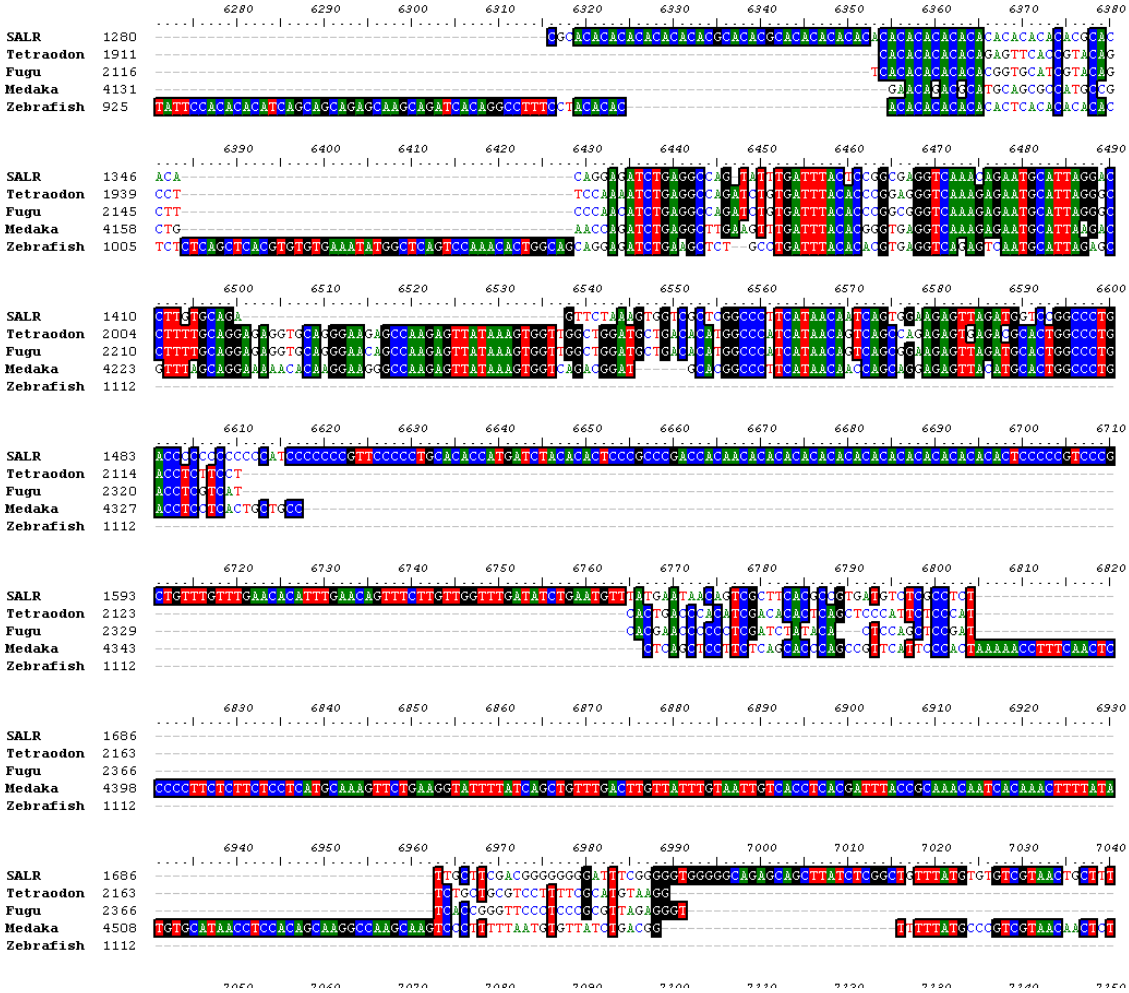
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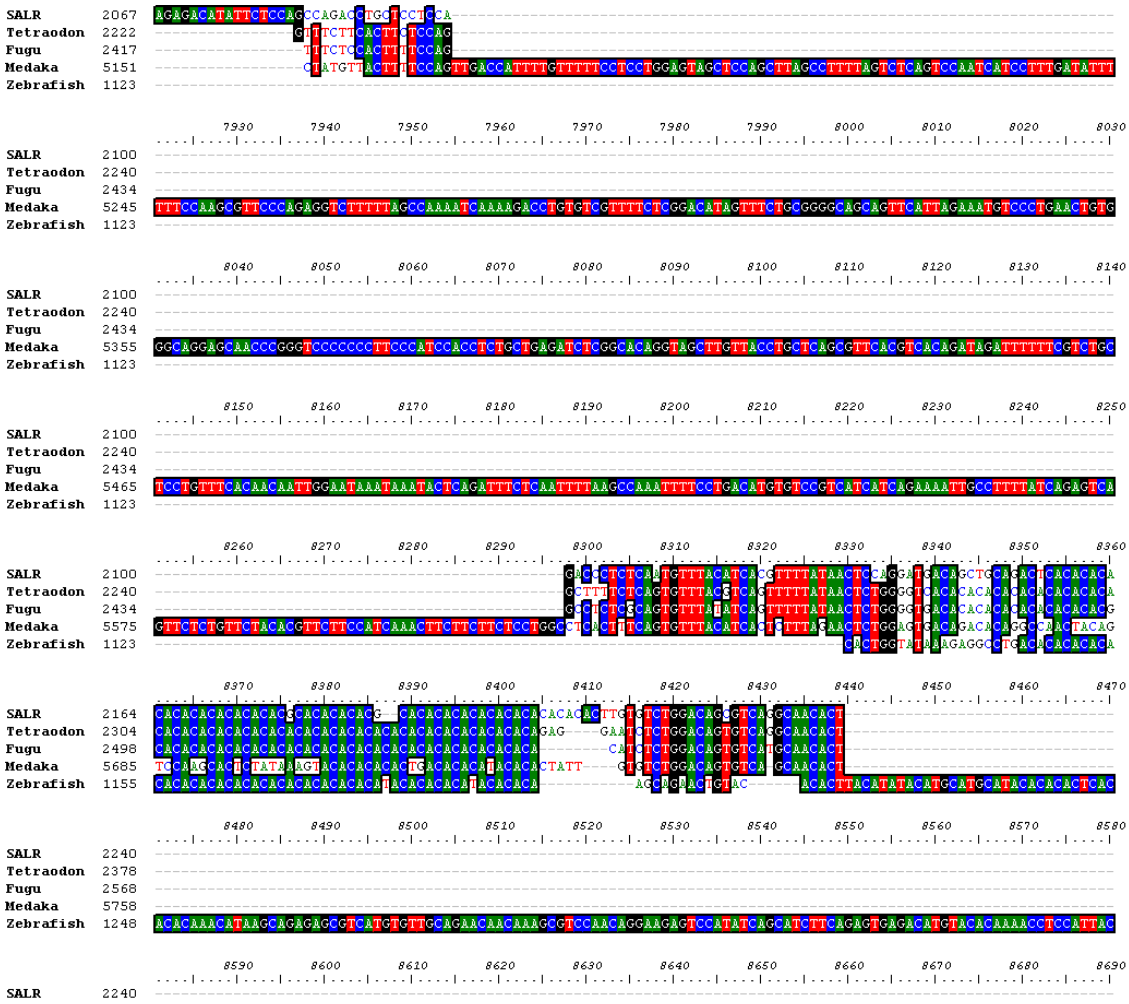
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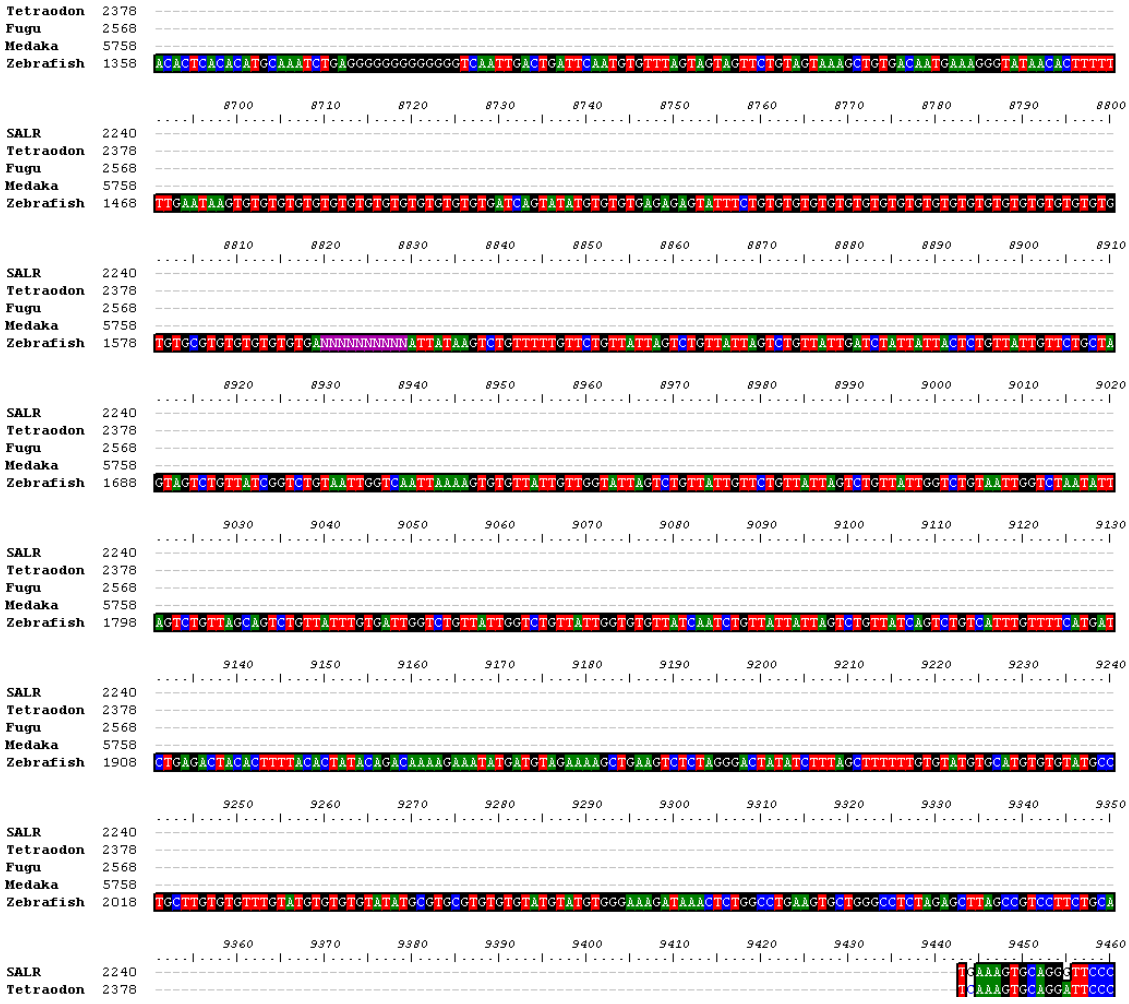
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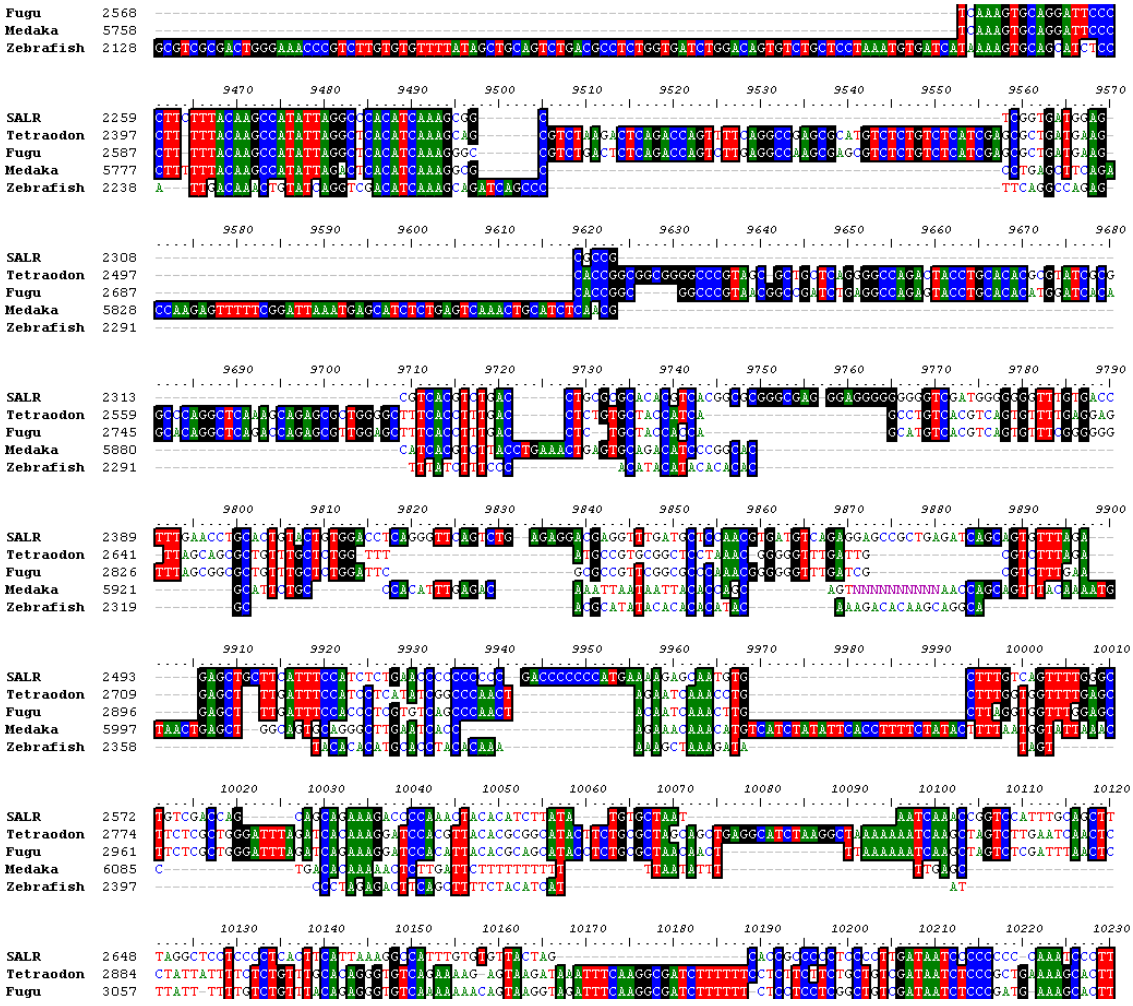
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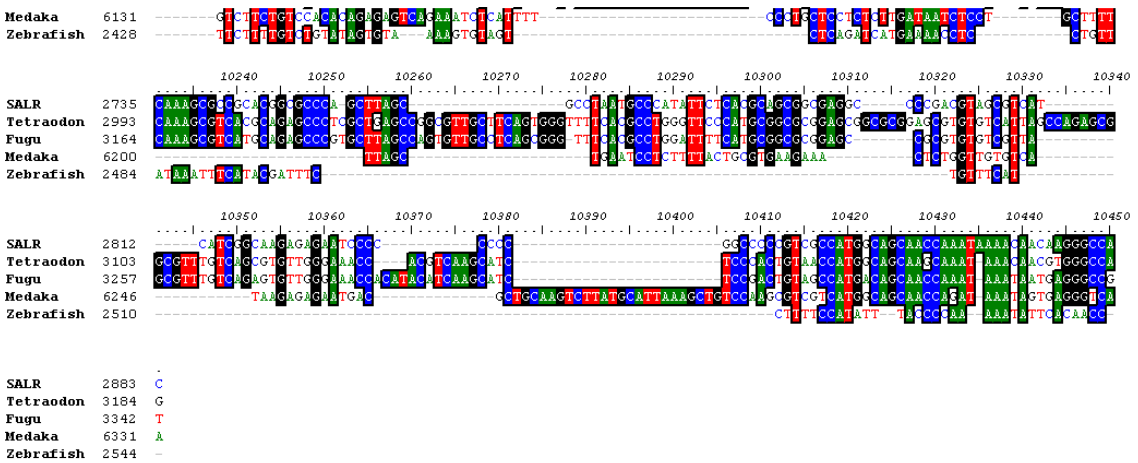


Fig. S3. Multiple sequence alignment between stickleback *Pel*-2.5kb^{SALR} sequence (Fig. S7) and the corresponding region from other teleost fish. Some sequences conserved between sticklebacks and zebrafish show sequence changes in pelvic-reduced pufferfish (fugu and tetraodon). The sequences conserved between distantly-related teleost species can not yet be aligned to most tetrapod genomes tested to date, including chicken, mouse, human, and various other mammals.

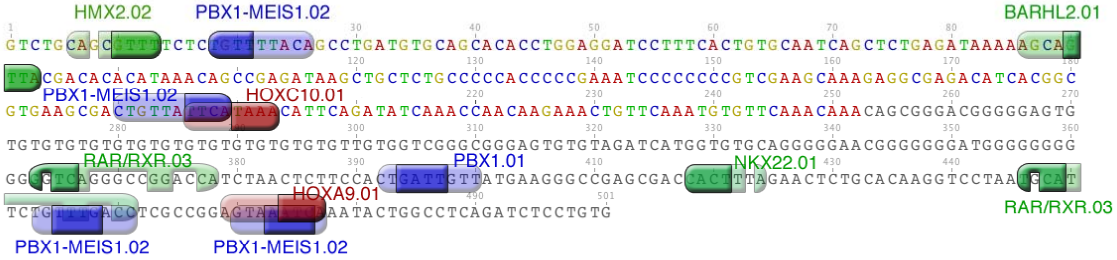


Fig. S4. Predicted binding sites of major developmental regulators located within the *Pel*-501bp (SALR) sequence**.** Predicted transcription factor binding sites are indicated by their names and bars above the sequence. The wider bars indicate sequences matching the position weight matrices as predicted by MatInspector (see methods). Strong bars indicate the core of the matrix. Greyed out sequences indicate overlap with the 488bp area shared between pelvic-reduced populations (see Fig. 4A, S7).

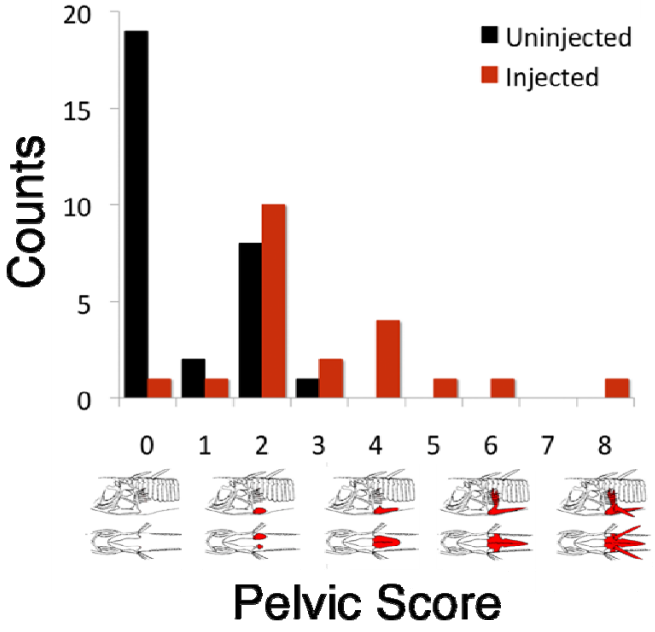


Fig. S5. Distribution of pelvic phenotypes in injected sticklebacks and uninjected control siblings. Transgenic animals were generated as described in Methods, and pelvic scores were assessed according to (S*21*).

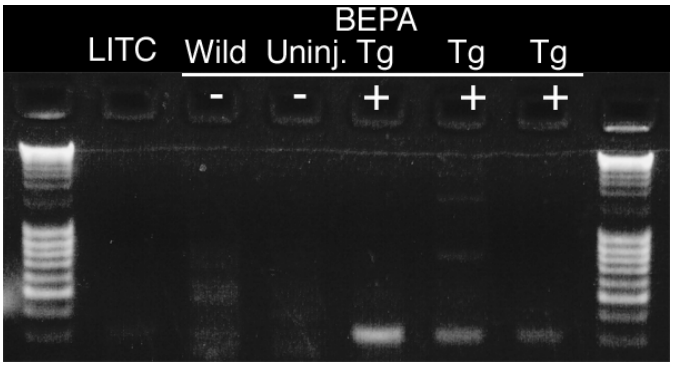


Fig. S6. PCR validation of transgenesis. Primers Geno-F and Geno-R (see Transgenic Rescue Vector in methods) were used to amplify the fragment containing the transgenic *Pitx1* minigene. The transgene (Tg+) yields a product of ~300bp.

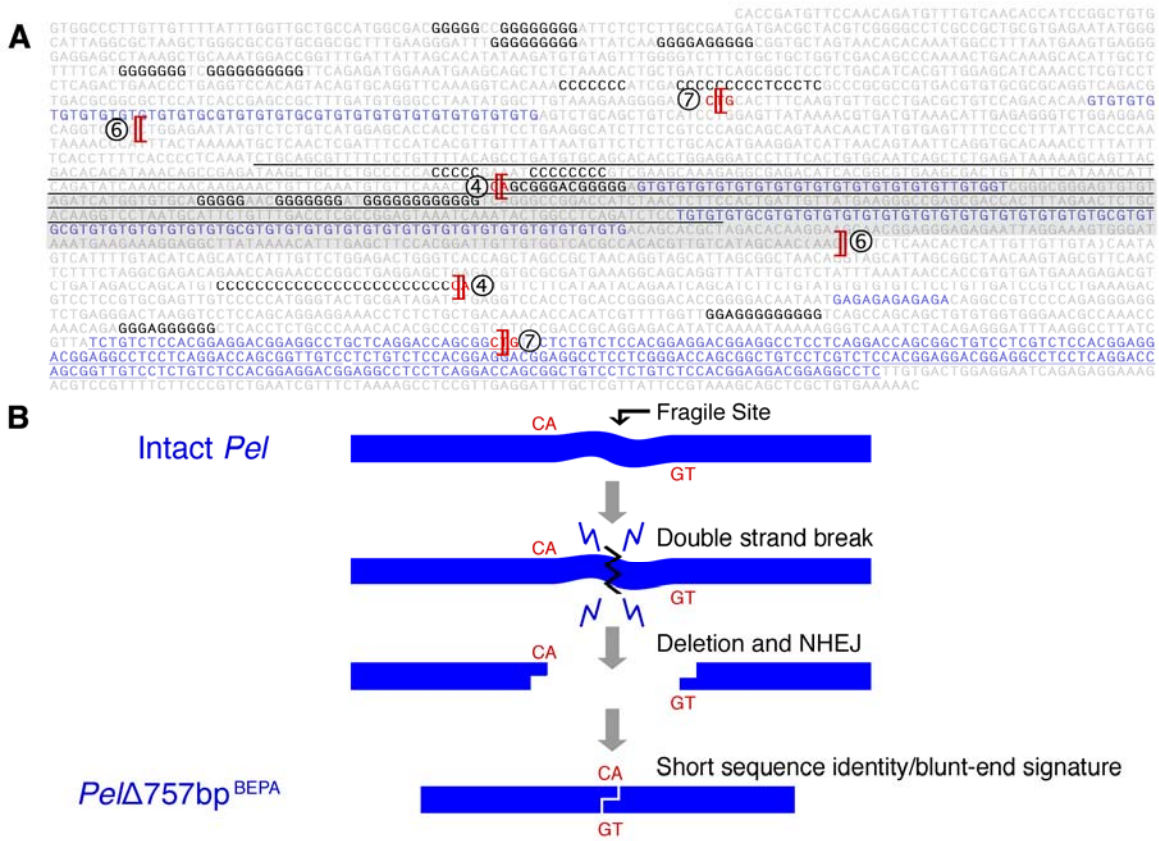


Fig. S7. Sequence of *Pel* enhancer and deletion end points in pelvic-reduced populations. **(A)** The sequence of the region located within the *Pel*-2.5kb^{SALR} is displayed in background grey color. Homopolymer tracks are indicated by black letters, with repeats, alternating purine/pyrimidine tracts, and microsatellites indicated in blue. The underlined blue sequence indicates a 48bp sub-telomeric repeat motif that is specific to threespine sticklebacks. Red brackets and numberings indicate cloned breakpoints, with the red letters underneath each breakpoint indicating micro-homologies. ④ indicates BEPA, with a CA micro-homology. ⑥ indicates HUMP. ⑦ indicates PAXB, with a CTG micro-homology. Shaded sequence indicates minimal region of 488bp shared in multiple deletions. This region shows substantial overlap with the *Pel*-501bp^{SALR} fragment (black underlined sequence). **(B)** A model of a possible deletion event. A fragile site (indicated by wavy line) induces a spontaneous deletion via double strand break during replication fork stalling. To resolve breakage, re-ligation is attempted, with chew-back at the deletion boundary, according to the non-homologous end joining (NHEJ) pathway. Short identical sequences at the junction can assist the annealing and ligation process, indicated here as CA overhangs. After repair, the deleted sequence shows a single instance of CA, whereas it appears at both mapped boundaries in the ancestral sequence.

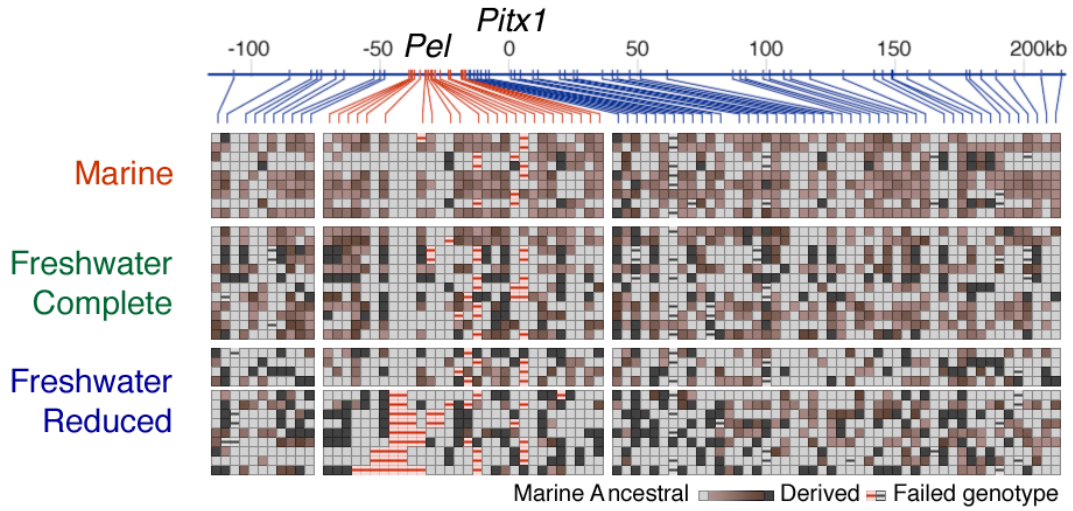


Fig. S8. SNP allele frequencies in the *Pitx1* region by population. Each column corresponds to an individual SNP, and each row shows genotyping results collected from 12 individuals of a particular population. Only markers with at least 10% minor allele frequency in marine populations are included in the analysis. The positions of these SNPs are marked by lines above each column, indicating the physical location of the SNP with respect to the transcription start site of *Pitx1* (0 kb). Each genotype tab is colored according to the ancestral-derived gradient, as indicated, with the most common allele among marine populations taken as the marine ancestral allele, and the alternate allele as the derived allele. Markers that are polymorphic within a population are shown with intermediate reddish tints. Failed genotypes are indicated by "-" dashes. Red lines and dashes indicate locations falling within the 23kb candidate interval defined by association studies in Fig. S1B. The order of populations plotted is as follows: BIGR_3.63, TYNE_1, RABS, NVRO_1, KODK, GORT, GJÖG, LITC, BJDG for marine pelvic-complete populations. WALC, PAXL, PRIB, PRIL, WMSO, TYNE_8, OLNY, FTC, FRIL, FRIC, FLCK_12.8, BIGR_20.33 for freshwater pelvic-complete populations. ORPH, BOUL, SCAD, DOLO, [break], HUMP, BOOT, BEPA, WHAL, PAXB, MVRO, VIFI, FADA, WALR for freshwater pelvic-reduced populations. The small break between pelvic-reduced populations separates populations with and without detected deletions.

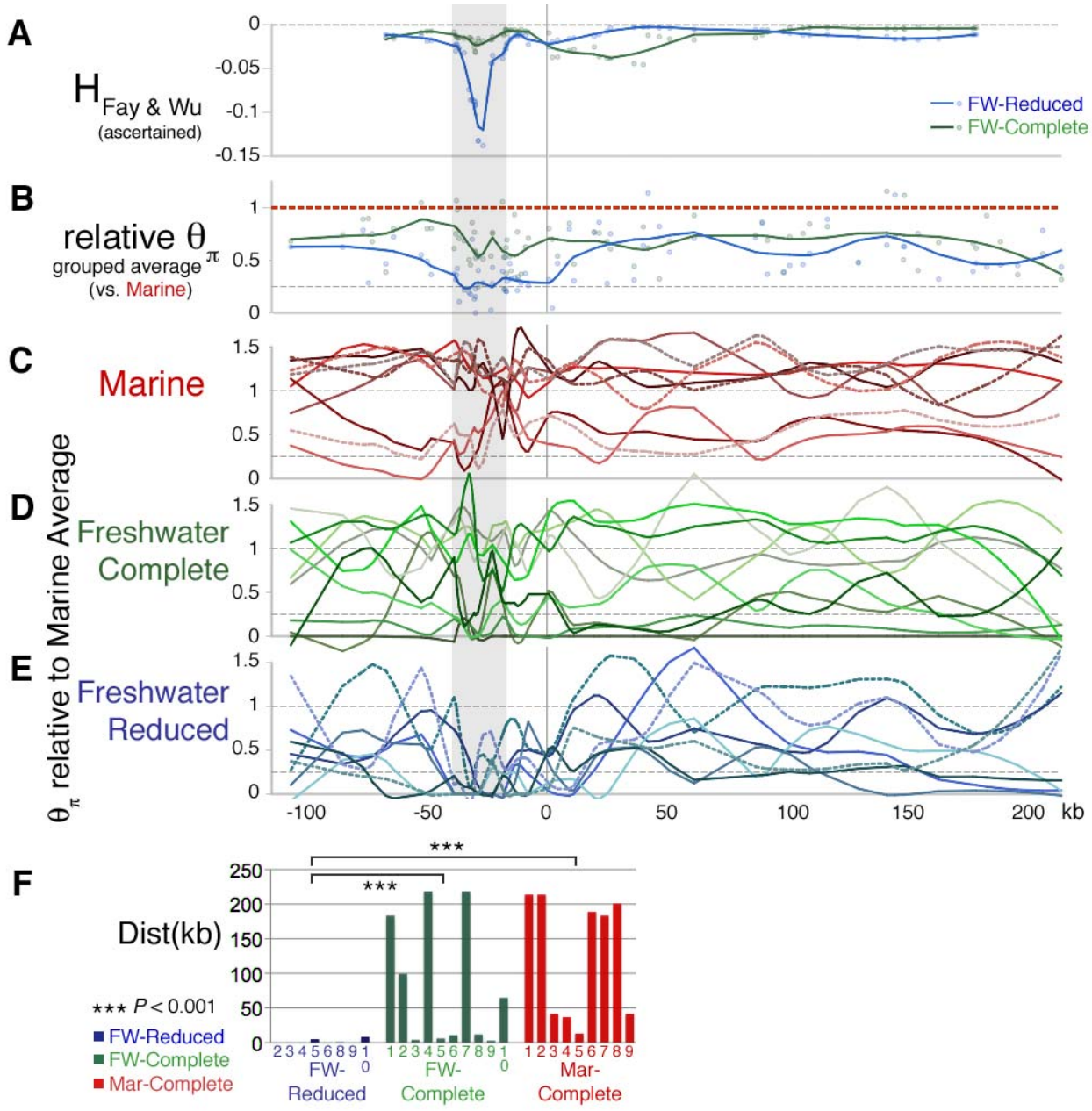
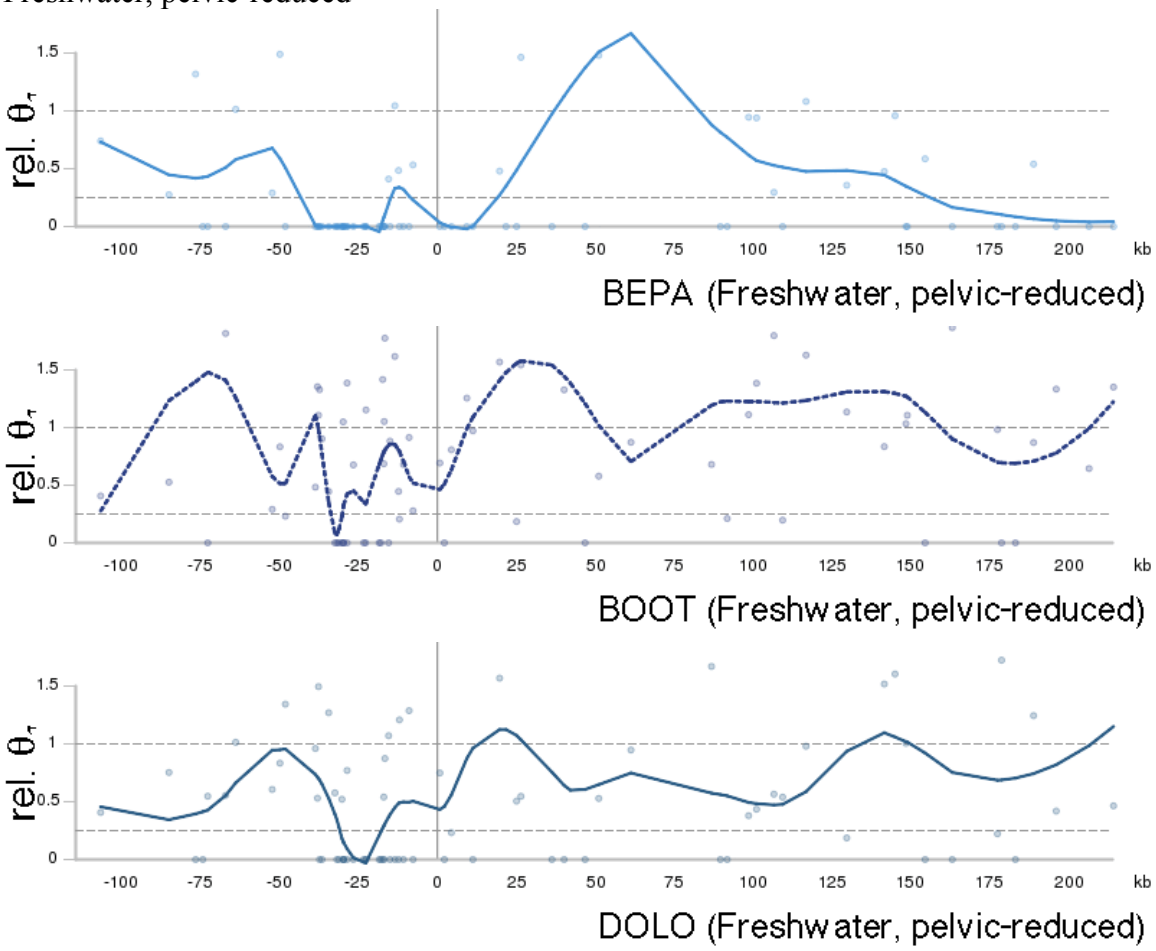
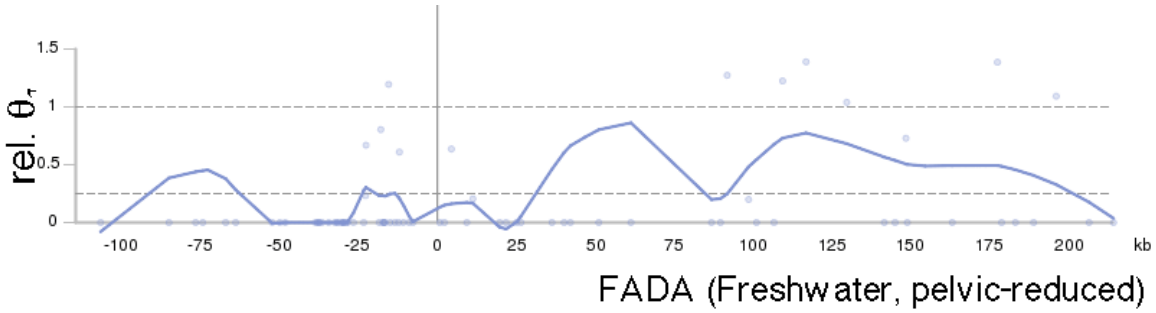


Fig. S9. Signatures of positive selection in *Pel* enhancer region of pelvic reduced fish. **(A and B)** Expanded view of Fig. 5A & B. Fay and Wu's H and relative heterozygosity (θπ) statistics across the *Pitx1* region. Blue (freshwater pelvic-reduced) and green (freshwater pelvic-complete) data points and LOESS smoothed (α=0.2) line represent the phenotypic mean-averaged summary statistics calculated initially by stickleback populations. The *Pel*-containing regulatory region of *Pitx1* (grey candidate region from Fig. S1B) shows both negative H values, indicating an excess of derived alleles; and reduced heterozygosity in pelvic-reduced fish, consistent with positive selection (see main text). θπ values are plotted relative to the grouped marine mean (per SNP) to control for variation in ascertainment between SNPs. **(C, D and E)** Relative

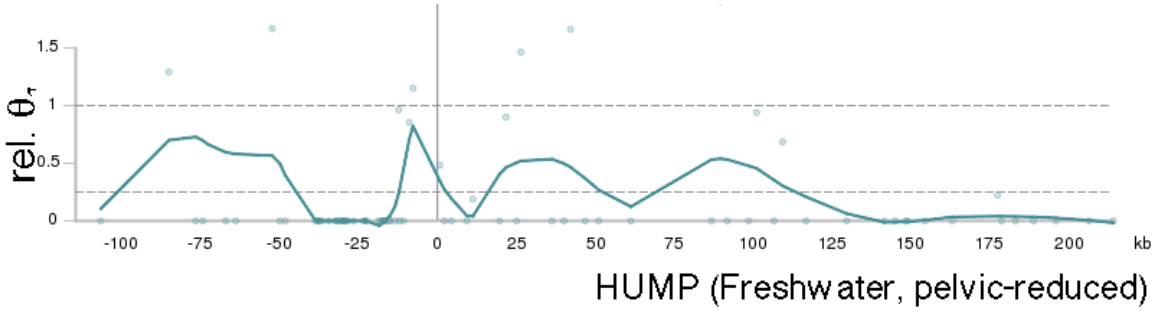
heterozygosity ($\theta\pi$), normalized to grouped marine mean value. Each panel represents the heterozygosity of the group indicated plotted along the *Pitx1* region. Curves correspond to LOESS regressions by population (α=0.2, split plots shown below main figure). Red dotted line indicates the marine mean value (normalized to 1). The lower dotted line in each panel indicates a relative heterozygosity of 0.25. **(F)** Position of minimum heterozygosity relative to *Pel* enhancer, shown for individual freshwater and marine populations. Minimum heterozygosity colocalizes with the *Pel* region in most pelvic reduced populations, but not in freshwater or marine populations with a complete pelvis. *** indicates $P < 0.001$.

Signatures of selection displayed as individual population plots:
Freshwater, pelvic-reduced





FADA (Freshwater, pelvic-reduced)



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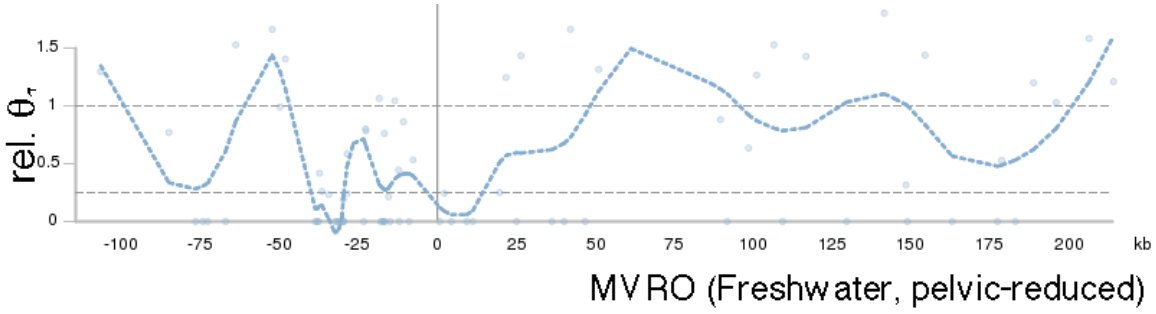
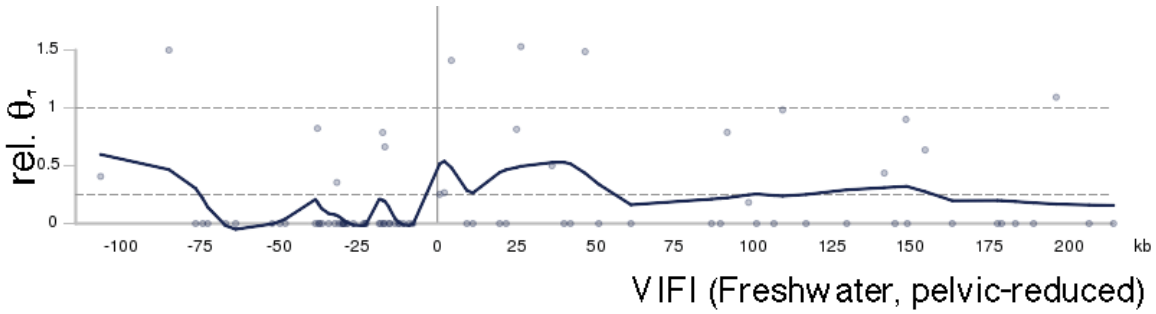
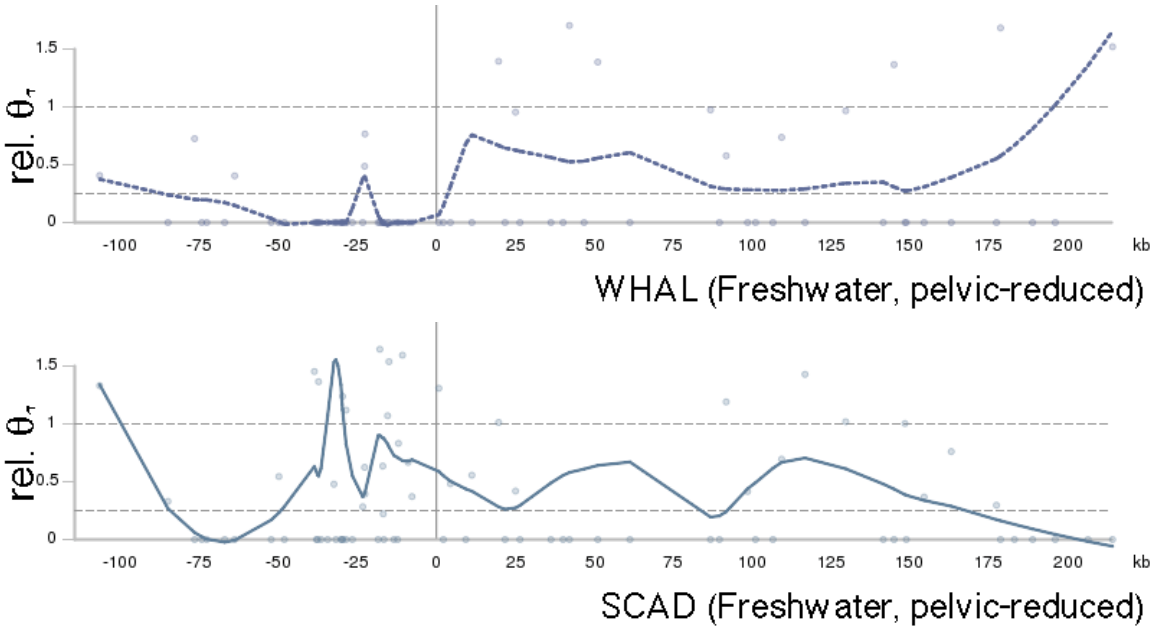


MVRO (Freshwater, pelvic-reduced)



VIFI (Freshwater, pelvic-reduced)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175
200
kb
WHAL (Freshwater, pelvic-reduced)
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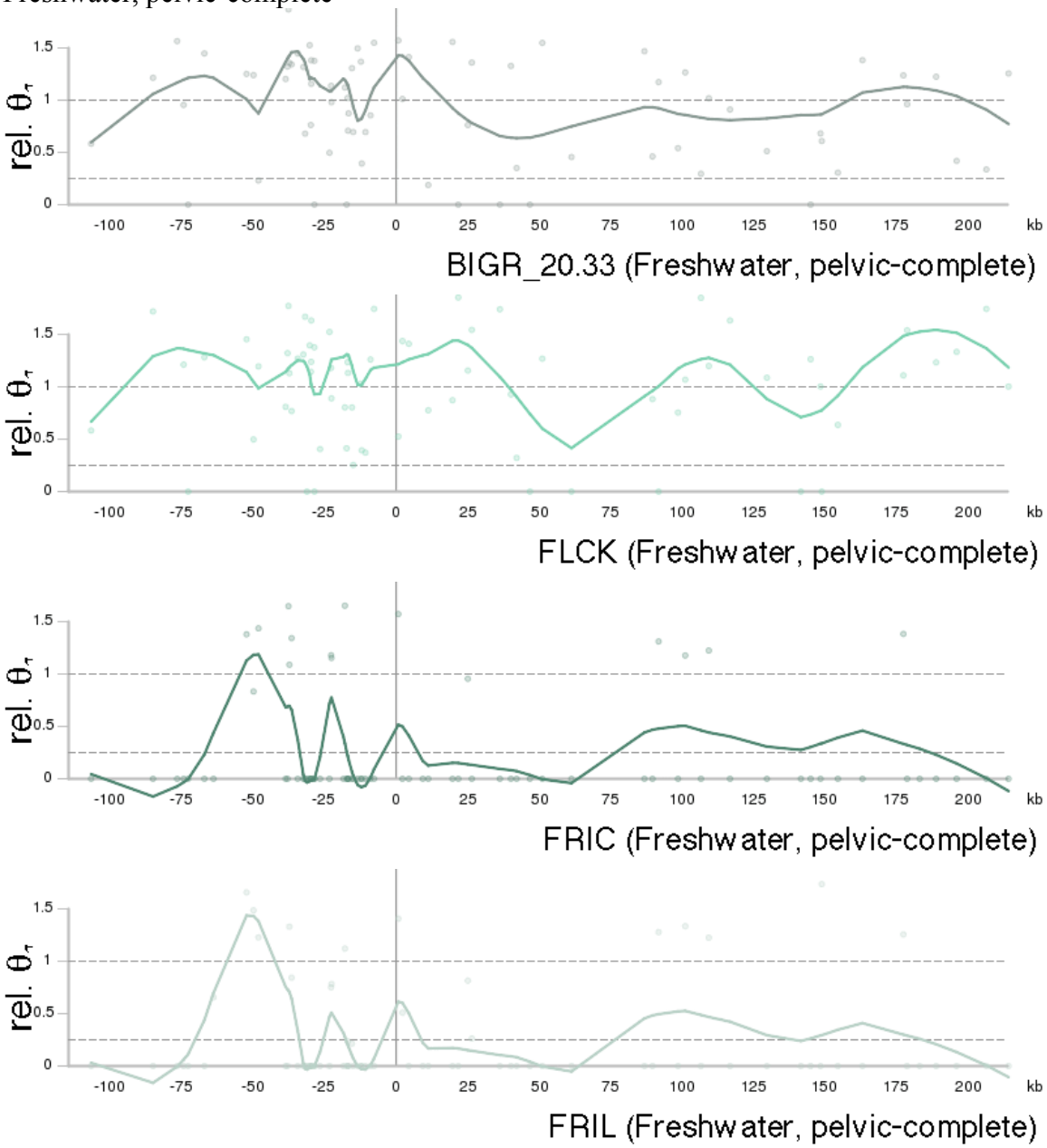
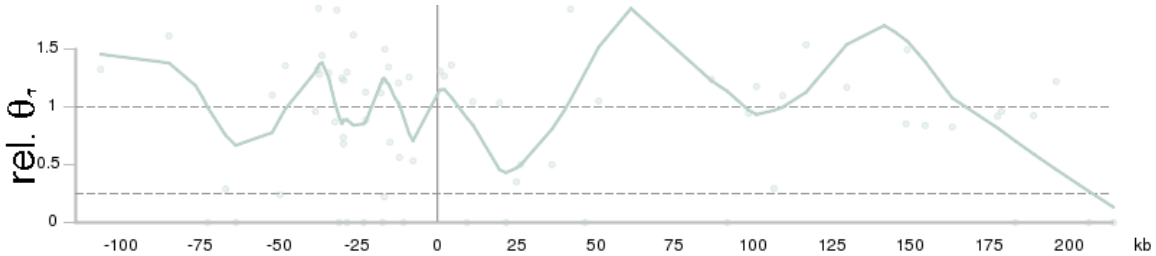
kb
SCAD (Freshwater, pelvic-reduced)

Freshwater, pelvic-complete





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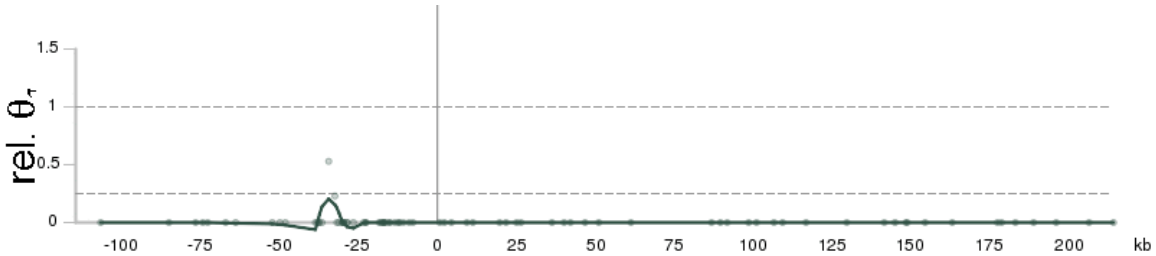

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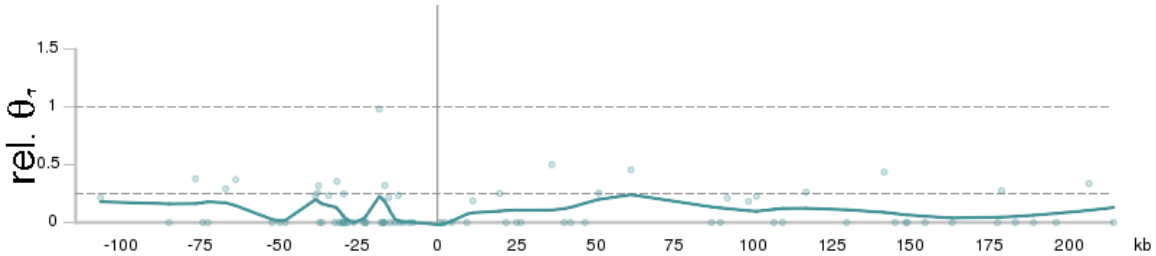
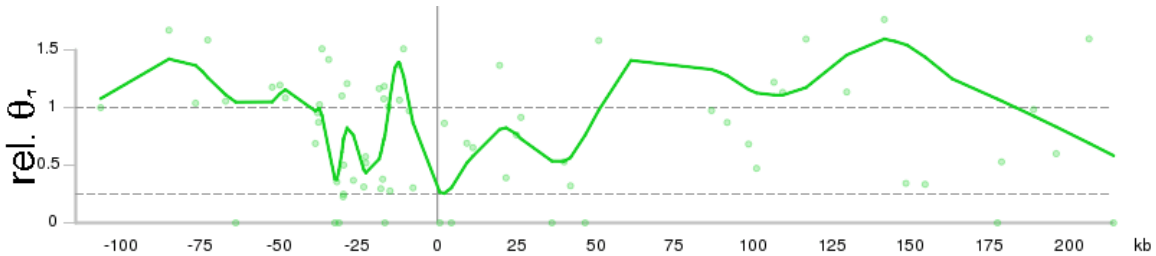


OLNY (Freshwater, pelvic-complete)



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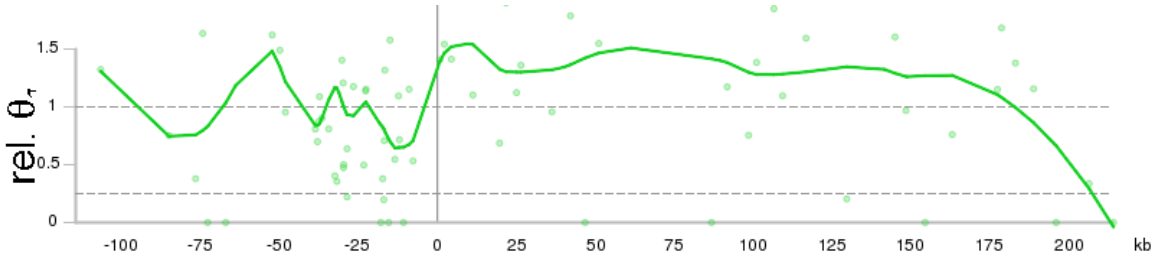

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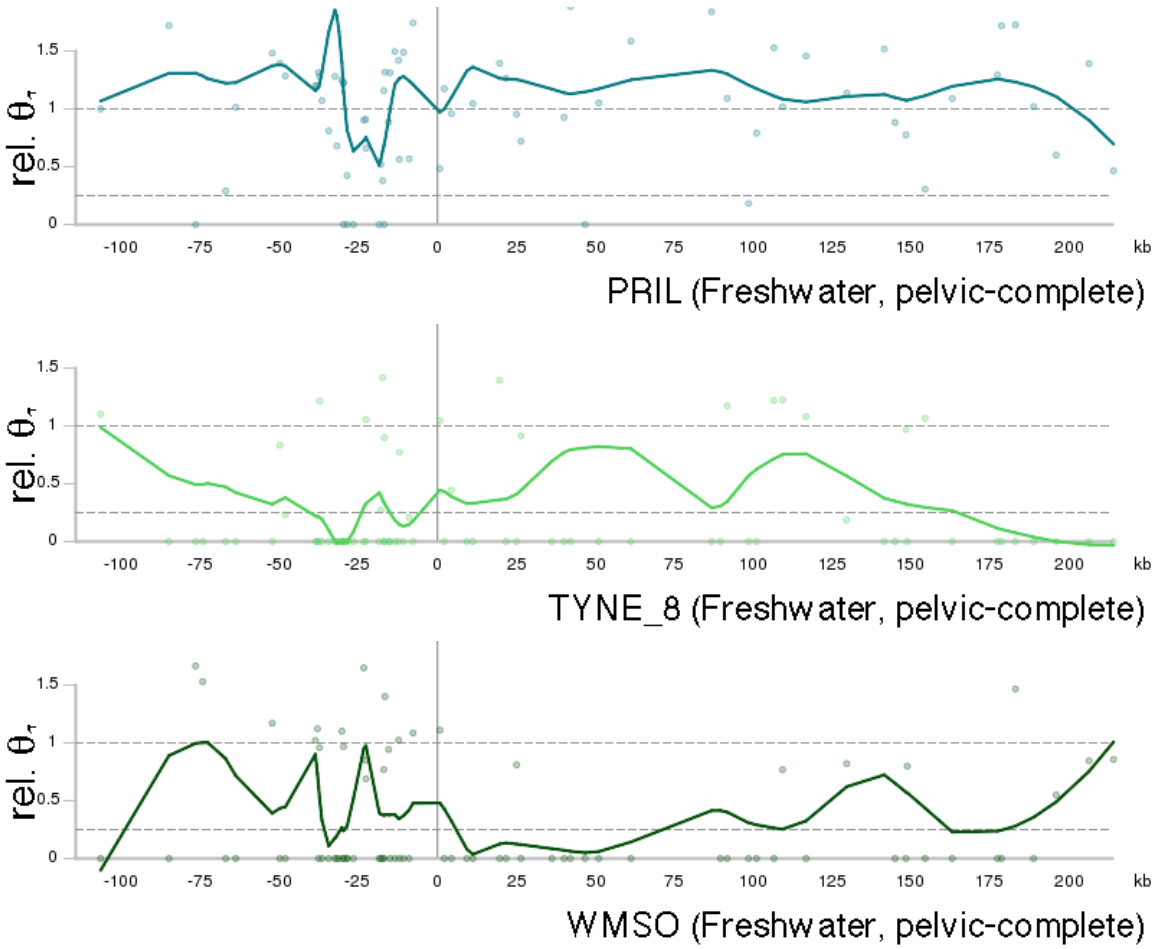
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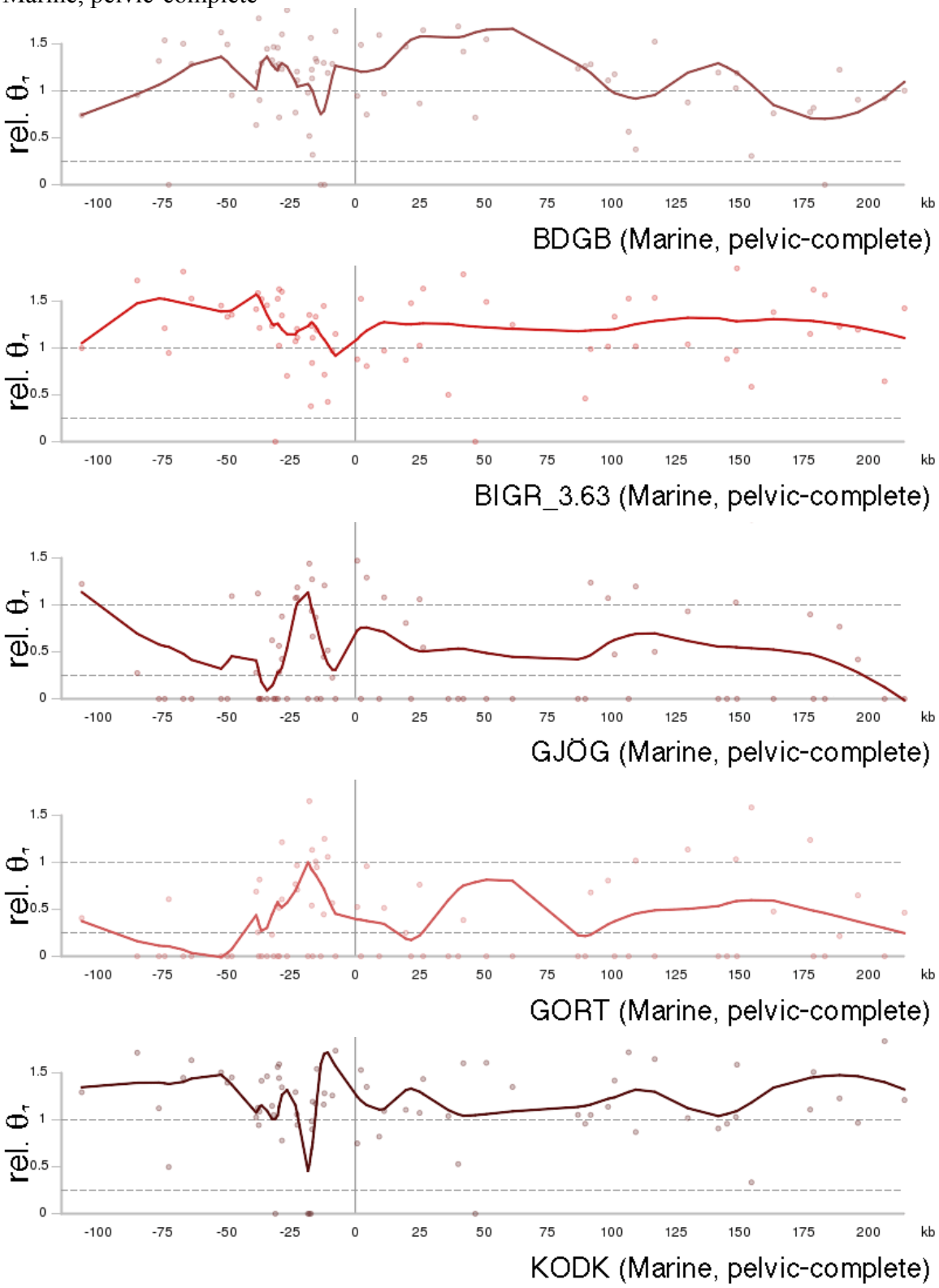
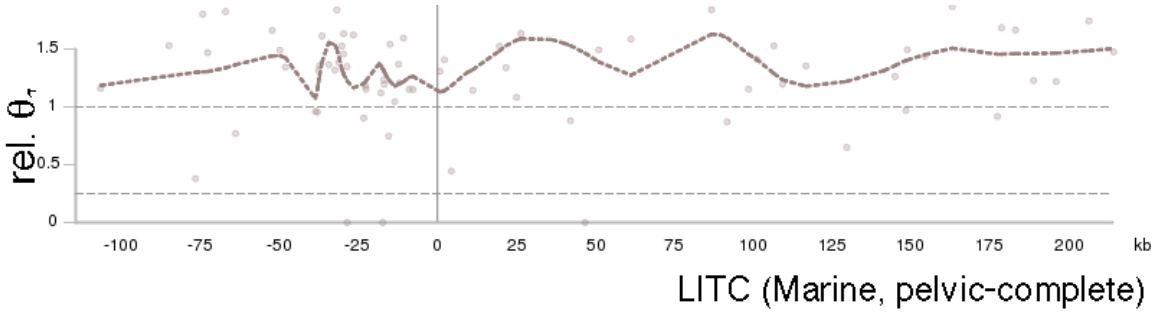
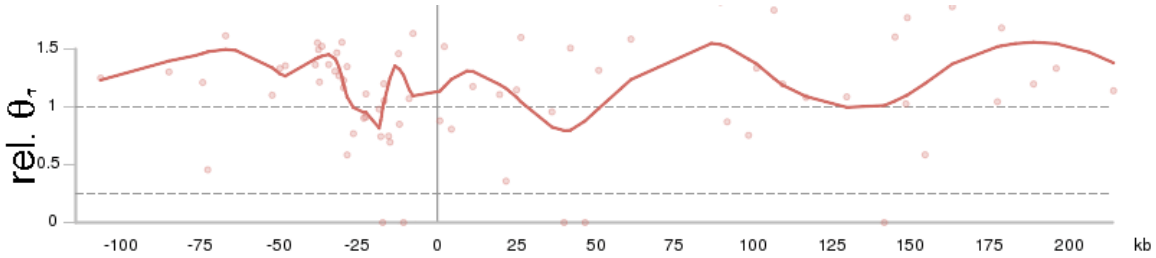
TYNE_8 (Freshwater, pelvic-complete)
WMSO (Freshwater, pelvic-complete)

Marine, pelvic-complete

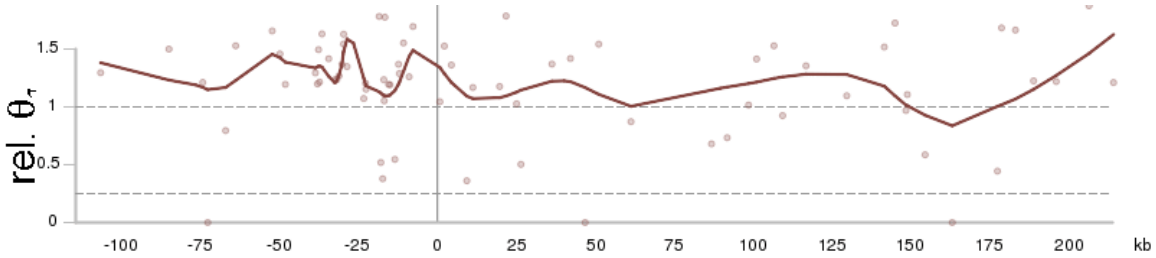




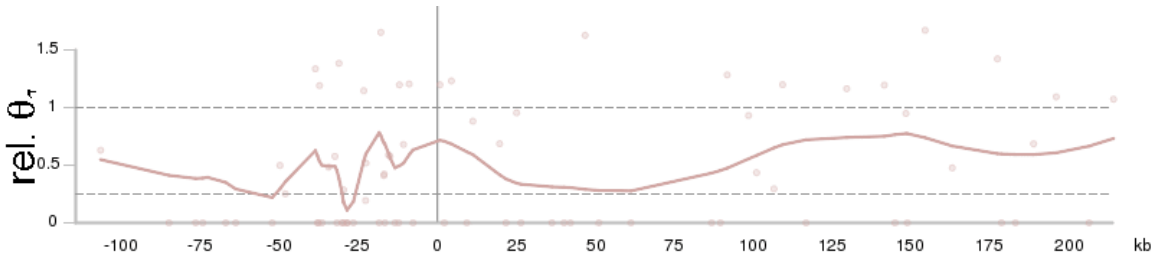
LITC (Marine, pelvic-complete)



NVRO_1 (Marine, pelvic-complete)



RABS (Marine, pelvic-complete)



TYNE_1 (Marine, pelvic-complete)

Supporting tables

Table S1. Stickleback populations used in this study.

Population	Country	Code	Habitat	Class	Phenotype	Lat	Long	Year	Collectors	Pop # in Figs. 4 and 5	Collecting Ref.
Bear Paw Lake, AK	USA	BEPA	Lake	Freshwater	Reduced	61.615	-149.757	2006	M.A. Bell	FW/R-4	This study
Big River (Site 20.33), CA	USA	BIGR_20.33	River	Freshwater	Complete	39.317	-123.686	2007	F.C. Jones & C. Brown	FW/C-1	This study
Big River (Site 3.63), CA	USA	BIGR_3.63	Estuary	Marine	Complete	39.304	-123.780	2007	F.C. Jones & C. Brown	Mar-2	This study
Bodega Bay, CA	USA	BDGB	Brackish water	Marine	Complete	38.325	-123.041	2005	H. Zhang	Mar-1	This study
Boot Lake, AK	USA	BOOT	Lake	Freshwater	Reduced	61.718	-150.130	2006	M.A. Bell	FW/R-5	This study
Boulton Lake, BC	Canada	BOUL	Lake	Freshwater	Reduced, atypical	53.783	-132.098	2003 - 2006	M.E. Marks, B.R. Summers & Y.F. Chan	FW/R-12	This study
Dolomite Lake, AK	USA	DOLO	Lake	Freshwater	Reduced, atypical	60.721	-151.136	2006	M.A. Bell	FW/R-10	This study
Fish Trap Creek, WA	USA	FTC	River	Freshwater	Complete	48.931	-122.487	2005	C.T. Miller	FW/C-4	(S*24*)
Flynn Creek (Site 128), CA	USA	FLCK_128	River	Freshwater	Complete	39.161	-123.583	2007	F.C. Jones & C. Brown	FW/C-2	This study
Friant Dam (Complete-plated), CA	USA	FRIC	River	Freshwater	Complete	36.980	-119.731	1994 - 2007	Kingsley Lab	FW/C-3	(S*25*)
Friant Dam (Low-plated), CA	USA	FRIL	River	Freshwater	Complete	36.980	-119.731	1994 - 2007	Kingsley Lab	FW/C-11	(S*25*)
Gasterosteus	USA	WMSO	River	Freshwater	Complete	34.435	-118.198	2001	C. Peichel	FW/C-10	(S*25*)

williamsonii								& 2002	& D.M. Kingsley		
Gjögur	Iceland	GJÖG	Fjord	Marine	Complete	65.980	-21.440	2003	E. Elfarsdóttir & B. Jónsson	Mar-3	(S*25*)
Gorten Sands	UK	GORT_4	Tidal beach	Marine	Complete	56.909	-5.852	2001	F.C. Jones & C. Brown	Mar-4	This study
Hump Lake, AK	USA	HUMP	Lake	Freshwater	Reduced	60.768	-151.170	2006	M.A. Bell	FW/R-6	This study
Kodiak Island, AK	USA	KODK	Open sea	Marine	Complete	56.967	-151.350	2004	M. Wilson (NOAA)	Mar-5	This study
Little Campbell River, BC	Canada	LITC	Estuary	Marine	Complete	49.018	-122.779	2004	C.T. Miller & Y.F. Chan	Mar-6	(S*25*)
Loch Fada	UK	FADA	Lake	Freshwater	Reduced	57.613	-7.211	2001	C. Peichel & D.M. Kingsley	FW/R-9	(S*25*)
Loch Scadavay	UK	SCAD	Lake	Freshwater	Reduced	57.589	-7.225	2001	C. Peichel & D.M. Kingsley	FW/R-11	(S*25*)
Matadero Creek, CA	USA	MATA	River	Freshwater	Complete	37.386	-122.165	2004 - 2008	Y.F. Chan & F.C. Jones	FW/C-12	This study
Morvoro Lake, AK	USA	MVRO	Lake	Freshwater	Reduced	61.602	-149.786	2006	M.A. Bell	FW/R-3	This study
Navarro River (Site 1), CA	USA	NVRO_1	Estuary	Marine	Complete	39.193	-123.761	2003	Kingsley Lab	Mar-7	(S*25*)
Olney Creek, CA	USA	OLNY	River	Freshwater	Complete	40.528	-122.383	2007	B.R. Summers	FW/C-6	This study
Orphia Lake, AK	USA	ORPH	Lake	Freshwater	Reduced, atypical	60.388	-151.199	2006	M.A. Bell	FW/R-13	This study, also (S. *26*)
Paxton Lake (Benthic), BC	Canada	PAXB	Lake	Freshwater	Reduced	49.712	-124.525	2003	D. Schluter	FW/R-7	(S*1*, S*27*)

Paxton Lake (Limnetic), BC	Canada	PAXL	Lake	Freshwater	Complete	49.712	-124.525	2003	D. Schluter	FW/C-13	(S*27*)
Priest Lake (Benthic), BC	Canada	PRIB	Lake	Freshwater	Complete	49.746	-124.567	2003	D. Schluter	FW/C-7	(S*27*)
Priest Lake (Limnetic), BC	Canada	PRIL	Lake	Freshwater	Complete	49.746	-124.567	2003	D. Schluter	FW/C-8	(S*27*)
Rabbit Slough, AK	USA	RABS	River	Marine	Complete	61.537	-149.166	2005	M.A. Bell	Mar-8	(S*28*)
River Tyne (Site 1)	UK	TYNE_1	Tidal rock pools	Marine	Complete	55.999	-2.520	2003	F.C. Jones & C. Brown	Mar-9	(S*29*)
River Tyne (Site 8)	UK	TYNE_8	River	Freshwater	Complete	55.943	-2.785	2003	F.C. Jones & C. Brown	FW/C-9	(S*29*)
Vifisstaðavatn	Iceland	VIFI	Lake	Freshwater	Reduced	64.080	-21.873	2002 & 2004	E. Elfarsdóttir & B. Jónsson	FW/R-8	(S*29*)
Wallace Lake (Complete), AK	USA	WALC	Lake	Freshwater	Complete	61.573	-149.576	2004	M.A. Bell	FW/C-14	This study
Wallace Lake (Reduced), AK*	USA	WALR	Lake	Freshwater	Reduced	61.573	-149.576	2004	M.A. Bell	FW/R-1	This study
Whale Lake, AK	USA	WHAL	Lake	Freshwater	Reduced	61.542	-149.751	2006	M.A. Bell	FW/R-2	This study

FW/R – Freshwater, pelvic-reduced
FW/C – Freshwater, pelvic-complete
Mar – Marine, pelvic-complete

Table S2. Microsatellite Primer Sequences. Position from TSS — Position of the microsatellite marker relative to the *Pitx1* transcription start site (base pair 163184 in the 5' BAC clone CH213-164F21).

Name	GenBank ID	Position from TSS	Sequence
Stn446-F	GF100639	-162540	GATGAACTTGCCATGAAAGG
Stn446-R	GF100639	-162540	CTTCCTCTGGAAACGACAGC
Stn447-F	GF100640	-158901	GAGAACCAACAGGCAGTATCG
Stn447-R	GF100640	-158901	CCACCGACAGTAAGTTAGACG
Stn448-F	GF100641	-149303	CGAAGCTCTCGCTGTTCC
Stn448-R	GF100641	-149303	CTCAGACTGCGTGGTAATGC
Stn449-F	GF100642	-142358	AATTGAGCTGGATGATGACG
Stn449-R	GF100642	-142358	TTTGATTCATCTCGGACTCG
Stn482-F	GF101813	-140195	TTTCTCAGAGGCATGTTTCC
Stn482-R	GF101813	-140195	GGCCAGAGACACTGAGTCC
Stn450-F	GF100643	-131074	GGTCCCATTTAAGTCCAATCC
Stn450-R	GF100643	-131074	GACAGAGACGTGGAAACACC
Stn451-F	GF100644	-126002	CCACTACATCCCTGTTTGAGC
Stn451-R	GF100644	-126002	ACGTGTGTCTTGCCTCTCC
Stn452-F	GF100645	-122910	ATCCTCCTCTTCCTCAGTCC
Stn452-R	GF100645	-122910	CTGGGCAGACGATATTTGC
Stn453-F	GF100646	-118775	AACCTGCTACATGTCGTCTCC
Stn453-R	GF100646	-118775	CTGACTGATGTCGGATAGATGC
Stn454-F	GF100647	-108135	TGGTCGAACATTTGGTTCC
Stn454-R	GF100647	-108135	CTACCATCACACCCAACAGG
Stn455-F	GF100648	-82405	CTGTTCTTTCGACACGTTGC
Stn455-R	GF100648	-82405	TGAATGAGGTCCTGTTACCC
Stn456-F	GF100649	-66428	CAGTTCAGAGACGACAATGAGG
Stn456-R	GF100649	-66428	CATCAGATGCAGAACAGACG
Stn445-F	GF089698	-60520	TCTTCCGCTCTGTGATTGG
Stn445-R	GF089698	-60520	CTTCACGCAACACGTTTACC
Stn457-F	GF100650	-42385	CCATCGTTAGATTCTCTTCTCC
Stn457-R	GF100650	-42385	CGGGTCCAGGACTCTAGG
Stn458-F	GF100651	-42139	CCCACATTGTGAAACTAACACC
Stn458-R	GF100651	-42139	CAGATAGTTGTGTTGCCTTTCG
Stn459-F	GF100652	-39713	TGCACACATTTCCTACATGC
Stn459-R	GF100652	-39713	ACAGGAAGCAGCAGAGTCG
Stn460-F	GF100653	-39602	ACGGGAAGGTCCCTAACG
Stn460-R	GF100653	-39602	AGCTGATAAGAGCTGAGAAAGC
Stn461-F	GF100654	-38595	TTCTACGTCGCCACTAAACG
Stn461-R	GF100654	-38595	CCGTGAAGTCTTCTCACAGC
Stn462-F	GF100655	-37233	TCAGCAGTGTTTGAGCTTCC
Stn462-R	GF100655	-37233	TGATTCACTAAACGCCATCG
Stn463-F	GF100656	-35661	AGACCTGCTCCTCCAGACC
Stn463-R	GF100656	-35661	CTTTCAAGTGTTGCCTGACG
Stn464-F	GF100657	-30046	CACATCTGCTGCACAAGC
Stn464-R	GF100657	-30046	TTCTTTCCTCCATCGATCC
Stn465-F	GF100658	-23735	GGAGAGGCTTTGATCTGAGG

Stn465-R	GF100658	-23735	CATCGTCAGTCAGAGATGTGG
Stn466-F	GF100659	-22723	GTGGGAACCTTCTCTGTGC
Stn466-R	GF100659	-22723	CCCTGTCCTTTCATCTCTGG
Stn467-F	GF100660	-20477	GAATATCCGCCTCGTTAGC
Stn467-R	GF100660	-20477	GGTTCTTTCTCCAACATCAGG
Stn468-F	GF100661	-16787	AACTTGAGAAGGACGCTTCG
Stn468-R	GF100661	-16787	GGTTAAACCTGCAGTCAAGC
Stn469-F	GF100662	-16197	CTGTCAATCACCGTTGTCG
Stn469-R	GF100662	-16197	ATGGGTGGAGCATTGACC
Stn470-F	GF100663	7567	TCTGCACGTGTCTCTCACG
Stn470-R	GF100663	7567	CCTGAGTCTCTTTCAGCATCC
Stn431-F	GF089697	8914	ACCCAGAGGAGGAACATGG
Stn431-R	GF089697	8914	AGCTTCACCTCCTAATTCTGC
Stn471-F	GF100664	15604	TTCACTGGACGACGTTATGC
Stn471-R	GF100664	15604	TCTCCGTGCTCTTCATCG
Stn472-F	GF100665	31503	TGAAGGTCTGCGTCTGTCC
Stn472-R	GF100665	31503	GCTCTGCAGATCACCAAGG
Stn473-F	GF100666	44744	AGCATGTAGCATCTCACTATCG
Stn473-R	GF100666	44744	GCATAGTGACTGGGAGCAGA
Stn474-F	GF100667	45612	AGACGAGACGCAAATGTGG
Stn474-R	GF100667	45612	AGGAAGTGACCTCACAGTCG
Stn475-F	GF100668	52160	TCCTTATTGGTGAGTGTTCTCC
Stn475-R	GF100668	52160	GGTGTGCAGAAATCCCAATT
Stn476-F	GF100669	58077	ACTGTTCCCACCGAGAGC
Stn476-R	GF100669	58077	GCACACACACACCAACACAC
Stn477-F	GF100670	60065	GAATCCAGAGCCGTGAGC
Stn477-R	GF100670	60065	TTCTGATCTGAGGAGCATCG
Stn478-F	GF100671	65042	CATCATCAGCTCCGTACTTCC
Stn478-R	GF100671	65042	TCTCGTCGGTCCGTTAGC
Stn479-F	GF100672	79311	GTGTGGACGACACAATGC
Stn479-R	GF100672	79311	CATCAAACGACAACAGAACC
Stn480-F	GF100673	103540	TTTCAACCAACACACGTTCC
Stn480-R	GF100673	103540	AATCCCTCCATTCAGACTGC

Table S3. SNPs used for high density genotyping of the *Pitx1* region.

SNP	Chr	Position	Alleles	"Anc"	10% MAF?	Used in θ_π?	*Pel*	Sequence
ss158145849	chrIII	6586435	A/C	n.d.	N	Y	N/A	GATGAGCCG[A/C]AGGGCTTTT
ss158145672	chrIII	6596376	A/G	n.d.	N	Y	N/A	ACACAGACA[A/G]CAAAGGAAG
ss158145673	chrIII	6599283	A/G	n.d.	N	Y	N/A	ATGGTCATC[A/G]AATTTGGTA
ss158145674	chrIII	6602429	A/C	n.d.	N	Y	N/A	TAGCTGCAT[A/C]TATTCCCTC
ss158145675	chrIII	6618228	A/G	n.d.	N	Y	N/A	TTAAAATGC[A/G]CGACTTGTT
ss158145676	chrIII	6625171	A/C	n.d.	N	Y	N/A	CACGTGTTT[A/C]GAATATAGC
ss158145677	chrIII	6630535	A/T	n.d.	N	Y	N/A	GTCTACATA[A/T]GGGAGCCAT
ss158145678	chrIII	6638976	A/C	n.d.	N	Y	N/A	ATGCCTTCC[A/C]CTTGCCATT
ss158145679	chrIII	6641414	A/C	n.d.	N	Y	N/A	CTGAAACTA[A/C]AATTATAGC
ss158145680	chrIII	6645878	A/G	n.d.	N	Y	N/A	TAACAACAC[A/G]CGCAAATGT
ss158145681	chrIII	6650410	A/C	n.d.	N	Y	N/A	TGTTATCTA[A/C]CATACGTGT
ss158145682	chrIII	6651054	C/G	n.d.	N	Y	N/A	CCCTGAAAT[C/G]TCCCTGTTT
ss158145683	chrIII	6661551	A/G	n.d.	N	Y	N/A	TAATTTTGC[A/G]TGTCGTTTT
ss158145684	chrIII	6675980	A/G	n.d.	N	Y	N/A	ACCCAGTGC[A/G]ACTTTCATG
ss158145685	chrVII-*Pitx1*	-106860	A/G	G	Y	N	N	CGTGTAGTC[A/G]GCTGGAGTC
ss158145686	chrVII-*Pitx1*	-104344	C/G	N/A	N	N	N	ATACGCTAA[C/G]TGTACCTTT
ss158145687	chrVII-*Pitx1*	-103213	A/C	N/A	N	N	N	TTGCCAATT[A/C]CTAAGCTAA
ss158145688	chrVII-*Pitx1*	-100714	A/T	N/A	N	N	N	TTTGTCCTC[A/T]GCTCATACT
ss158145689	chrVII-*Pitx1*	-95697	C/G	N/A	N	Y	N	TGAGCTCCA[C/G]CAGTGCTCC
ss158145690	chrVII-*Pitx1*	-87996	A/G	N/A	N	N	N	AAAGACATT[A/G]TAACTTCTG
ss158145691	chrVII-*Pitx1*	-85185	C/G	G	Y	N	N	GATCTCTGT[C/G]TGTGGCTCA
ss158145692	chrVII-*Pitx1*	-83383	A/G	N/A	N	N	N	GAAATTCAC[A/G]CCAACGTTT
ss158145693	chrVII-*Pitx1*	-76733	A/T	A	Y	N	N	GTAAATATT[A/T]AACACAAGT
ss158145694	chrVII-*Pitx1*	-74467	A/C	C	Y	Y	N	ATATAATTA[A/C]TTTGACTCC
ss158145695	chrVII-*Pitx1*	-72879	A/C	A	Y	N	N	GCTAGCTGC[A/C]GCTCGCTTT
ss158145696	chrVII-*Pitx1*	-69944	A/C	N/A	N	Y	N	TGGCATGCG[A/C]GCACACACA
ss158145697	chrVII-*Pitx1*	-67201	A/G	G	Y	Y	N	CAGGAGACA[A/G]AAGGTCTGT
ss158145698	chrVII-*Pitx1*	-65636	A/G	N/A	N	N	N	AAAGCAGAC[A/G]GACGTTCAC

ss158145699	chrVII-*Pitx1*	-64004	A/G	G	Y	N	N	ACATTAAAG[A/G]TGGATTCAT
ss158145700	chrVII-*Pitx1*	-57072	C/G	N/A	N	N	N	GCATCAGGA[C/G]CTCAAACCA
ss158145701	chrVII-*Pitx1*	-52419	A/G	G	Y	N	N	TAATCGATT[A/G]GTTGAACAA
ss158145702	chrVII-*Pitx1*	-49974	A/C	C	Y	N	N	TCTAACGGG[A/C]CTTTTTGTT
ss158145703	chrVII-*Pitx1*	-48246	A/C	C	Y	N	N	AATCAATAA[A/C]GTACGTTTT
ss158145704	chrVII-*Pitx1*	-42886	A/G	N/A	N	N	N	GGCGAGGAA[A/G]ACGCATTTA
ss158145705	chrVII-*Pitx1*	-38724	A/G	A	Y	N	N	GTCTTTATG[A/G]GGAGGAAGT
ss158145706	chrVII-*Pitx1*	-38648	A/G	N/A	N	N	N	GGATGAGGA[A/G]ATGAGATAT
ss158145707	chrVII-*Pitx1*	-38322	C/G	N/A	N	N	N	ACATTTACA[C/G]GTATACTGT
ss158145708	chrVII-*Pitx1*	-38056	A/G	A	Y	Y	N	CTCAATTAA[A/G]CCTGGACGT
ss158145709	chrVII-*Pitx1*	-37729	A/G	A	Y	N	Y	CAACTAGAG[A/G]GCTCAAAAA
ss158145710	chrVII-*Pitx1*	-37554	A/C	N/A	N	N	Y	ACACCCTCC[A/C]GGAGATATT
ss158145711	chrVII-*Pitx1*	-37401	A/C	A	Y	N	Y	GCGCTAAAC[A/C]AACGTACAG
ss158145712	chrVII-*Pitx1*	-36628	A/T	A	Y	Y	Y	CTTGACTTG[A/T]GGACGACAG
ss158145713	chrVII-*Pitx1*	-36045	A/G	N/A	N	N	Y	GGCGCTGGC[A/G]AGCGATGAG
ss158145714	chrVII-*Pitx1*	-34903	C/G	N/A	N	N	Y	GAGTGAAGC[C/G]CCACAGAAA
ss158145715	chrVII-*Pitx1*	-34497	A/G	A	Y	Y	Y	AACCGGTCC[A/G]TTTGCAGCT
ss158145716	chrVII-*Pitx1*	-33286	A/T	N/A	N	N	Y	AGAGTTCTA[A/T]AGTGGTCGC
ss158145717	chrVII-*Pitx1*	-33268	A/T	N/A	N	N	Y	TGCATTAGG[A/T]CCTTGTGCA
ss158145718	chrVII-*Pitx1*	-32530	A/G	N/A	N	N	Y	TCTGGGGAC[A/G]GCCTGTCTC
ss158145719	chrVII-*Pitx1*	-32520	A/G	A	Y	N	Y	GTCCCCAGA[A/G]GGAGGTCTG
ss158145720	chrVII-*Pitx1*	-31905	A/G	G	Y	Y	Y	CAAATCCTC[A/G]ACGGAGGCT
ss158145721	chrVII-*Pitx1*	-31771	A/G	N/A	N	N	Y	ATGTCATTT[A/G]TCTAATTGT
ss158145722	chrVII-*Pitx1*	-31460	A/C	N/A	N	N	Y	GGATGAAAT[A/C]AAACAATTA
ss158145723	chrVII-*Pitx1*	-31251	A/G	N/A	N	N	Y	CCTTAAAGA[A/G]ACACTGAAC
ss158145724	chrVII-*Pitx1*	-31238	A/C	C	Y	N	N	ATAATAATC[A/C]TTTGTTCAG
ss158145725	chrVII-*Pitx1*	-30351	A/C	N/A	N	N	N	TGATCGAAT[A/C]CCACGTGAT
ss158145726	chrVII-*Pitx1*	-30297	A/T	T	Y	N	N	CCAAAAATT[A/T]GTTTAATCG
ss158145727	chrVII-*Pitx1*	-30172	C/G	N/A	N	N	N	TTTTAAAGT[C/G]TGAAAACAT
ss158145728	chrVII-*Pitx1*	-29968	A/G	N/A	N	N	N	AACCTAATC[A/G]TTTAAAACG
ss158145729	chrVII-*Pitx1*	-29919	A/T	N/A	N	N	N	TGTTTAAAA[A/T]AAAACGTTT

ss158145730	chrVII-*Pitx1*	-29900	A/T	T	Y	N	N	AGAGAATTT[A/T]AAAAGCAAA
ss158145731	chrVII-*Pitx1*	-29796	C/G	G	Y	N	N	TGTTTCTAT[C/G]CATGTTTAA
ss158145732	chrVII-*Pitx1*	-29795	C/G	N/A	N	N	N	TTTAAACAT[C/G]GATAGAAAC
ss158145733	chrVII-*Pitx1*	-29755	A/G	G	Y	N	N	AAACCATCT[A/G]AAGAATTTT
ss158145734	chrVII-*Pitx1*	-28795	A/G	N/A	N	N	N	GAATATAAT[A/G]AACGTGTTG
ss158145735	chrVII-*Pitx1*	-28703	A/C	N/A	N	N	N	TATACAATA[A/C]ATTGTAAAT
ss158145736	chrVII-*Pitx1*	-28665	A/G	A	Y	N	N	GTACATAAT[A/G]CTTACACAT
ss158145737	chrVII-*Pitx1*	-28652	A/C	C	Y	N	N	ATGTACACA[A/C]TTTCCATTT
ss158145738	chrVII-*Pitx1*	-26659	A/C	A	Y	Y	N	CAATTATGA[A/C]TTCCCTGAT
ss158145739	chrVII-*Pitx1*	-24803	A/G	N/A	N	N	N	CTGACTGAC[A/G]ATGTATACG
ss158145740	chrVII-*Pitx1*	-24377	A/G	N/A	N	N	N	ACCAGCACC[A/G]CGGCCCTGA
ss158145741	chrVII-*Pitx1*	-24237	C/G	N/A	N	N	N	ACAACATTT[C/G]TGACAATTT
ss158145742	chrVII-*Pitx1*	-24124	A/C	N/A	N	N	N	TTGGTCAGA[A/C]GCACAAACG
ss158145743	chrVII-*Pitx1*	-23975	A/G	N/A	N	N	N	TGGAGGCCG[A/G]CAGGTTGAT
ss158145744	chrVII-*Pitx1*	-23894	A/G	N/A	N	N	N	CTCTGGAAA[A/G]GATTAACTA
ss158145745	chrVII-*Pitx1*	-23372	A/C	C	Y	N	N	TGTAATTAA[A/C]CAGTTCCGT
ss158145746	chrVII-*Pitx1*	-23320	A/G	N/A	N	N	N	ATTAAAACC[A/G]GCACATGCA
ss158145747	chrVII-*Pitx1*	-22821	A/T	N/A	N	N	N	TATCAGATT[A/T]TGCTCTCCA
ss158145748	chrVII-*Pitx1*	-22780	A/C	A	Y	N	N	CATCGTACG[A/C]TCAATCTTA
ss158145749	chrVII-*Pitx1*	-22662	A/G	G	Y	N	N	AGCTAACAC[A/G]GTGACACAC
ss158145750	chrVII-*Pitx1*	-21285	A/C	N/A	N	N	N	ATGAATATC[A/C]GCCTCGTTA
ss158145751	chrVII-*Pitx1*	-19464	A/G	N/A	N	N	N	GTTTATGGA[A/G]CAAATCTCT
ss158145752	chrVII-*Pitx1*	-18485	A/G	G	Y	Y	N	AATCATTCC[A/G]CGTGCCGGA
ss158145753	chrVII-*Pitx1*	-17958	A/G	A	Y	N	N	AATGACAGT[A/G]ACAGTGACC
ss158145754	chrVII-*Pitx1*	-17357	A/G	G	Y	N	N	CCAGTTAAA[A/G]TGTGAATAA
ss158145755	chrVII-*Pitx1*	-17014	A/G	G	Y	Y	N	CACGACAAC[A/G]GTGATTGAC
ss158145756	chrVII-*Pitx1*	-16878	A/G	G	Y	N	N	GGATTAGAA[A/G]CTCATGGAC
ss158145757	chrVII-*Pitx1*	-16661	A/G	A	Y	N	N	GAGTTAAAG[A/G]CCTCCAAAG
ss158145758	chrVII-*Pitx1*	-15801	A/G	N/A	N	N	N	TGGTGTCCC[A/G]GGTGAGCTG
ss158145759	chrVII-*Pitx1*	-15479	C/G	C	Y	N	N	GACCGGCTT[C/G]AGACGCCTG
ss158145760	chrVII-*Pitx1*	-15044	A/G	?	Y	N	N	GTGCAGAGA[A/G]TCGTCTCCA

ss158145761	chrVII-*Pitx1*	-13498	A/G	A	Y	N	N	GAGTTCAGC[A/G]CGTTACGCT
ss158145762	chrVII-*Pitx1*	-12328	A/T	T	Y	N	N	AAGCACAGA[A/T]CTGTGAGCT
ss158145763	chrVII-*Pitx1*	-12124	A/C	N/A	N	N	N	AGGACCGAA[A/C]AATGCTGCT
ss158145764	chrVII-*Pitx1*	-12056	A/G	G	Y	N	N	TACACCTGC[A/G]GCGAGGAAC
ss158145765	chrVII-*Pitx1*	-11709	A/G	N/A	N	N	N	GCTTCAATA[A/G]ATAAACTGT
ss158145766	chrVII-*Pitx1*	-10768	A/G	G	Y	N	N	GAGCTTCCT[A/G]CCCGCCGCC
ss158145767	chrVII-*Pitx1*	-8984	A/C	C	Y	N	N	TTCACGTGA[A/C]GTTTATTAG
ss158145768	chrVII-*Pitx1*	-7742	C/G	C	Y	N	N	TTCCAATCA[C/G]AGCATGTTA
ss158145769	chrVII-*Pitx1*	-5241	A/C	N/A	N	N	N	AGACGGAGA[A/C]GATCGGCCG
ss158145770	chrVII-*Pitx1*	767	A/T	T	Y	N	N	TGTTTTATT[A/T]ATTGGACCG
ss158145771	chrVII-*Pitx1*	2203	A/C	A	Y	N	N	GAGTTAACA[A/C]AGTGACTGC
ss158145772	chrVII-*Pitx1*	4435	A/G	G	Y	N	N	ACTGTCCAC[A/G]CGGACGTGA
ss158145773	chrVII-*Pitx1*	7632	A/C	N/A	N	N	N	CTTTTATGT[A/C]ATTGGGGTC
ss158145774	chrVII-*Pitx1*	9336	A/C	C	Y	N	N	TGTGGTTCA[A/C]GGTTCCATC
ss158145775	chrVII-*Pitx1*	11255	A/G	G	Y	N	N	CACCCTCTC[A/G]TGTTACAAG
ss158145776	chrVII-*Pitx1*	19740	A/G	A	Y	N	N	TAAAAAAAC[A/G]TGACCTCAC
ss158145777	chrVII-*Pitx1*	21779	A/G	?	Y	N	N	AGTACATCG[A/G]TGCAGGTGA
ss158145778	chrVII-*Pitx1*	25121	A/G	G	Y	N	N	AAACCGGTA[A/G]GTAGTGTCC
ss158145779	chrVII-*Pitx1*	26504	A/T	A	Y	N	N	AAATAGTTT[A/T]CGGTCCACG
ss158145780	chrVII-*Pitx1*	29448	A/C	N/A	N	N	N	ATCTGTGAG[A/C]CAAAGAAAA
ss158145781	chrVII-*Pitx1*	32012	C/G	N/A	N	N	N	CAAGATCAA[C/G]CGCACCAGT
ss158145782	chrVII-*Pitx1*	36350	C/G	C	Y	Y	N	TGATCTATA[C/G]CCGGTAATC
ss158145783	chrVII-*Pitx1*	40204	A/G	A	Y	N	N	TTCATCCTC[A/G]TCTTCCAAA
ss158145784	chrVII-*Pitx1*	42215	A/G	G	Y	Y	N	TGTTCTATC[A/G]ATGAATGAA
ss158145785	chrVII-*Pitx1*	46864	A/G	G	Y	N	N	ACTTTAGGA[A/G]GAGATTGTT
ss158145786	chrVII-*Pitx1*	51203	A/G	A	Y	N	N	TTGGATCAC[A/G]TATGCGGTT
ss158145787	chrVII-*Pitx1*	58571	A/T	N/A	N	N	N	ACATTAGTA[A/T]CCAGTAAGC
ss158145788	chrVII-*Pitx1*	59124	A/G	N/A	N	N	N	GTTGGATTG[A/G]AGATAAAAT
ss158145789	chrVII-*Pitx1*	61420	A/C	C	Y	N	N	CTTGAGCTC[A/C]TGAAAGGCT
ss158145790	chrVII-*Pitx1*	65085	C/G	N/A	N	N	N	TTTTAAATC[C/G]CTGGCTGAT
ss158145791	chrVII-*Pitx1*	66469	A/G	N/A	N	N	N	TGTGGAACT[A/G]TCGGTGGAA

ss158145792	chrVII-*Pitx1*	71346	A/G	N/A	N	N	N	ACGCCTCGT[A/G]AGAGAAGGC
ss158145793	chrVII-*Pitx1*	74302	A/G	N/A	N	N	N	TATTTATCT[A/G]CAGCAGATA
ss158145794	chrVII-*Pitx1*	84162	A/G	N/A	N	N	N	GAGTTCATG[A/G]GTTTAGTTT
ss158145795	chrVII-*Pitx1*	86984	A/G	G	Y	Y	N	CTGAATATT[A/G]CTCATTATT
ss158145796	chrVII-*Pitx1*	89813	A/G	G	Y	N	N	TTTCACGCG[A/G]CATCAGAAT
ss158145797	chrVII-*Pitx1*	91956	A/T	A	Y	N	N	TCTGTGGAG[A/T]CCAGTTTTC
ss158145798	chrVII-*Pitx1*	97195	A/C	N/A	N	N	N	AAAGAACAT[A/C]ATGTCGTGG
ss158145799	chrVII-*Pitx1*	98750	C/G	G	Y	Y	N	AGTACATTA[C/G]AAGATTCTT
ss158145800	chrVII-*Pitx1*	101274	A/C	C	Y	N	N	ACCTGCAGG[A/C]GGAGAGACA
ss158145801	chrVII-*Pitx1*	104731	A/T	N/A	N	N	N	AACAATAAA[A/T]TCACCCCTA
ss158145802	chrVII-*Pitx1*	106793	A/G	A	Y	Y	N	AAAGCCGTT[A/G]TAGTCTTTT
ss158145803	chrVII-*Pitx1*	109545	A/T	A	Y	N	N	ACAATGTGC[A/T]GCTCAGGTT
ss158145804	chrVII-*Pitx1*	113259	A/G	N/A	N	N	N	AAACTTCTA[A/G]GTTGTAAGA
ss158145805	chrVII-*Pitx1*	116974	A/C	C	Y	Y	N	ACCAAGATC[A/C]GAGGTTCAA
ss158145806	chrVII-*Pitx1*	120540	A/C	N/A	N	N	N	TCCTGGTTC[A/C]GTTAAAAAG
ss158145807	chrVII-*Pitx1*	121789	A/G	N/A	N	N	N	ACTGGAAAT[A/G]AAACGCTGC
ss158145808	chrVII-*Pitx1*	124456	C/G	N/A	N	N	N	TTGAGTTTG[C/G]CGATTTTGG
ss158145809	chrVII-*Pitx1*	126604	A/G	N/A	N	N	N	CCTTTAGAC[A/G]TTTATTCAG
ss158145810	chrVII-*Pitx1*	129893	A/G	G	Y	N	N	CCTGTGAGG[A/G]AACGTTTTA
ss158145811	chrVII-*Pitx1*	132309	A/G	N/A	N	N	N	TTTAACGTC[A/G]TTGATGTGA
ss158145812	chrVII-*Pitx1*	134152	A/T	N/A	N	N	N	CCTTTGCAT[A/T]TGGCTGTTA
ss158145813	chrVII-*Pitx1*	141805	A/C	C	Y	Y	N	CTCATTCTA[A/C]GATTACCAC
ss158145814	chrVII-*Pitx1*	145217	A/C	C	Y	Y	N	GTCTTGGTG[A/C]TAAATGAAT
ss158145815	chrVII-*Pitx1*	148679	A/G	?	Y	Y	N	GTGTTTGGT[A/G]TTACTGCCG
ss158145816	chrVII-*Pitx1*	149112	A/C	C	Y	Y	N	CCTCCGGTG[A/C]TGAAGTCGC
ss158145817	chrVII-*Pitx1*	152310	A/G	N/A	N	Y	N	ACTGTCAGG[A/G]CGCTTATCT
ss158145818	chrVII-*Pitx1*	154762	A/G	A	Y	N	N	TCGTGTTCC[A/G]CCATCTTTA
ss158145819	chrVII-*Pitx1*	163377	A/G	G	Y	Y	N	TGAGAAGTC[A/G]GTCCAGATG
ss158145820	chrVII-*Pitx1*	165511	A/G	N/A	N	Y	N	TTAACAAGT[A/G]TAAAATTGC
ss158145821	chrVII-*Pitx1*	166416	A/G	N/A	N	Y	N	TTCTCGTTT[A/G]TTACGCTA
ss158145822	chrVII-*Pitx1*	168923	C/G	N/A	N	N	N	AGCATGAAA[C/G]CAGGATACG

ss158145823	chrVII-*Pitx1*	172127	A/C	N/A	N	Y	N	TGTCCAACA[A/C]ATGGATGTA
ss158145824	chrVII-*Pitx1*	177714	A/G	G	Y	Y	N	CTCTCATAC[A/G]CGCTGCAGA
ss158145825	chrVII-*Pitx1*	179019	A/G	G	Y	Y	N	AGCAAAACA[A/G]CACCAAATA
ss158145826	chrVII-*Pitx1*	183425	A/C	A	Y	Y	N	TGCTGTTTG[A/C]ATTTAAAAT
ss158145827	chrVII-*Pitx1*	184436	C/G	N/A	N	Y	N	ATTTACAAT[C/G]AGACAAGAA
ss158145828	chrVII-*Pitx1*	186351	A/G	N/A	N	N	N	AATCATCAC[A/G]TGAAGATAA
ss158145829	chrVII-*Pitx1*	189178	A/G	G	Y	Y	N	AGAATATAA[A/G]GAAAAACAC
ss158145830	chrVII-*Pitx1*	196364	A/G	A	Y	Y	N	TGTTGGCAA[A/G]CCAAACTTT
ss158145831	chrVII-*Pitx1*	204135	A/C	N/A	N	N	N	ATACTTTAC[A/C]GTTACATGC
ss158145832	chrVII-*Pitx1*	206784	A/C	A	Y	Y	N	AGAAGACTC[A/C]CAACAAGCT
ss158145833	chrVII-*Pitx1*	214543	A/C	A	Y	Y	N	CTGAACATA[A/C]TATATGTGT
ss158145834	chrXI	1669455	A/G	n.d.	N	Y	N/A	CATTTGGCT[A/G]AATTTGTGA
ss158145835	chrXI	1675661	A/C	n.d.	N	Y	N/A	GCTTTCAAA[A/C]GTGGAACTG
ss158145836	chrXI	1683557	A/C	n.d.	N	Y	N/A	CTATATATT[A/C]CATCCAACC
ss158145837	chrXI	1699906	A/T	n.d.	N	Y	N/A	CGTATTGTA[A/T]AGTGGGTGG
ss158145838	chrXI	1705579	A/C	n.d.	N	Y	N/A	CATAGAGAG[A/C]GCGGGCTCT
ss158145839	chrXI	1710629	A/G	n.d.	N	Y	N/A	TTCACCGCA[A/G]GACCACATT
ss158145840	chrXI	1712239	A/G	n.d.	N	Y	N/A	TTTGGTTGC[A/G]AGCCATGAA
ss158145841	chrXI	1713968	A/T	n.d.	N	Y	N/A	GTACAAGGA[A/T]GTAAAAGAG
ss158145842	chrXI	1725724	A/C	n.d.	N	Y	N/A	CAGAGAAAA[A/C]ATTTGGGTA
ss158145843	chrXI	1727375	A/G	n.d.	N	Y	N/A	TGACTCTGT[A/G]TCTTTGGAG
ss158145844	chrXI	1739125	A/G	n.d.	N	Y	N/A	TGATGTGTT[A/G]CTATTATTT
ss158145845	chrXI	1744258	A/C	n.d.	N	Y	N/A	AAACTGAAT[A/C]AGAAAAACA
ss158145846	chrXI	1780060	A/T	n.d.	N	Y	N/A	TTTTACATG[A/T]GTCATTTTC
ss158145847	chrXI	1828565	C/G	n.d.	N	Y	N/A	AAATATTTG[C/G]CGAGGCATA
ss158145848	chrXI	1829666	A/C	n.d.	N	Y	N/A	GGTATGAAT[A/C]ATATTTTTC
ss158145849	chrXI	1831726	A/G	n.d.	N	Y	N/A	TTTTTTCAG[A/G]TTATTATGG

Anc — Ancestral allele as determined by most frequent allele in Pacific Marine populations.
10% MAF — At least 10% minor allele frequency. “Y” indicates that the SNP was used in population genetics analyses
Used in θπ — SNPs marked with “Y” indicate that they have similar ascertainment and were used in the quantitative θπ analysis
Pel — “SNPs” marked with “Y” indicate that were indel markers and occur within the candidate region containing *Pel*

n.d. — Not determined
Position column refers to base pair in stickleback genome assembly (chrIII and chrXI), or relative to the TSS of *Pitx1*.

Table S4. Statistical analysis at SNP ss158145716. Grouping genotypes by population and then by the indicated grouping methods, the mean frequency of successful genotypes was compared between groups using two-tailed t-tests.

Group Statistics

	Grouping methods	N	Mean	Std. Deviation	Std. Error Mean
% Successful ss158145716	Pelvic-reduced (All)	13	0.3526	0.44767	0.12416
	Pelvic-complete (Mar/FW)	21	0.9782	0.06126	0.01337

Equal variances not assumed, df = 12.279, $P < 0.0004$

% Successful ss158145716	Pelvic-reduced (Deletion-only)	9	0.0741	0.12805	0.04268
	All others	25	0.9783	0.05784	0.01157

Equal variances not assumed, df = 9.202, $P < 1\text{x}10^{-8}$

% Successful ss158145716	Pelvic-reduced (Deletion-only)	9	0.0741	0.12805	0.04268
	Pelvic-complete (Mar/FW)	21	0.9782	0.06126	0.01337

Equal variances not assumed, df = 9.610, $P < 1\text{x}10^{-8}$

Table S5. Genotyping results at ss158145716 and ss158145717, located within the core of *Pel*.

			ss158145716				ss158145717			
Population	**Phenotype**	**Habitat**	**Failed**	**Successful**	**Total**	**% Successful**	**Failed**	**Successful**	**Total**	**% Successful**
Completes	Pelvic-complete	Mixed	5	239	244	98%	3	241	244	99%
Reduced	Pelvic-reduced	Freshwater	100	55	155	35%	102	54	156	35%
Pel-deleted	Pelvic-reduced	Freshwater	99	8	107	7.5%	99	9	108	8.3%
Non-*Pel*-deleted	Pelvic-reduced	Freshwater	1	47	48	98%	3	45	48	94%
BDGB	Pelvic-complete	Marine	0	12	12	100%	0	12	12	100%
LITC	Pelvic-complete	Marine	0	12	12	100%	0	12	12	100%
GJOG	Pelvic-complete	Marine	0	12	12	100%	0	12	12	100%
GORT_4	Pelvic-complete	Marine	0	12	12	100%	1	11	12	92%
KODK	Pelvic-complete	Marine	0	11	11	100%	0	11	11	100%
NVRO_1	Pelvic-complete	Marine	0	12	12	100%	0	12	12	100%
RABS	Pelvic-complete	Marine	0	12	12	100%	0	12	12	100%
TYNE_1	Pelvic-complete	Marine	0	12	12	100%	1	11	12	92%
BIGR_3_63	Pelvic-complete	Marine	0	12	12	100%	0	12	12	100%
BIGR_20_33	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
FLCK_128	Pelvic-complete	Freshwater	1	7	8	88%	0	8	8	100%
FRIC	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
FRIL	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
FTC	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
OLNY	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
TYNE_8	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
WMSO	Pelvic-complete	Freshwater	0	9	9	100%	0	9	9	100%
PRIL	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
PRIB	Pelvic-complete	Freshwater	0	12	12	100%	0	12	12	100%
PAXL	Pelvic-complete	Freshwater	1	11	12	92%	0	12	12	100%
WALC	Pelvic-complete	Freshwater	3	9	12	75%	1	11	12	92%
WALR*	Pelvic-reduced	Freshwater	8	4	12	33%	7	5	12	42%
FADA	Pelvic-reduced	Freshwater	12	0	12	0%	12	0	12	0%
VIFI	Pelvic-reduced	Freshwater	11	0	11	0%	12	0	12	0%
MVRO	Pelvic-reduced	Freshwater	11	1	12	8.3%	11	1	12	8.3%
PAXB	Pelvic-reduced	Freshwater	9	3	12	25%	9	3	12	25%
WHAL	Pelvic-reduced	Freshwater	12	0	12	0%	12	0	12	0%

BEPA	Pelvic-reduced	Freshwater	12	0	12	0%	12	0	12	0%
BOOT	Pelvic-reduced	Freshwater	12	0	12	0%	12	0	12	0%
HUMP	Pelvic-reduced	Freshwater	12	0	12	0%	12	0	12	0%
DOLO†	Pelvic-reduced	Freshwater	0	12	12	100%	0	12	12	100%
SCAD	Pelvic-reduced	Freshwater	0	12	12	100%	3	9	12	75%
BOUL‡	Pelvic-reduced	Freshwater	1	11	12	92%	0	12	12	100%
ORPH‡	Pelvic-reduced	Freshwater	0	12	12	100%	0	12	12	100%

* Represent a mixture of two pelvic-reduced haplotypes, one of which has *Pel* deletion.

† Genetic and morphological information suggest *Pitx1*-mediated pelvic reduction

‡ Genetic and morphological information suggest *Pitx1*-independent pelvic reduction

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