Supporting Online Material for:

Partially repeatable genetic basis of benthic adaptation in threespine sticklebacks

Priscilla A. Erickson[1], Andrew M. Glazer[1], Emily E. Killingbeck[1], Rachel M. Agoglia[1], Jiyeon Baek[1], Sara M. Carsanaro[1], Anthony M. Lee[1], Phillip A. Cleves[1], Dolph Schluter[2], and Craig T. Miller[1*]

[1]Department of Molecular and Cell Biology, University of California-Berkeley, California, USA

[2]Biodiversity Research Centre and Zoology Department, University of British Columbia, Vancouver, BC, Canada

Contents:

Supplementary Methods:

Phenotyping

Standard length was measured with digital calipers twice on all fish and averaged. All fish were fixed in 10% neutral buffered formalin overnight, stained with 0.008% Alizarin red in 1% potassium hydroxide and cleared in 50% glycerol, 0.5% potassium hydroxide. Most traits phenotyped were previously shown to have a strong genetic basis in a PAXB x marine F2 cross (Miller et al. 2014). Body depth, dorsal spines (DS1-3), left and right pelvic spines (PSL and PSR), and premaxilla height (PMH) were measured using digital calipers, and lateral plates were counted on the left side. Additional external craniofacial phenotypes [frontal width (FW), jaw width (JW), premaxilla length (PML), articular length (AL), dentary length (DL) opercle length (OPL), and opercle width (OPW)] were measured using a Leica M125 microscope with reticule as described in Miller et al. 2014. Branchial skeletons were dissected and flat-mounted to measure branchial bone length [the basihyal (BH), all five ceratobranchials (CB1-5), and the anterior-most epibranchial (EB1)] and to count ventral and dorsal pharyngeal teeth (VTP, DTP1, and DTP2), ventral gill rakers in rows 1-9 along the ceratobranchials (R1C-R9C), and the total number of row 1-9 ventral rakers (C). See Miller et al. 2014 for more details on branchial skeletal phenotypes. For DTP1 and VTP, the total number of teeth on the right and left tooth plates was counted; for DTP2, the left only was counted. For rakers and branchial bones, the left side was counted or measured. Bone lengths were measured using NIH Image J as described in Erickson et al. 2014.

Phenotypes were tested for significant association with sex and standard length using ANOVA (sex), linear regression (standard length), or general linear model (both sex and standard length) in R (version 3.1.2, http://www.r-project.org/) and corrected for any variable

significant at $P \leq 0.05$. Outliers more than 3 standard deviations from the mean were removed and the residuals were recalculated. Each phenotype was tested for increasing variances by a Levene test and deviation from normality by an Anderson-Darling test, and log-transformed if doing so corrected either of these deviations (see Miller et al. 2014 for details). Corrections were performed on phenotypes within each F2 family, and all residuals were back transformed to a 40 mm standard length fish for QTL mapping. See Table S2 for corrections applied to each phenotype in each F2 family. Phenotype correlation plots (Figure S1) were generated with the *Ellipse* package in R (version 0.3-8, http://CRAN.R-project.org/package=ellipse) using code adapted from https://hlplab.wordpress.com/.

DNA Isolation and Genotyping-By-Sequencing (GBS)

DNA was extracted from pectoral fins using the Qiagen DNAEasy 96 Blood & Tissue kit according to manufacturer's instructions. Concentration was quantified using the Quant-iT PicoGreen kit and 50 ng genomic DNA was used for each sample. Thirteen F2 fish were excluded from GBS due to low DNA concentration. Genomic DNA was digested with ApeKI and barcoded libraries were prepared as described in Glazer et al. 2015 using a set of 96 unique barcodes and four unique adapters. A total of 384 barcoded samples were sequenced in each of two Illumina HiSeq2000 lanes using paired-end 100 bp reads. The grandparents of the crosses were also genotyped with GBS. For the grandparental DNA samples, multiple barcodes were used to generate higher coverage for SNP calling (11 barcodes for the LCM grandfather, 9 barcodes for the PRIB grandmother, and 6 barcodes each for the PAXB and ENOB grandmothers). All GBS reads are deposited in the Sequence Read Archive # SRP070856.

Reads were mapped to the stickleback reference genome with Samtools, and SNPs that had opposite homozygous states in the grandparents were called as described in Glazer et al. 2015. Genotypes were assigned using a custom pipeline to combine individual SNP genotypes into bins with a maximum length of 500 kb. Markers that failed in over 25% of fish (n_{PAXB} =98, n_{PRIB} = 105, n_{ENOB} = 83) or were outliers in the expected 1:2:1 distribution by a chi-square test were dropped (n_{PAXB} = 68, n_{PRIB} = 58, n_{ENOB} = 41). F2 fish in which over 50% of markers failed (n_{PAXB} = 1, n_{PRIB} = 3, n_{ENOB} = 4,) were also excluded. Sex was assigned based on coverage of the X chromosome relative to the rest of the genome as in Glazer et al. 2015. See Table S3 for a summary of GBS data processing.

Constructing Linkage Maps

A set of 823 binned markers that were informative in all three crosses was initially used to construct linkage maps in Joinmap 4.0 (Kyazma) using the cross-pollination setting. Plots of genetic distance versus physical distance (G x P) were generated using the revised genome assembly described in Glazer et al. 2015, and 18 markers that appeared to be strong outliers in at least one G x P plot were removed from each cross, resulting in a final set of 805 shared markers used for mapping in all three crosses.

Mapping QTL in R/qtl

Initial QTL mapping was performed in R/qtl (version 1.33-7, http://www.rqtl.org/, Broman et al. 2003; Broman and Sen 2009) using the *stepwiseqtl* mapping function. Penalties were calculated for a set of 22 representative phenotypes (see Table S2) in each cross using the *scantwo* function

with 100 permutations of each phenotype. The penalties of the 22 phenotypes were then averaged across all three crosses to determine a final penalty of 3.7, which was used as a genome-wide significance threshold in a *stepwiseqtl* scan. Following this initial mapping, *addqtl*, *fitqtl* and *refineqtl* were used to search for additional QTL and to calculate the LOD scores and percent variance explained. In cases where two QTL for the same phenotype had overlapping 1.5-LOD intervals, the lower LOD score QTL was dropped (n = 5). QTL with a LOD score less than 3.7 after the *refineqtl* calculation were dropped (n = 8). The *addqtl*/*refineqtl*/*fitqtl* process was repeated to search for additional QTL. No additional QTL were identified, so the QTL and LOD scores from the second round of analysis were used in the final data set (see Table S4). LOD scores at every marker for every phenotype were calculated with *refineqtl* for chromosomes with a QTL and *scanone* for chromosomes without any QTL.

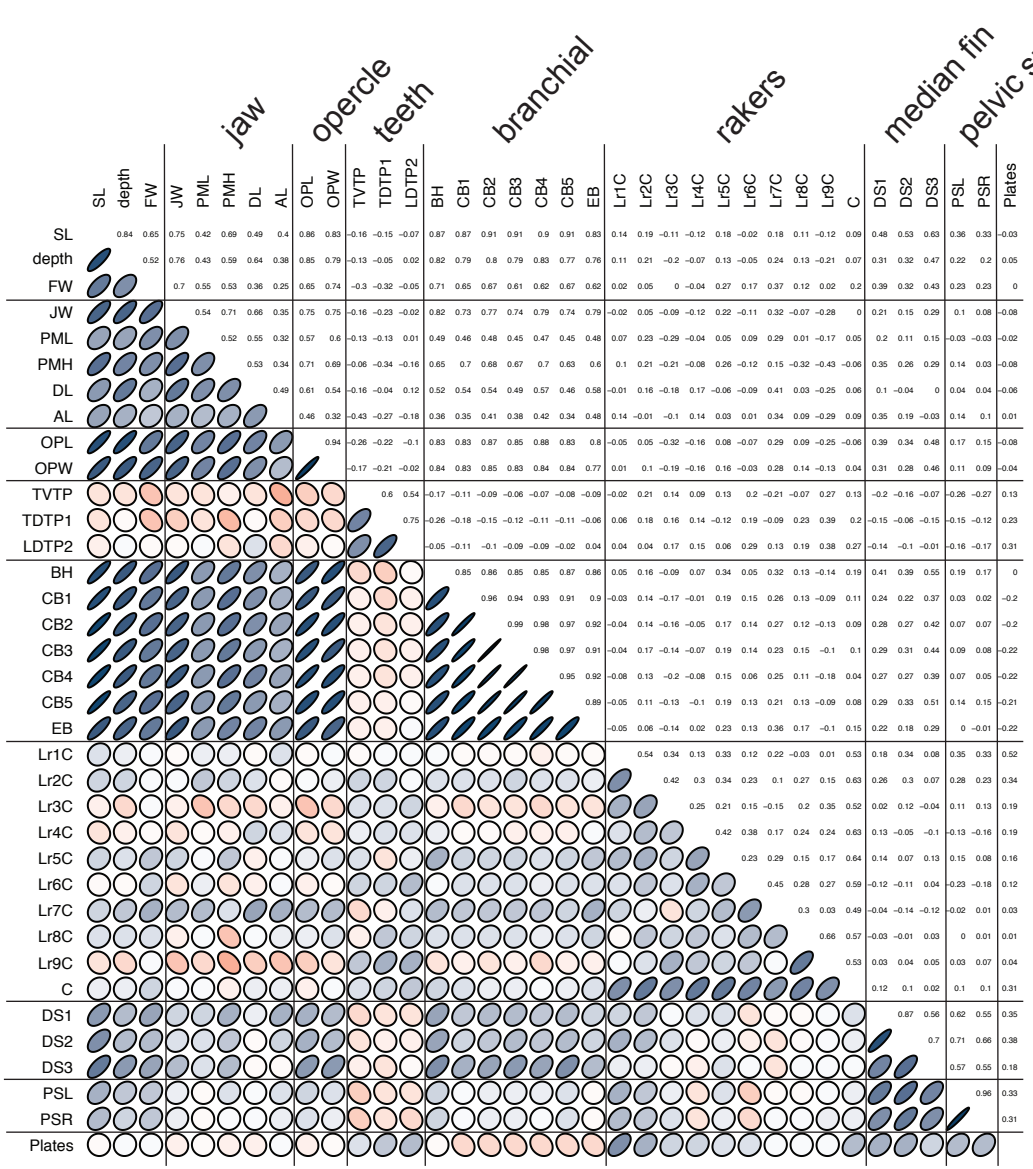
Analysis of potential pleiotropy

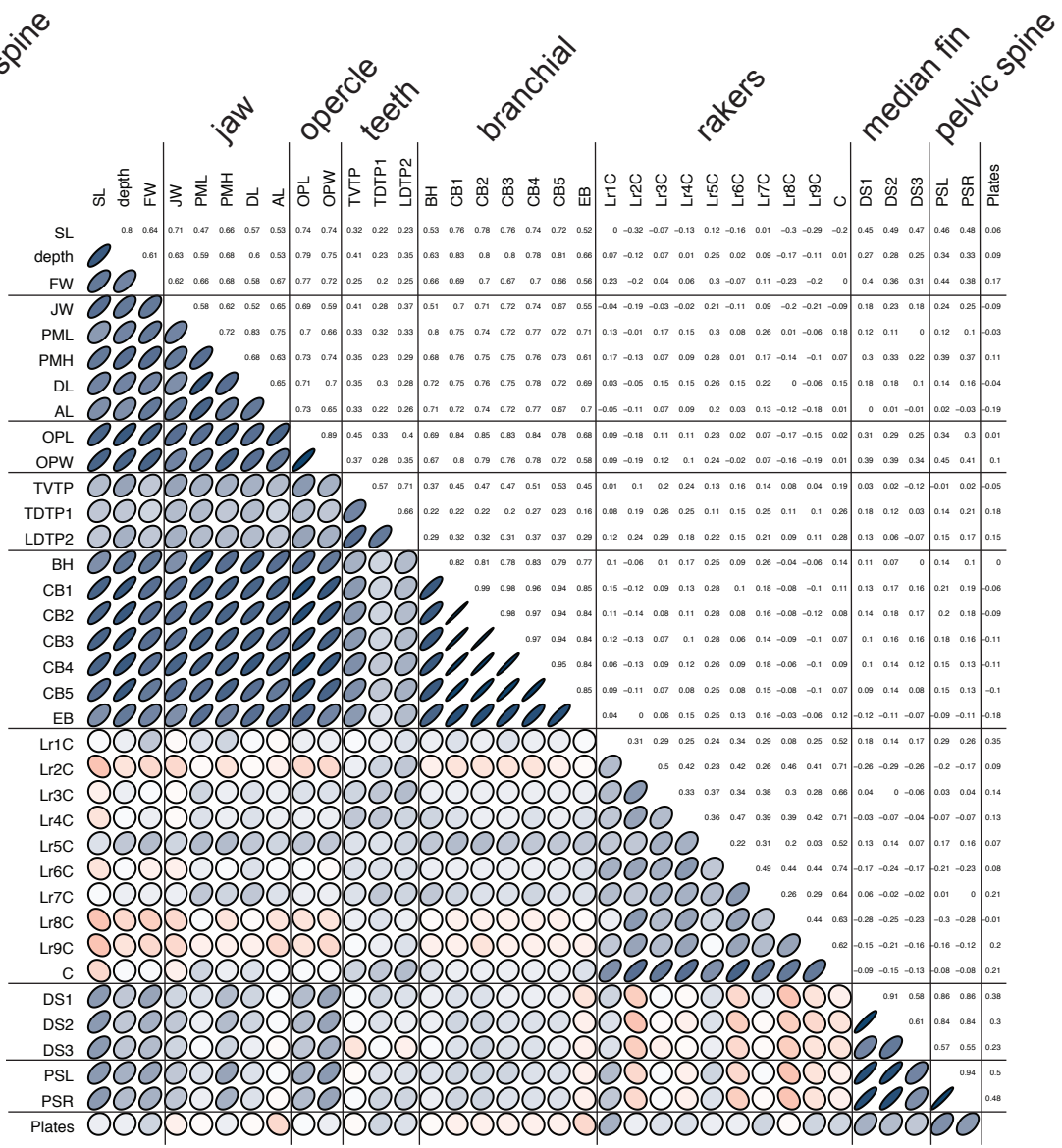
Given that overlapping QTL affecting different trait classes in different crosses could be the result of the same underlying parallel genetic change affecting multiple phenotypes, we performed a second overlap analysis, not classifying QTL into any trait classes. Starting with the list of all filtered, genome-wide QTL, we chose the largest effect (highest PVE) QTL for each chromosome in each cross. Then, in order of decreasing PVE, we added any additional non-overlapping QTL. This process generated a single list of QTL that represented all skeletal QTL regions in the cross, with any given genomic position represented at most once per cross. We then performed an overlap analysis and simulations as described above, but did not consider the trait category when counting overlaps.

Supplemental Figures and Legends:

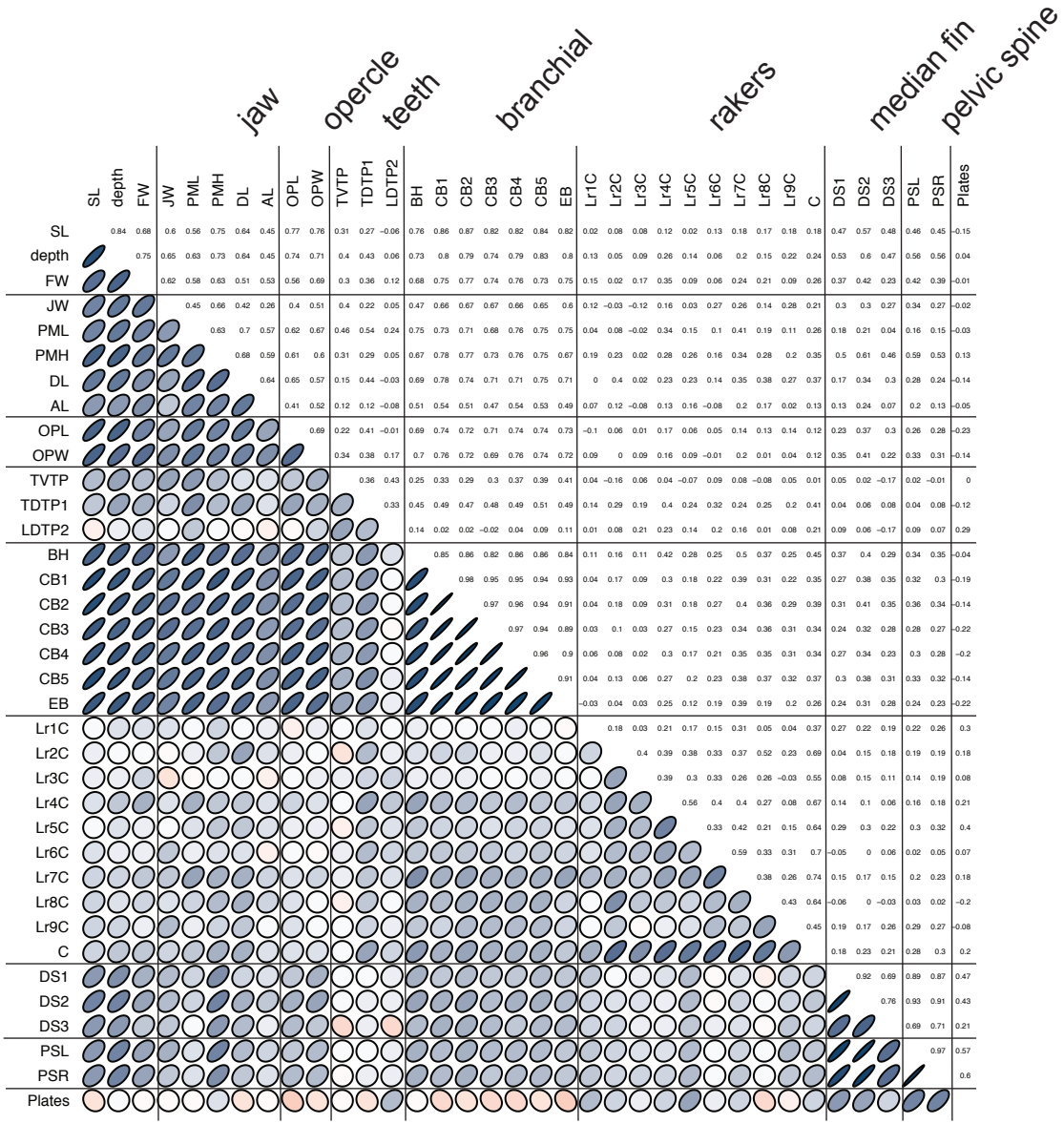
Figure S1: Correlation matrix of all raw phenotypes measured. (begins on next page) Below the diagonal, the color, shape, and orientation of the ellipse indicate the strength of the correlation (darker blue and upward sloping indicates a more positive correlation, darker red and downward sloping represents a more negative correlation; narrower ellipses indicate stronger correlations, more circular ellipses indicate weak correlations). The correlation coefficient is presented above the diagonal. Horizontal and vertical lines separate trait categories, which are labeled above.

Figure S1


PAXB phenotype correlations
jaw
opercle
teeth
branchial
rakers
median fin
pelvic spine
SL
depth
FW
JW
PML
PMH
DL
AL
OPL
OPW
TVTP
TDTP1
LDTP2
BH
CB1
CB2
CB3
CB4
CB5
EB
Lr1C
Lr2C
Lr3C
Lr4C
Lr5C
Lr6C
Lr7C
Lr8C
Lr9C
C
DS1
DS2
DS3
PSL
PSR
Plates


PRIB phenotype correlations
jaw
opercle
teeth
branchial
rakers
median fin
pelvic spine
SL
depth
FW
JW
PML
PMH
DL
AL
OPL
OPW
TVTP
TDTP1
LDTP2
BH
CB1
CB2
CB3
CB4
CB5
EB
Lr1C
Lr2C
Lr3C
Lr4C
Lr5C
Lr6C
Lr7C
Lr8C
Lr9C
C
DS1
DS2
DS3
PSL
PSR
Plates

ENOB phenotype correlations



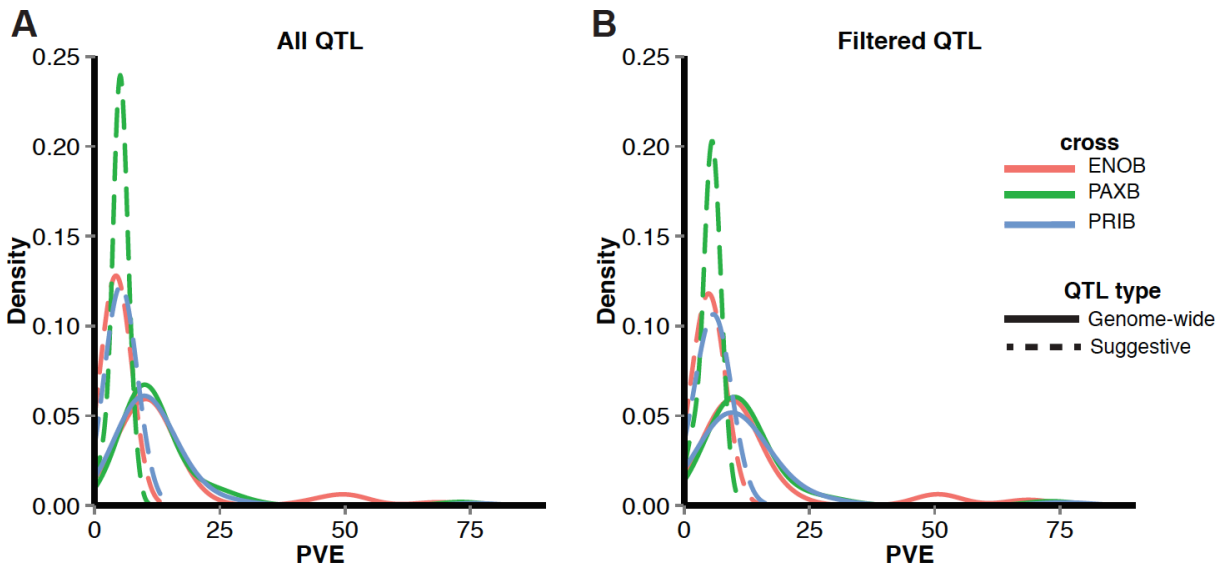


Figure S2: Distribution of QTL effect sizes. Density curves for percentage of phenotypic variance explained (PVE) in each cross. Genome-wide QTL are indicated with solid lines and suggestive parallel QTL are indicated with dashed lines. (A) All QTL for all phenotypes. (B) Filtered QTL. Filtering QTL did not dramatically change the PVE distribution. Red = ENOB, green = PAXB, blue = PRIB.

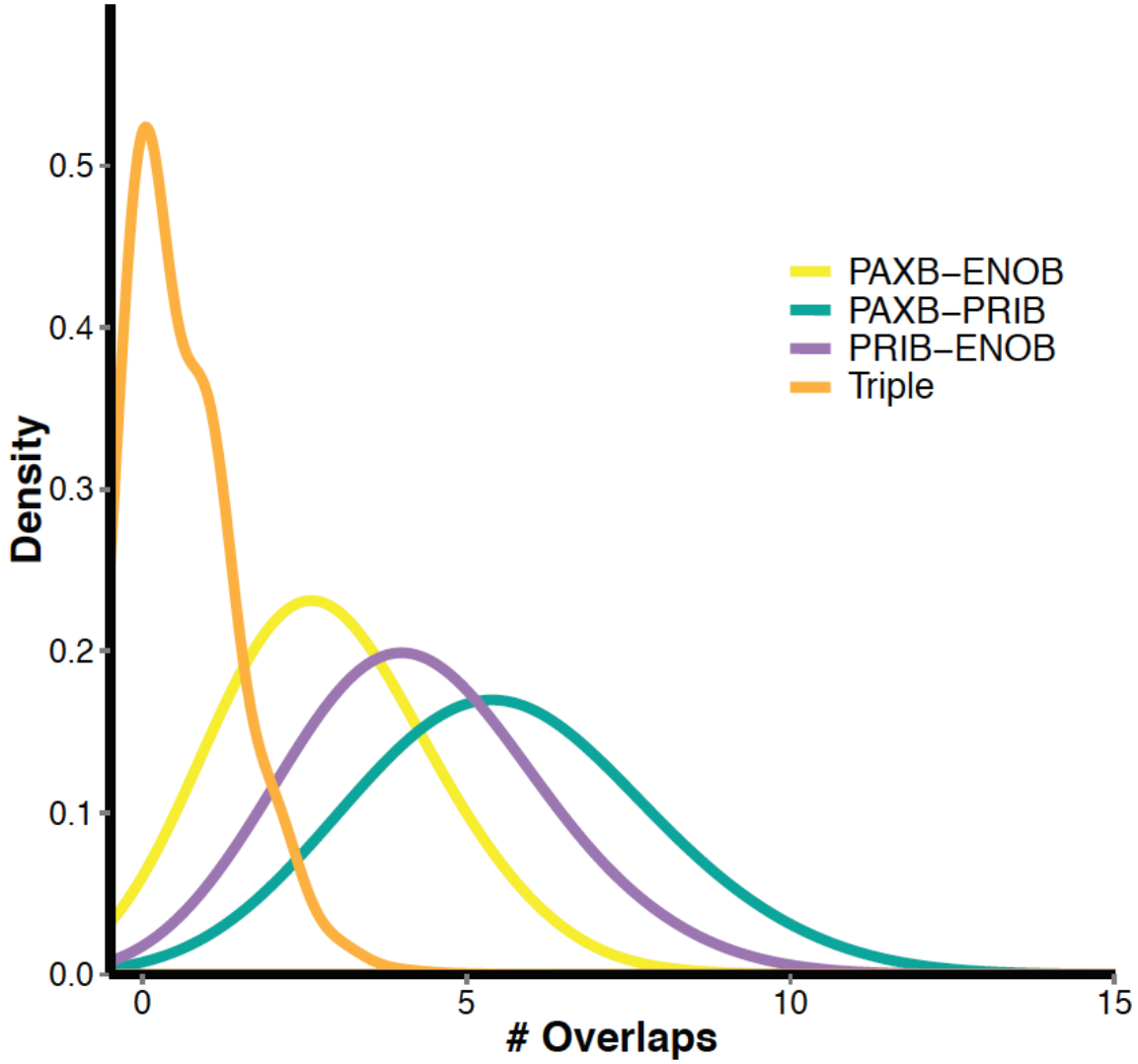


Figure S3: Results of QTL overlap simulations. Density plots of the number of simulated overlaps for all QTL at a genome wide significance threshold (LOD = 3.7) are shown based on 10,000 permutations without replacement for each trait category. These distributions were used to calculate the statistical significance of the detected overlapping QTL.

Figure S4: Overview of all suggestive parallel QTL relative to genome-wide QTL. (begins on next page). Chromosomes 1-21 are separated by vertical lines and numbered below. On each chromosome, QTL for PAXB, PRIB, and ENOB are indicated from left to right and labeled on top. Horizontal lines separate trait categories. Color intensity indicates the detection threshold: dark colors indicate genome-wide QTL; light colors indicate suggestive parallel QTL, and white indicates no detected QTL (see key). Red indicates skeletal gain QTL (freshwater allele confers more bone), blue indicates skeletal loss QTL (freshwater allele confers less bone), and grey indicates QTL with no difference between homozygous genotypes.

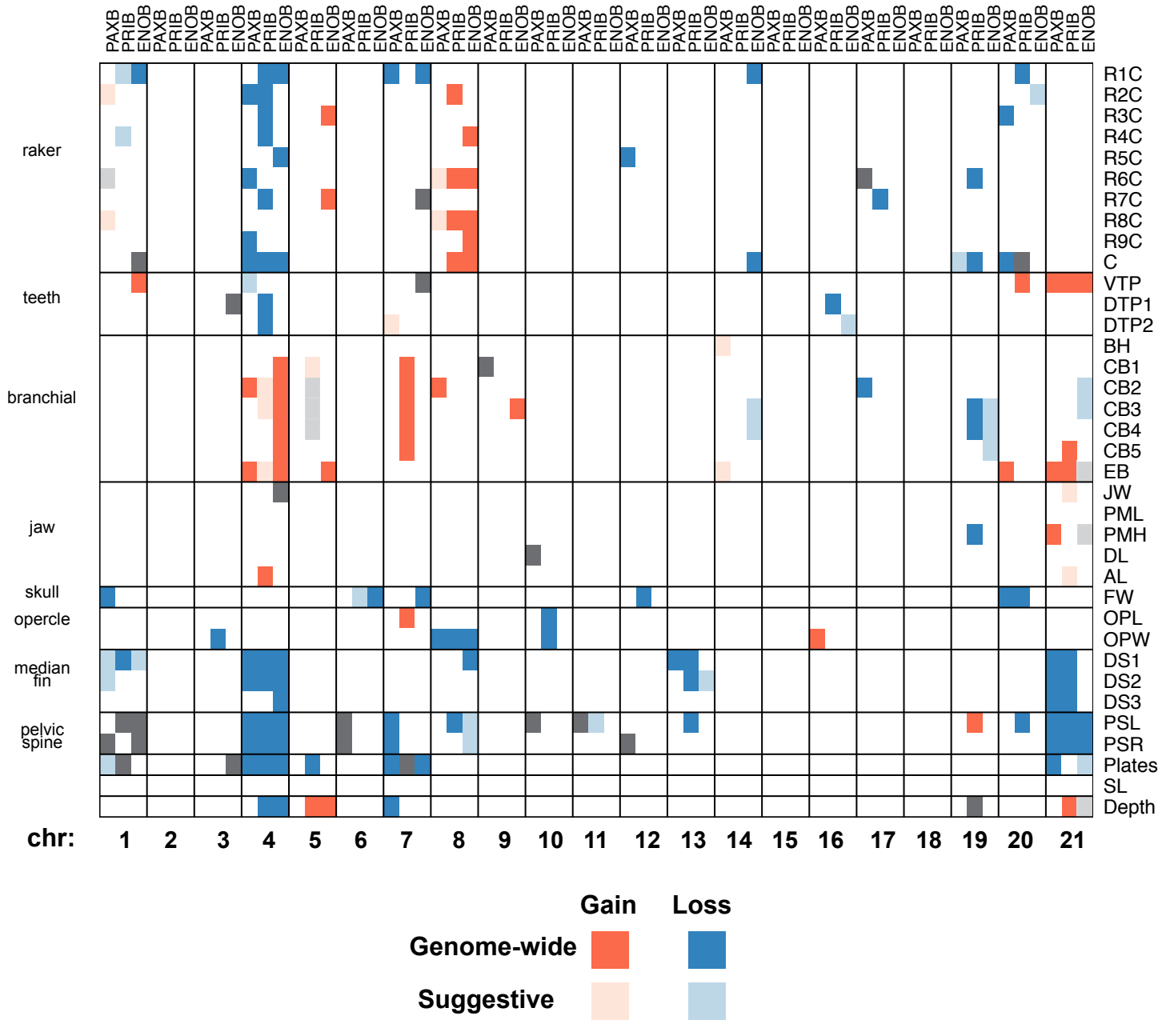
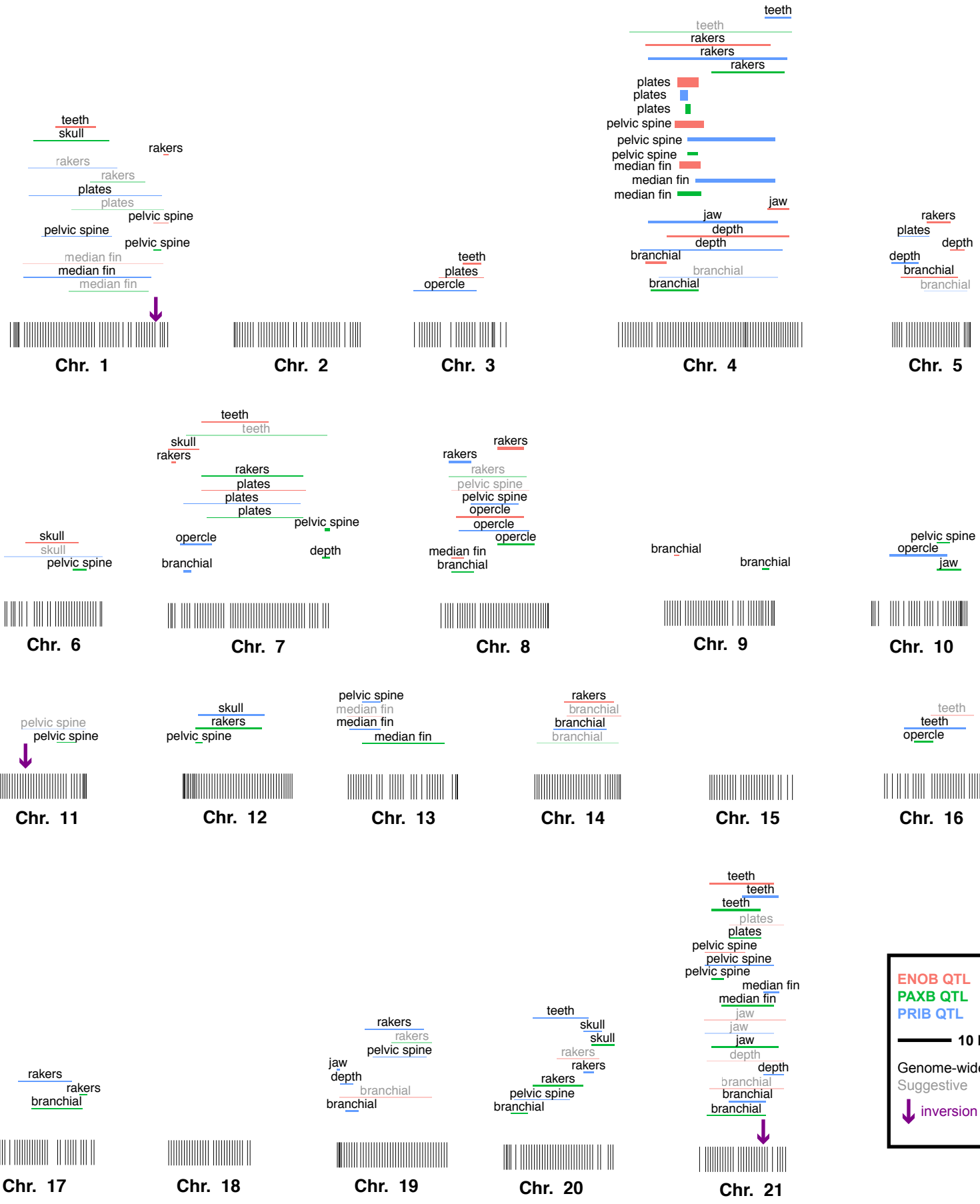

PAXB
PRIB
ENOB
raker
teeth
branchial
jaw
skull
opercle
median fin
pelvic spine
R1C
R2C
R3C
R4C
R5C
R6C
R7C
R8C
R9C
C
VTP
DTP1
DTP2
BH
CB1
CB2
CB3
CB4
CB5
EB
JW
PML
PMH
DL
AL
FW
OPL
OPW
DS1
DS2
DS3
PSL
PSR
Plates
SL
Depth
chr:
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Gain
Loss
Genome-wide
Suggestive

Figure S5: Location of all filtered QTL. (begins on next page) The physical position of each GBS marker is indicated as a vertical grey line. The physical size of the 1.5-LOD interval for each QTL is indicated with a horizontal line. The thickness of the line is proportional to the PVE of each QTL and each QTL is labeled with its trait category. Genome-wide QTL are indicated with black text and bold colors and suggestive parallel QTL are indicated with grey text and light colors. Red = ENOB, green = PAXB, blue = PRIB. The positions of three previously identified chromosomal inversions showing marine-freshwater ecotype-specific allele frequencies (Jones et al. 2012b) are marked with purple arrows.

Figure S5


teeth
skull
rakers
rakers
rakers
plates
plates
pelvic spine
pelvic spine
pelvic spine
median fin
median fin
median fin
Chr. 1
Chr. 2
teeth
plates
opercle
Chr. 3
teeth
teeth
rakers
rakers
rakers
plates
plates
plates
pelvic spine
pelvic spine
pelvic spine
median fin
median fin
median fin
jaw
jaw
depth
depth
branchial
branchial
branchial
Chr. 4
rakers
plates
depth
depth
branchial
branchial
Chr. 5
skull
skull
pelvic spine
Chr. 6
teeth
teeth
skull
rakers
rakers
plates
plates
plates
pelvic spine
opercle
depth
branchial
Chr. 7
rakers
rakers
rakers
pelvic spine
pelvic spine
opercle
opercle
opercle
median fin
branchial
Chr. 8
branchial
branchial
Chr. 9
pelvic spine
opercle
jaw
Chr. 10
pelvic spine
pelvic spine
Chr. 11
skull
rakers
pelvic spine
Chr. 12
pelvic spine
median fin
median fin
median fin
Chr. 13
rakers
branchial
branchial
branchial
Chr. 14
Chr. 15
teeth
teeth
opercle
Chr. 16
rakers
rakers
branchial
Chr. 17
Chr. 18
rakers
rakers
pelvic spine
jaw
depth
branchial
branchial
Chr. 19
teeth
skull
skull
rakers
rakers
rakers
pelvic spine
branchial
Chr. 20
teeth
teeth
teeth
plates
plates
pelvic spine
pelvic spine
pelvic spine
median fin
median fin
jaw
jaw
jaw
depth
depth
branchial
branchial
branchial
Chr. 21
ENOB QTL
PAXB QTL
PRIB QTL
10 Mb
Genome-wide
Suggestive
inversion

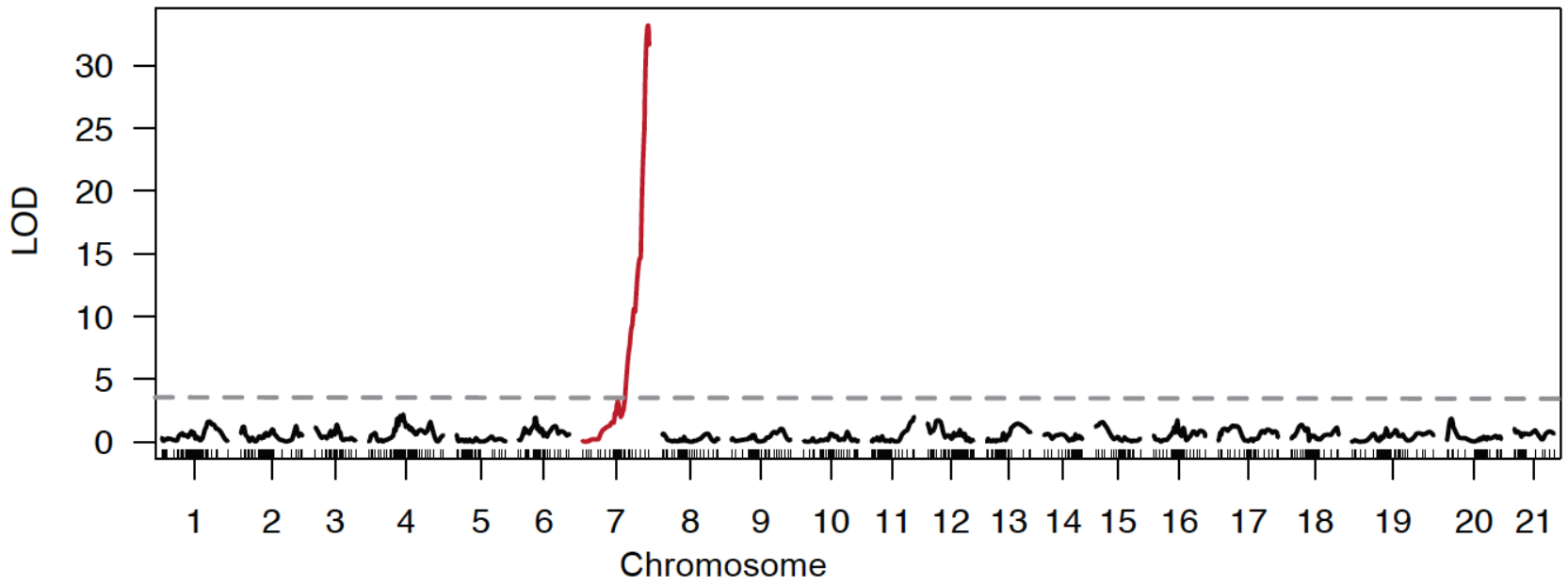


Figure S6: Pelvic spine presence/absence maps to chromosome 7 in PAXB. Spine presence or absence was mapped as a binary trait using the *scanone* function in R/qtl. A single QTL (LOD = 32.4) was detected on the right end of chromosome 7 (red line). Grey dashed line indicates the genome-wide significance threshold of 3.7. *Pitx1,* shown to control pelvic spine presence/absence, is located at this right end of chromosome 7 (Shapiro et al. 2004; Chan et al. 2010)

Supplemental Tables:

Cross	F2 family	# F2s	mean SL (mm)	st. dev. SL (mm)
ENOB	G1-5	180	40.8	3.2
PAXB	G3-2	94	43.5	3.8
PAXB	G3-4	92	42.2	3.6
PRIB	G2-13	90	40.8	2.7
PRIB	G2-18	90	43.3	3

Table S1: Description of F2 families included in analysis. Phenotypes were corrected for size and standard length within families, and then families were combined within each cross for QTL analysis. Ten fish with either more than 50% failed genotypes (8) or duplicate genotypes (2) were excluded from the phenotypic analysis.

Table S2 (continued on next page):

Phenotype	Description	Category	ENOB	PAXB G3-2	PAXB G3-4	PRIB G2-13	PRIB G2-18	penalty
SL	standard length (mm)	SL	NA	NA	NA	NA	NA	yes
depth	body depth (mm)	depth	Size, Log	Size, Sex	Size	Size, Sex	Size	yes
FW	frontal width (mm)	skull	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size	yes
JW	jaw width (mm)	jaw	Size	Size	Size, Sex	Size, Sex	Size, Sex	yes
PML	premaxillary length (mm)	jaw	Size, Sex, Log	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
DL	dentary length (mm)	jaw	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
AL	articular length (mm)	jaw	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
PMH	premaxillary height (mm)	jaw	Size	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
OPL	opercle length (mm)	opercle	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
OPW	opercle width (mm)	opercle	Size, Sex, Log	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
Plates	left lateral plate count	plates	NA	NA	NA	NA	NA	yes
VTP	total ventral pharyngeal tooth count	teeth	Sex	NA	NA	Size	Size, Sex	yes

Phenotype	Description	Category	ENOB	PAXB G3-2	PAXB G3-4	PRIB G2-13	PRIB G2-18	penalty
DTP1	total dorsal pharyngeal tooth plate 1 count	teeth	Sex	NA	Sex	Size, Sex	Size, Sex	yes
DTP2	left dorsal pharyngeal tooth plate 2 count	teeth	Sex	NA	NA	Size	Size, Sex	yes
BH	basihyal length (mm)	branchial	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
CB1	ceratobranchial 1 length (mm)	branchial	Size, Sex	Size, Sex	Size	Size, Sex	Size, Sex	no
CB2	ceratobranchial 2 length (mm)	branchial	Size, Sex	Size, Sex	Size	Size, Sex	Size, Sex	no
CB3	ceratobranchial 3 length (mm)	branchial	Size, Sex	Size, Sex	Size	Size, Sex	Size, Sex	no
CB4	ceratobranchial 4 length (mm)	branchial	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size, Sex	no
CB5	ceratobranchial 5 length (mm)	branchial	Size, Sex	Size	Size	Size, Sex	Size, Sex	yes
EB	epibranchial 1 length	branchial	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size, Sex	yes
R1C	left row 1 cerato gill raker count	rakers	NA	Size	NA	NA	NA	no
R2C	left row 2 cerato gill raker count	rakers	NA	Sex	NA	NA	Sex	no
R3C	left row 3 cerato gill raker count	rakers	NA	NA	NA	NA	Sex	no
R4C	left row 4 cerato gill raker count	rakers	Sex	Sex	Sex	Sex	Sex	no
R5C	left row 5 cerato gill raker count	rakers	NA	Sex	Sex	NA	Sex	no
R6C	left row 6 cerato gill raker count	rakers	NA	Size	Sex	NA	NA	no
R7C	left row 7 cerato gill raker count	rakers	Sex	Size, Sex	Sex	NA	Sex	no
R8C	left row 8 cerato gill raker count	rakers	NA	Size	NA	NA	Sex	no
R9C	left row 9 cerato gill raker count	rakers	NA	NA	NA	NA	NA	no
C	total left row 1 through 9 cerato gill raker count	rakers	NA	Size, Sex	Sex	NA	Sex	yes
DS1	dorsal spine 1 length (mm)	median fin	Size, Sex	Size	Size	Size	Size	yes
DS2	dorsal spine 2 length (mm)	median fin	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size	yes
DS3	dorsal spine 3 length (mm)	median fin	Size, Sex	Size, Sex	Size, Sex	Size, Sex	Size, Sex, Log	yes
PSL	left pelvic spine length (mm)	pelvic spine	Size, Sex	NA	Size, Sex	Size	Size	yes
PSR	right pelvic spine length (mm)	pelvic spine	Size, Sex	NA	Size, Log	Size	Size, Sex	no

Table S2. Statistical corrections applied to each phenotype in each F2 family. Phenotypes were tested for a significant association with sex and standard length (size) in a linear model. All size-corrected residuals were back-transformed to a 40 mm fish. NA indicates that no statistical corrections were applied and raw phenotypic values were used. "Penalty" indicates whether the phenotype was used to calculate *scantwo* penalties via 100 permutations of the phenotype data.

	PAXB	PRIB	ENOB	LCM
Number of barcodes used for grandparents	6	9	6	11
Grandparent mapped reads	12,488,892	15,963,364	20,486,268	21,305,328
SNPs called in grandparents	133,228	140,604	157,821	-
Homozygous different SNPs in grandparents	81,396	81,618	93,722	-
F2s sequenced	188	183	184	-
F2 mapped reads	133,029,878	130,366,646	124,438,779	-
Average reads per F2	707,606	716,300	676,298	-
High quality SNPs in F2s	60,715	59,358	64,061	-
Initial number of sex chromosome bins	36	35	35	-
Initial number of autosomal bins	1,063	1,060	1,049	-
High quality binned markers (including sex chromosomes)	933	932	960	-
Final shared markers in analysis	805	805	805	-
Number F2s dropped (failed or duplicate genotypes)	2	3	4	-

Table S3. Data for processing of Illumina reads into genotypes for 3 crosses. Genomic DNA of grandparents and F2s was digested with ApeKI and barcoded with unique barcodes for genotyping-by-sequencing (GBS). Libraries were sequenced in two Illumina HiSeq2000 lanes with 384 barcodes per lane. Reads were mapped to the stickleback genome and processed using a custom pipeline (Glazer et al., 2015) to create binned markers with a maximum bin length of 500 kb. A shared set of markers informative in all three crosses was used for QTL mapping. LCM = Little Campbell Marine grandfather used to call SNPs in all three crosses.

Table S4 (.xlsx file) All QTL detected at genome wide LOD cutoff (3.7). Marker names follow Glazer et al. 2015 and indicate scaffold number and bin number. Physical positions of the peak and 1.5 LOD interval markers are based on the revised assembly. "Filtered" indicates that the QTL was the highest PVE QTL for a trait category on the chromosome. MM, MF, and FF indicate fish that were homozygous marine, heterozygous, and homozygous freshwater, respectively, for the peak marker. SD = standard deviation.

Table S5 (.xlsx file) All QTL including suggestive QTL. All QTL locations and effect sizes were recalculated with suggestive QTL included in the model. Marker names follow Glazer et al. 2015 and indicate scaffold number and bin number. Physical positions of the peak and 1.5 LOD interval markers are based on the revised assembly. "QTL Type" indicates whether QTL was detected at the genome wide LOD threshold (3.7) or was suggestive. "Filtered" indicates that the QTL was the highest PVE QTL for a trait category on the chromosome. MM, MF, and FF indicate fish that were homozygous marine, heterozygous, and homozygous freshwater, respectively, for the peak marker. SD = standard deviation.

QTL type	observed	mean simulated	2.5% simulated	97.5% simulated	*P*(simulated ≥ observed)
PAXB unique	5	5.6	2	9	0.7286
PRIB unique	2	4.3	1	8	0.9679
ENOB unique	5	3.8	1	7	0.3241
PAXB-PRIB overlapping	7	6.6	3	11	0.5057
PAXB-ENOB overlapping	2	4.1	1	8	0.9485
PRIB-ENOB overlapping	3	3.8	1	7	0.7818
triple overlapping	5	3.3	1	6	0.1888

Table S6: Results of QTL overlap simulations without respect to QTL category. QTL were filtered so that only the largest effect QTL covering any given chromosome region was counted. The physical locations of the filtered QTL were randomly simulated 10,000 times and tested for overlap between crosses in each simulation, regardless of trait category of the QTL. The total number of pairwise overlaps and triple overlaps was counted. The mean, 2.5 and 97.5 percentiles of permuted overlapping QTL are presented. The *P*-value was calculated based on the number of simulations with an equal or greater number of overlaps than the actual observed overlaps.

	Signals of Selection	
QTL set:	**Marine-freshwater**	**Benthic-limnetic**
PAXB unique (n=15)	1.24 (0.26)	2.26 (**0.0078**)
PRIB unique (n=15)	1.35 (0.18)	1.16 (0.0555)
ENOB unique (n=11)	0.55 (0.75)	1.03 (0.3676)
all double (n=23)	2.00 (**0.0001**)	1.59 (**0.0053**)
all triple (n=14)	2.68 (**<0.0001**)	2.04 (**0.0003**)

Table S7: Shared suggestive QTL are enriched for marine-freshwater and benthic-limnetic signals of selection. The number of overlaps between signals of selection and benthic QTL (including suggestive QTL) were counted and compared to 10,000 random simulations of the QTL locations. Values given are the fold enrichment followed by the *P*-value in parentheses. Marine-freshwater signals of selection based on Jones et al (2012b) and benthic-limnetic signals of selection based on Jones et al (2012a).

Table S8 (.xlsx file) Locations of all double and triple QTL detected at genome-wide cutoff. Phenotypes, peak markers, and 1.5 LOD intervals are indicated for each overlapping QTL. Additional information about each QTL can be found in Table S4. Note that two double overlapping QTL shared one QTL in common: the PAXB pelvic spine QTL on chromosome 1 overlapped with both PRIB and ENOB, but PRIB and ENOB did not overlap each other.

Table S9 (.xlsx file) Locations of all double and triple QTL detected including suggestive QTL. Phenotypes, peak markers, and 1.5 LOD intervals are indicated for each overlapping QTL. QTL type indicates whether QTL was detected at a genome-wide (3.7) or suggestive (2.0) LOD threshold. Additional information about each QTL can be found in Table S5.

Supplemental File 1 (.xlsx file) All data used for QTL mapping. Data are separated on tabs for each cross. Raw phenotypes are the actual measured phenotypes, corrected phenotypes were corrected for size, sex, normality of residuals, and increasing variance as described in Table S2. Corrected phenotypes were used for QTL mapping; raw phenotypes were used to generate Figure S1. Genotypes are in R/qtl format; the first row has marker names, the second row has marker position in cM, and the third row has the chromosome number, and each following row is an individual fish.