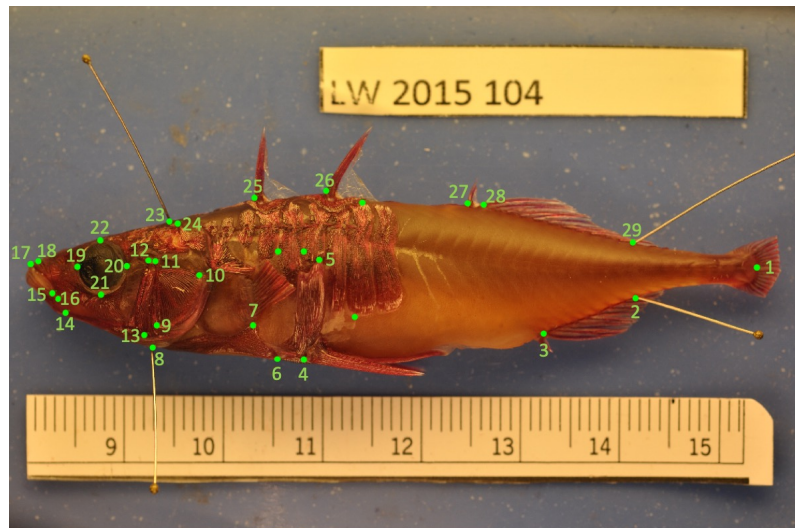


Figure S1. Twelve neuromast lateral lines were phenotyped using either DASPEI or alkaline phosphatase staining to identify and count neuromasts. (A) Lateral lines were identified following Wark and Peichel (2010). The twelve lines are: AP-anterior pit; CF-caudal fin; ET-ethmoid; IO-infraorbital; Ma-anterior main trunk; MD-mandibular; Mp-posterior main trunk; OR-oral; OT-otic; PO-preopercular; SO-supraorbital; ST-supratemporal. (B) Fish were stained with alkaline phosphatase in custom staining chambers made from 50mL conical tubes, nylon mesh and pipette tip refill wafers. These chambers fit 3 fish at a time with their ID tags, and washes were easily exchanged through the mesh without opening the tubes. (C) A representative photo of alkaline phosphatase staining of neuromasts (arrowheads) and Alizarin red staining of bone at the intersection of the PO and MD lines.



Landmark	Description
1	Posterior extent of caudal peduncle
2	Posterior insertion of anal fin
3	Anterior insertion of anal fin
4	Insertion point of pelvic spine into the pelvic girdle
5	Dorsal extent of the ascending branch of the pelvis
6	Posterior extent of ectocoracoid
7	Dorsal extent of ectocoracoid
8	Anterior extent of ectocoracoid
9	Ventral extent of operculum
10	Posteriordorsal extent of operculum
11	Anteriodorsal extent of operculum
12	Dorsal extent of preopercular
13	Posteroventral extent of preopercular
14	Anterioventral extent of preopercular
15	Posterior extent of premaxilla
16	Posterior extent of maxilla
17	Anterior-most extent of the premaxilla
18	Anterior extent of maxilla
19	Anterior extent of orbit
20	Posterior extent of orbit
21	Ventral extent of orbit
22	Dorsal margin of the orbit in line with the eye's midpoint
23	Supraoccipital notch lateral to the dorsal midline
24	Posterior extent of supraoccipital
25	Anterior insertion of first dorsal spine
26	Anterior insertion of second dorsal spine
27	Anterior insertion of third dorsal spine
28	Anterior insertion of the dorsal fin at the first soft ray
29	Posterior insertion of dorsal fin at the first soft ray

Figure S2. Landmark positions and descriptions used for geometric morphometrics. Landmarks were chosen based on previous QTL mapping studies between marine and freshwater fish (Table S2).

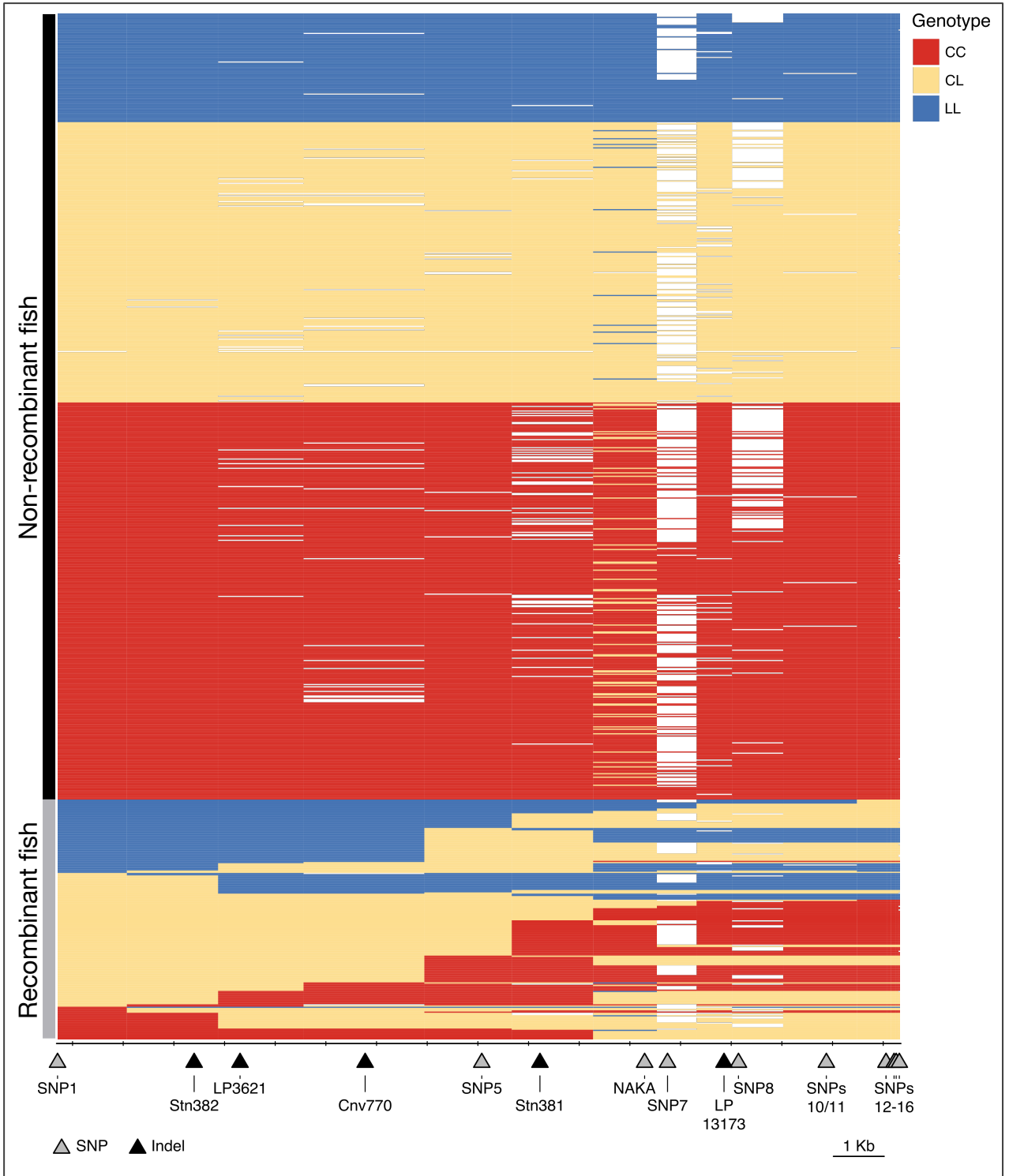


Figure S3. Visual genotypes of Lake Washington fish reveal historical recombination events and frequency of NAKA SNP. Wild-caught adult stickleback from Lake Washington (n = 885) were genotyped at a subset of 16 SNPs or indels across the haplotype and SNP1. Most fish showed no evidence of recombination within the 16 kb haplotype (top). However, we did find evidence of at least one historical recombination event within the haplotype in 198, or 22%, of the fish (bottom, same as Figure 5). In addition to visualizing recombination breakpoints, this figure highlights the frequency of the “L” allele of the NAKA SNP on an otherwise “C” haplotype. For example, we see complete correspondence between NAKA and the other genotypes within the LL non-recombinant fish (blue, top). However, within the generally CL non-recombinant fish, there are 12 blue LL genotypes at NAKA (n = 12 of 244 C haplotypes, 4.9%). Within the generally CC non-recombinant fish there are 56 fish CL at NAKA (n = 56 of 694 C haplotypes, 8.1%). The frequencies of the L allele at the NAKA SNP on generally C haplotype is 9.4% in Puget Sound (Figure 2) and 7.2% in Lake Washington. The markers are depicted as triangles at their physical location within the haplotype and labelled at the bottom of the figure. Tick marks start at the beginning of intron 1 of *Eda* (the presumed start of the haplotype) and are spaced every 1000 bases for scale. Genotypes are represented visually as CC (homozygous for the completely-plated allele), CL (heterozygous) or LL (homozygous for the low-plated, typically freshwater, allele).

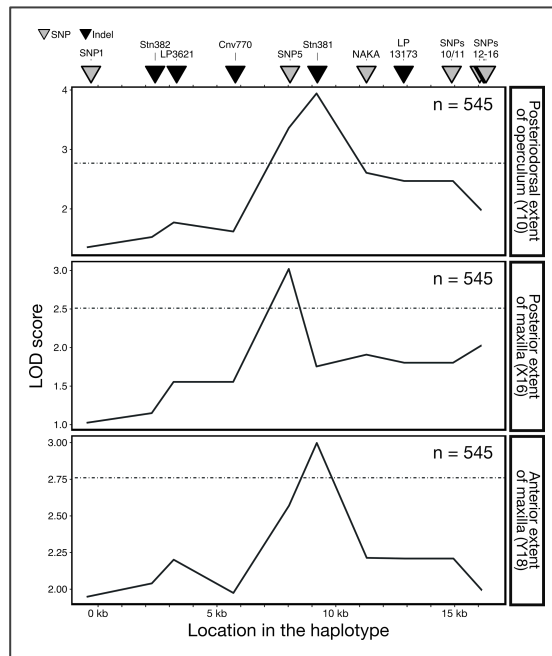


Figure S4. Association mapping of geometric morphometric traits in Lake Washington stickleback reveals three traits that are associated with the *Eda* haplotype. The strength of association between each marker and each phenotype was calculated as a log odds likelihood (LOD) score compared with the model of no association between marker and phenotype. These LOD curves are plotted for three geometric morphometric landmarks that are significant following correction for multiple comparisons (Figure S2). Posteriorodorsal extent of the operculum (Y10) and anterior extent of maxilla (Y18) map most strongly to Stn381. The posterior extent of the maxilla (X16) maps most strongly to SNP5. These patterns of association are consistent with either separate, linked causative mutations, or a single pleiotropic mutation between SNP5 and Stn381. The dashed lines represent the LOD threshold.

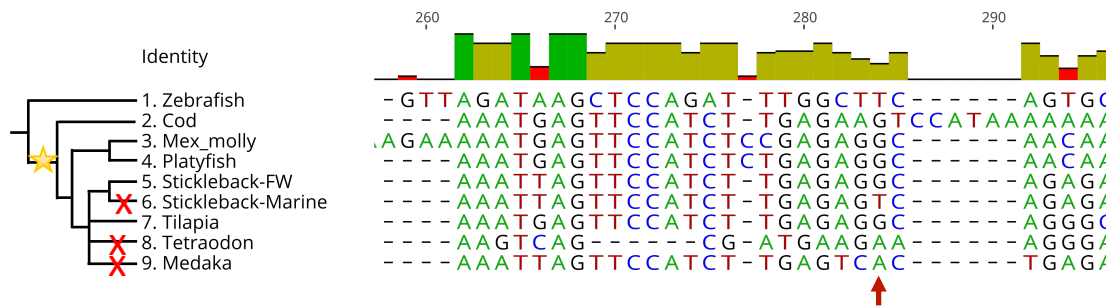


Figure S5. NAKA SNP near *Eda* is conserved across most percomorph species and likely lost in marine stickleback. Alignment of intergenic region downstream of *Eda*. Downstream genomic sequences were retrieved from NCBI using the stickleback *Eda* sequence as a query. Sequences were aligned using VISTA and corrected at the locus by eye. Zebrafish and cod are not members of the percomorph clade, yet cod shares a “G” at the NAKA SNP (red arrow) with other percomorphs (including freshwater, low-plated stickleback), suggesting that the NAKA SNP arose in the common ancestor of cod and percomorphs (yellow star). Three percomorphs do not have a “G” at the NAKA SNP position, suggesting it was lost in marine stickleback, tetraodon and medaka (red X’s).

A

Left plate count	SNP1	Stn382	LP3621	Cnv770	SNP5	Stn381	NAKA	SNP7	LP13173	SNP8	SNPs10/11	SNP12	SNP13	SNP14	SNP15	SNP16
7	CL	CL	LL	LL	LL	LL	LL	-	LL	LL	LL	LL	LL	LL	LL	LL
*	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
*	CL	CL	LL	LL	LL	LL	LL	-	LL	LL	LL	LL	LL	LL	LL	LL
7	LL	LL	LL	LL	LL	LL	LL	-	LL	LL	LL	CL	CL	CL	CL	CL
7	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
→	LL	LL	LL	CL	CL	CL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL
7	LL	LL	LL	LL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
7	CL	LL	LL	-	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
7	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	CL	CL	CL	CL
7	CC	LL	LL	LL	LL	LL	LL	-	-	LL	LL	LL	LL	LL	LL	LL
7	LL	LL	LL	LL	LL	LL	LL	-	LL	LL	LL	LL	CL	CL	CL	CL
7	LL	LL	LL	LL	CL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL	CL
8	LL	LL	LL	LL	LL	LL	LL	CL	CL	CL	CL	CL	CL	CL	CL	CL
7	LL	LL	LL	LL	LL	LL	LL	LL	CL	CL	CL	CL	CL	CL	CL	CL
7	LL	LL	LL	LL	LL	LL	LL	LL	CL	CL	CL	CL	CL	CL	CL	CL
8	LL	LL	LL	LL	LL	CL	CL	-	CL	-	CL	CL	CL	CL	CL	CL
7	CL	CL	LL	LL	LL	LL	LL	-	-	-	LL	LL	LL	LL	LL	LL
8	CL	LL	LL	LL	LL	LL	LL	-	LL	-	LL	LL	LL	LL	LL	LL
8	LL	LL	LL	LL	LL	LL	LL	-	LL	LL	LL	CL	CL	CL	CL	CL
8	LL	LL	LL	LL	LL	LL	LL	CL	CL	CL	CL	CL	CL	CL	CL	CL
8	LL	LL	LL	LL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
8	LL	LL	LL	LL	LL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	-
*	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
9	LL	LL	LL	LL	CL	CL	CL	-	CL	-	CL	CL	CL	CL	CL	CL
9	LL	LL	LL	LL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
*	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
9	LL	LL	LL	LL	LL	LL	LL	LL	CL	CL	CL	CL	CL	CL	CL	CL
9	LL	LL	LL	LL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
10	LL	LL	LL	LL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
9	CL	CL	LL	LL	LL	LL	LL	-	LL	LL	LL	LL	LL	LL	LL	LL
10	LL	LL	LL	LL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
10	LL	LL	LL	LL	LL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL	CL
11	LL	LL	LL	LL	LL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL	CL
10	LL	LL	LL	LL	LL	CL	CL	-	CL	CL	CL	CL	CL	CL	CL	CL
#	LL	LL	CL	CL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
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#	LL	LL	CL	CL	CL	CL	LL	LL	-	LL	LL	LL	LL	LL	LL	LL
9	LL	LL	CL	CL	CL	CL	LL	LL	LL	LL	-	LL	LL	LL	LL	LL
#	LL	LL	CL	CL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL
10	LL	LL	CL	CL	CL	CL	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL

B

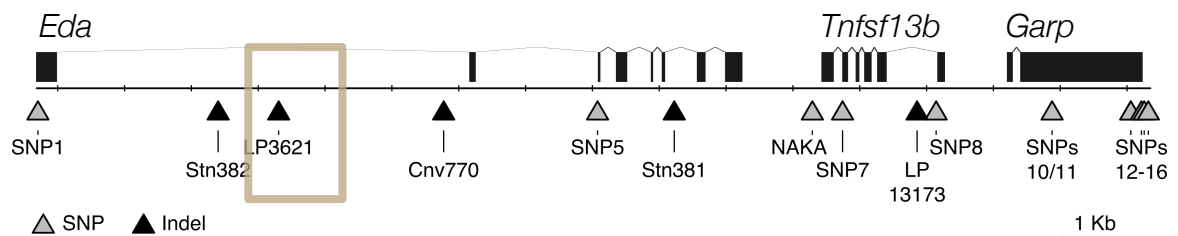


Figure S6. Recombination breakpoints in low-plated Lake Washington fish narrows the region containing the causative mutation to 1400 base pairs. Genotypes of low-plated fish at the 16 screened markers are shown for fish with evidence of at least one historical recombination. Together, these genotypes suggest that the causative allele is located between Stn382 and LP3621. Cloning and sequencing of five fish an asterisk next to their plate count identified the 5' recombination breakpoint at base pair 12,803,450 of the *gasAcu1* genome. Including the sequences of four fish with a pound sign next to their plate count found no mutation for which these fish all carried two copies of the freshwater allele. We therefore conclude that the six fish on the bottom of the table may carry a unique set of marine alleles and refocused on the region between LP3621 and *Cnv770*, since all other low-plated fish are homozygous for the freshwater allele at LP3621. A single fish (arrow) recombines between LP3621 and *Cnv770*. This recombination breakpoint was mapped to base pair 12,804,851 in the *gasAcu1* genome by cloning the recombinant allele. Therefore, the causative mutation lies within a region of 1,401 base pairs, marked with a box in (B).

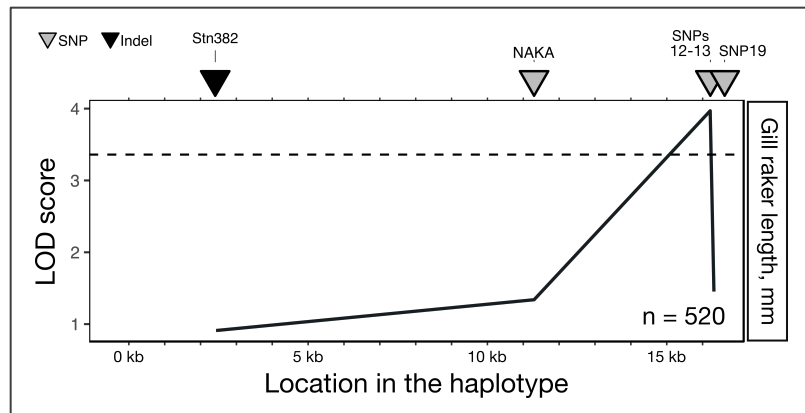


Figure S7. Gill raker length maps to SNPs12/13 in Puget Sound. Gill raker length was significantly associated with SNPs12/13 in the Puget Sound crosses when only Stn382 and SNPs12/13 were considered. In Lake Washington, the strongest signal of association between genotype and gill raker length was at the NAKA SNP (Figure 6). To assess the association between the NAKA SNP and gill raker length in Puget Sound, we genotyped the F1 offspring at the NAKA SNP, which was variable in two of the crosses, and reran the analysis. This confirmed the first analysis- the association of gill raker length and genotype is driven by the genotype at SNPs12/13 in Puget Sound, not the NAKA SNP or something outside of the haplotype (SNP19). The dashed line represents the LOD threshold.

Marker name	Description	Location on chr.IV of Broad/gasAcu1 genome assembly	Location in haplotype ²		Forward primer (5' - 3')	Reverse primer (5' - 3')	Genotyping method	Restriction enzyme	Population(s) screened
			Forward primer (5' - 3')	Reverse primer (5' - 3')					
Cnv76 ²	Intergenic FW insertion (68bp)	12,790,042-12,790,110	-	-	TGTTGTGCAGTCGGTGCCAGT	GGCTTCATTGTGCCTGGTGCCCTT	PCR	-	PS / -
SNP1 ¹	Nonsynonymous SNP in <i>Eda</i>	12,800,238	-	-	AGATGCCGAGGTAGAGAGCA	GTAGCAGACGAGCGTGACAG	Restriction digest	BtsCI	-- / LW
Stn382 ²	Intronic FW deletion (66bp)	12,802,847	2,400	-	CCCTTAGAGAATTTCTCAGCAG	CTTGCCCGGATCATACCC	PCR	-	PS / LW
LP3621	Intronic FW deletion (16bp)	12,803,862	3,306	-	CCTGGTGGACGGATAGAGCA	ATGTTGCTGTGACGACCA	PCR	-	-- / LW
Cnv770 ²	Intronic FW insertion (107bp)	12,806,052-12,806,138	5,775	-	AGGCATCGCCTCAGCTTGA	TCGCCCGTGAAGTATGCCCC	PCR	-	-- / LW
SNP5 ²	Nonsynonymous SNP in <i>Eda</i>	12,808,303	8,077	-	GTTCAGGAGAAGCTTTCAAGCT	GCCGCTTTTCCCTGTGAAG	Restriction digest	TaqI	-- / LW
Stn381 ²	Intronic FW insertion (19bp)	12,809,414-12,809,601	9,225	-	CGCTACACACGGACTTACA*	ATTGAGGGTTTCAAGCTTGG	PCR	-	-- / LW
NAKA.SNP ³	Intergenic SNP	12,811,481	11,291	-	TTGGACACTGCTGGCAGGG	TCACACTCTGTTAACCCTGGGA	Restriction digest	MnII	-- / LW
SNP7 ²	Nonsynonymous SNP in <i>Trnsf13b</i>	12,811,933	11,743	-	AGGATGTAGTCATCTGGACATTGT	CTTCAGAGAAGCTCAGACTGTTTGTG	MAMA PCR - FW allele	-	-- / LW
LP13173	Intronic FW insertion (20bp)	12,813,043-12,813,066	12,858	-	AGGATGTAGTCATCTGGACATTGT	TCAGAGAAGCTCAGACTGTTTGGC	MAMA PCR - Marine allele	-	-- / LW
SNP8 ²	Synonymous SNP in <i>Trnsf13b</i>	12,813,328	13,143	-	TACGTAGTTAGCTGCTGGC	AAATGAGGGAATGGGGCCTG	PCR	-	-- / LW
					CAAACAGCTTCAGAGACTGTGC	CCTGAAGCAGCTGCACAAGA	MAMA PCR - FW allele	-	-- / LW
					CAAACAGCTTCAGAGACTGTGC	CCTGAAGCAGCTGCACAAGA	MAMA PCR - Marine allele	-	-- / LW
SNPs10/11 ²	Nonsynonymous SNP in <i>Garp</i>	12,815,024	14,878	-	ACGACTTGGGTGATGATGCA	TCAAGCTGTACAACCTGGTCA	Restriction digest	AluI	-- / LW
SNP12 ²	Nonsynonymous SNP in <i>Garp</i>	12,816,201	16,055	-	TCTGCTACCAAGCAGTTTGA	AGTTTGCTATTACAGTTTCTCAC	Sequencing in LW	Mfe-HF in PS	PS / LW
SNP13 ²	Synonymous SNP in <i>Garp</i>	12,816,202	16,056	-	TCTGCTACCAAGCAGTTTGA	AGTTTGCTATTACAGTTTCTCAC	Sequencing in LW	Mfe-HF in PS	PS / LW
SNP14 ²	Nonsynonymous SNP in <i>Garp</i>	12,816,360	16,214	-	TCTGCTACCAAGCAGTTTGA	AGTTTGCTATTACAGTTTCTCAC	Sequencing	-	-- / LW
SNP15 ²	SNP in <i>Garp</i> 3' UTR	12,816,402	16,256	-	TCTGCTACCAAGCAGTTTGA	AGTTTGCTATTACAGTTTCTCAC	Sequencing	-	-- / LW
SNP16 ²	SNP in <i>Garp</i> 3' UTR	12,816,464	16,318	-	TCTGCTACCAAGCAGTTTGA	AGTTTGCTATTACAGTTTCTCAC	Sequencing	-	-- / LW
SNP19 ²	Nonsynonymous SNP in <i>Gjb1</i>	12,826,854	-	-	CAGCTCATCTGGTCTCCAC	TCCGGTGATCTGGAATCTCT	Restriction digest	EcoO109I	PS / -
IDH ⁴	Y-chr. deletion in 3'UTR of <i>Idh</i>	-	-	-	GGGACGACGAAAGTTATTG	TTATCGTAGCCAGGAGATGG	PCR	-	PS / LW

¹Lowe et al. 2018; ²Colosimo et al. 2005; ³O'Brown et al. 2015; ⁴Peichel et al. 2004
*Redesigned primer; previously published primer sat on a polymorphic site in Lake Washington
⁵Beginning of haplotype is set at start of *Eda* intron 1

Table S1. Summary of genotyping assays used in this study. PCR-based assays were used to genotype fish at the listed SNPs and indels. Information on the markers and the PCR-based assays used to genotype fish at each marker are listed. PCR = length polymorphism analysis; MAMA PCR = mismatch amplification mutation assay; PS = Puget Sound crosses; LW = Lake Washington wild-caught adults.

Table S2. Summary of mapping results performed in this study. The marker with the highest LOD score for each trait is listed in each mapping population with the corresponding LOD score, the covariates used in the analysis and the PVE explained by the listed marker. The p-value listed for each trait and marker combination is calculated from 5000 permutations of the data and represent the proportion of permutations that have an equal or higher LOD score at that marker. The p-value threshold was adjusted using a Bonferroni correction for the number of traits tested for each mapping population. PS = Puget Sound crosses; LW = Lake Washington wild-caught adults; NAKA = Puget Sound crosses between fish heterozygous at the NAKA SNP only; PVE = percent variation explained. This table is provided as a separate Excel file.

Mapping population	Trait type	Trait name	n	Cnv770		SNPs 12/13		Cnv770		SNPs 12/13		Cnv770		SNPs 12/13		Cnv770		SNPs 12/13		
				LC	CL	LC	CL	LC	CL	LC	CL	LC	CL	LC	CL	LC	CL	LC	CL	LC
Puget Sound crosses	Lateral line	Mp (posterior main trunk line) neuromasts per segment	497	2.37 ± 0.04		2.10 ± 0.05		2.31 ± 0.03		1.78 ± 0.12		1.44 ± 0.06		1.32 ± 0.06						
	Lateral line	Dorsal-Ventral (D-V) neuromast patterning	498	0.99 ± 0.01		0.94 ± 0.01		0.96 ± 0.01		0.35 ± 0.03		0.22 ± 0.01		0.08 ± 0.01						
	Meristic	Left plate count	578	34.1 ± 0.2		33.3 ± 0.3		33.3 ± 0.2		17.0 ± 0.8		13.3 ± 0.3		9.0 ± 0.3						
	Metric	Middle gill raker length	571	1.64 ± 0.01		1.69 ± 0.02		1.64 ± 0.01		1.70 ± 0.04		1.66 ± 0.02		1.56 ± 0.02						
	Lateral line	Mp (posterior main trunk line) neuromasts per segment	460	2.20 ± 0.05	1.95 ± 0.13	1.90 ± 0.08		1.87 ± 0.05		1.83 ± 0.21		2.08 ± 0.41		1.55 ± 0.09		1.45 ± 0.06				
Lake Washington wild-caught	Lateral line	Dorsal-Ventral (D-V) neuromast patterning	460	0.93 ± 0.02	0.80 ± 0.06	0.74 ± 0.04		0.66 ± 0.02		0.32 ± 0.10		0.65 ± 0.18		0.16 ± 0.04		0.09 ± 0.03				
	Meristic	Left plate count	696	33.2 ± 0.4	29.9 ± 0.9	27.7 ± 0.6		26.1 ± 0.4		12.0 ± 1.3		21.9 ± 3.9		12.1 ± 0.7		8.5 ± 0.4				
	Morphometric	Posterior extent of operculum (Y10)	545	0.013 ± 0.001	0.012 ± 0.001	0.012 ± 0.001		0.013 ± 0.001		0.011 ± 0.002		0.021 ± 0.005		0.012 ± 0.001		0.011 ± 0.001				
	Morphometric	Posterior extent of maxilla (X16)	545	-0.185 ± 0.000	-0.186 ± 0.001	-0.185 ± 0.000		-0.185 ± 0.000		-0.187 ± 0.001		-0.185 ± 0.003		-0.186 ± 0.001		-0.186 ± 0.000				
	Morphometric	Anterior extent of maxilla (Y18)	545	0.013 ± 0.001	0.014 ± 0.001	0.013 ± 0.001		0.013 ± 0.001		0.016 ± 0.003		0.005 ± 0.006		0.015 ± 0.001		0.015 ± 0.001				

Table S3. Estimated effects of Cnv770 and SNPs12/13 on traits that map to the haplotype. The R package *emmeans* was used to estimate the marginal mean trait values and standard deviation for fish with the specific combination of genotypes at Cnv770 and SNPs12/13. The linear model used included the covariates used in trait mapping. Figures 3 and 7 depict the data visually, though the points plotted are adjusted trait values, whereas the numbers represented here are estimates for real trait values.