

Reference-guided Gill Transcriptome Assembly  
(29295 XLOCS, 171620 TCONS)

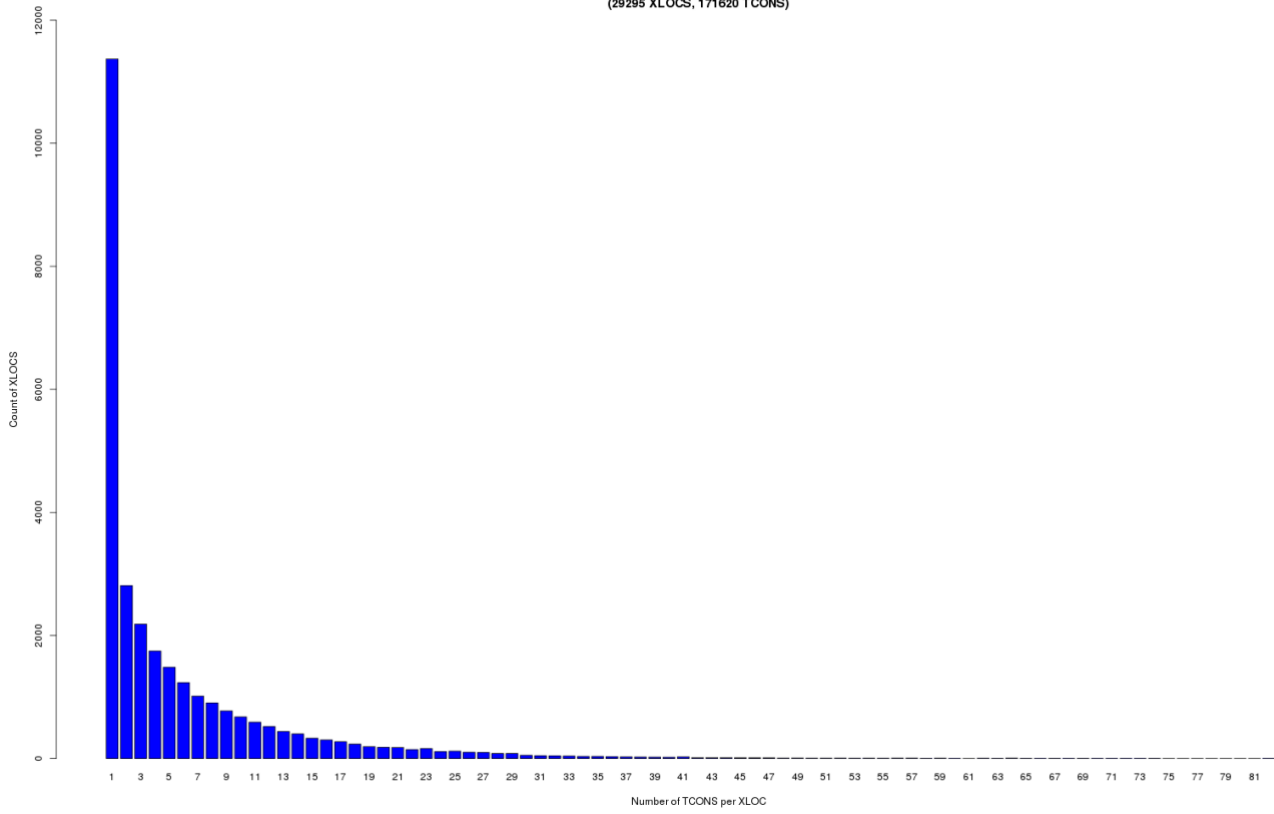


Fig S1. Number of isoforms (“TCONS”) per expressed locus (“XLOC”).

Hierarchical Clustering Ward's D2

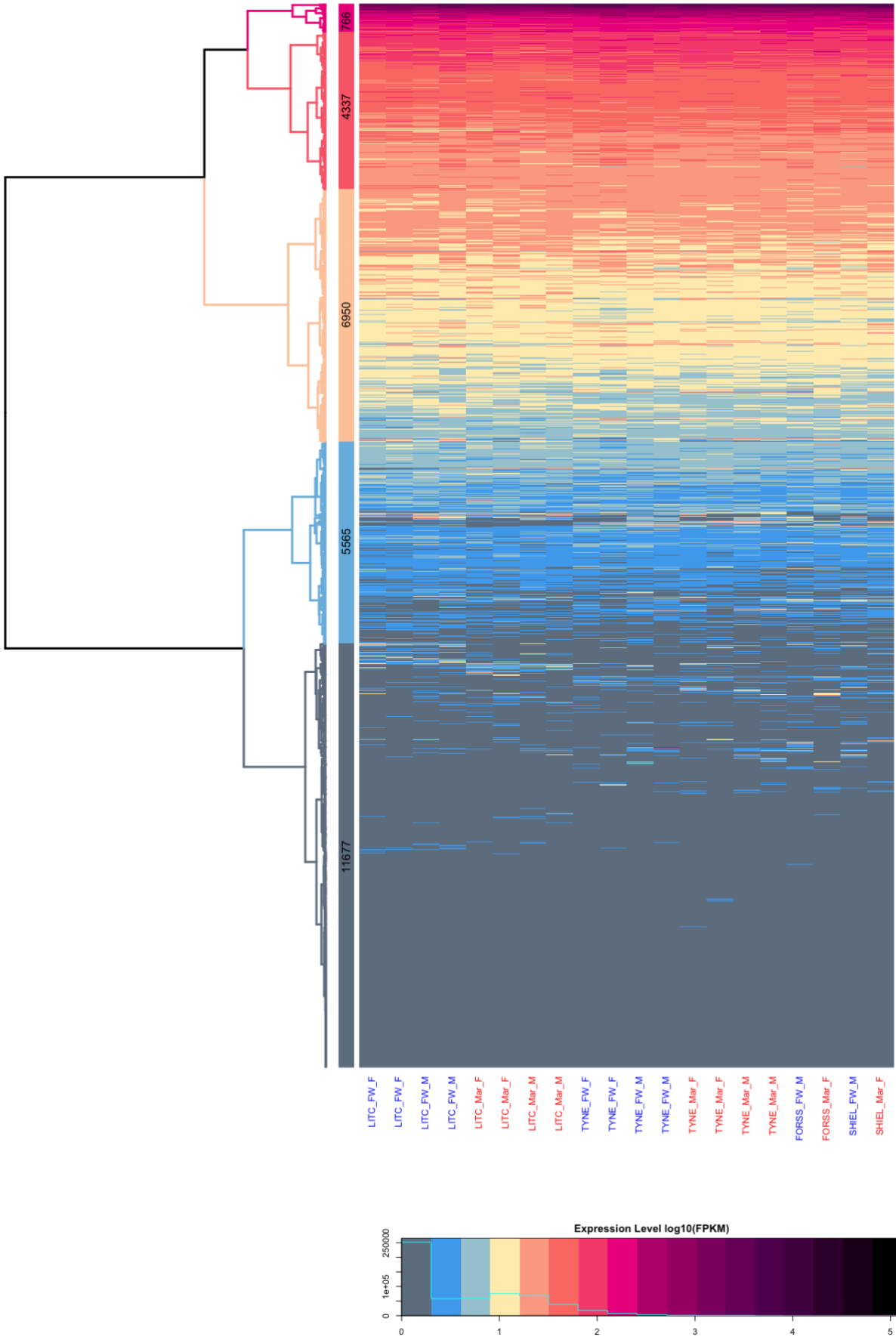


Fig S2. Hierarchical clustering of stickleback gill transcriptome by expression level.

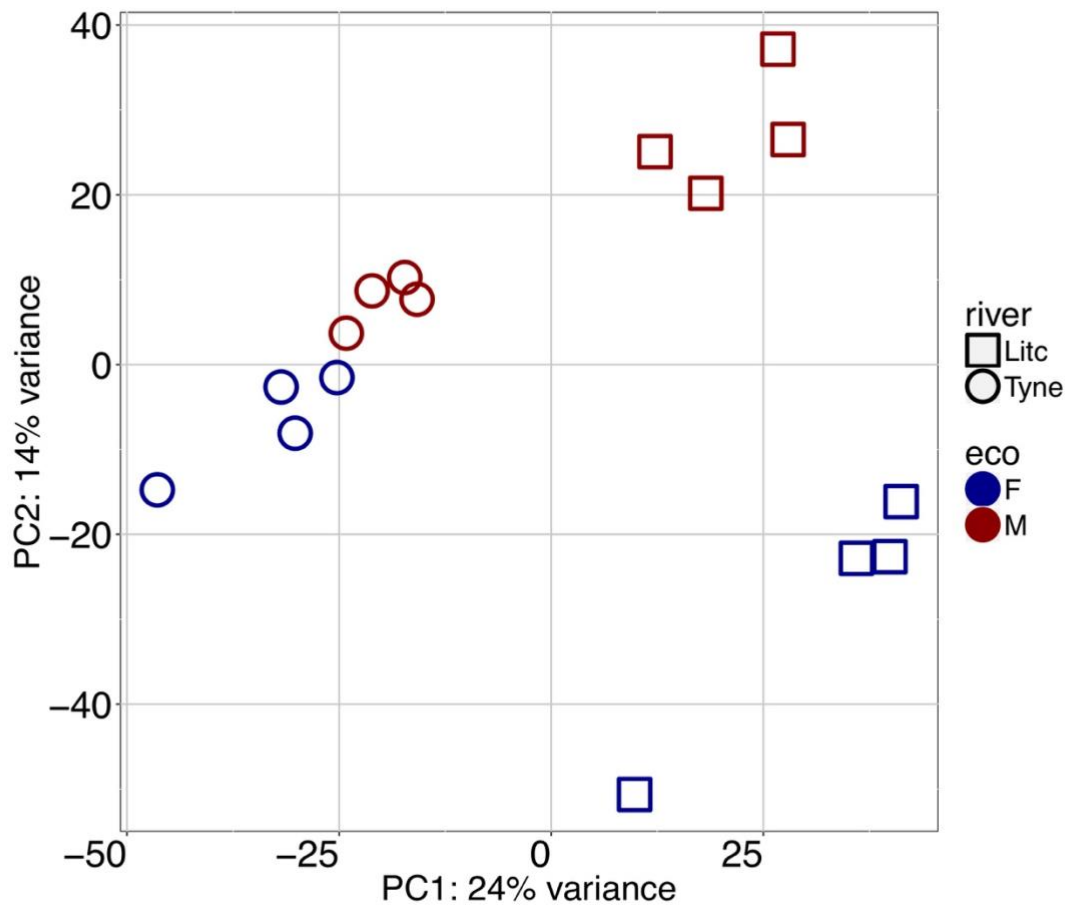


Fig S3. Principal component analysis of gill transcriptomes of Little Campbell and Tyne ecotypes (principal components 1 and 2).

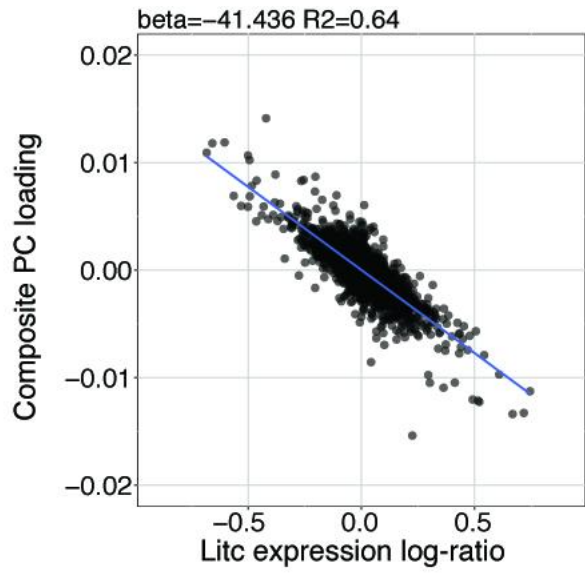
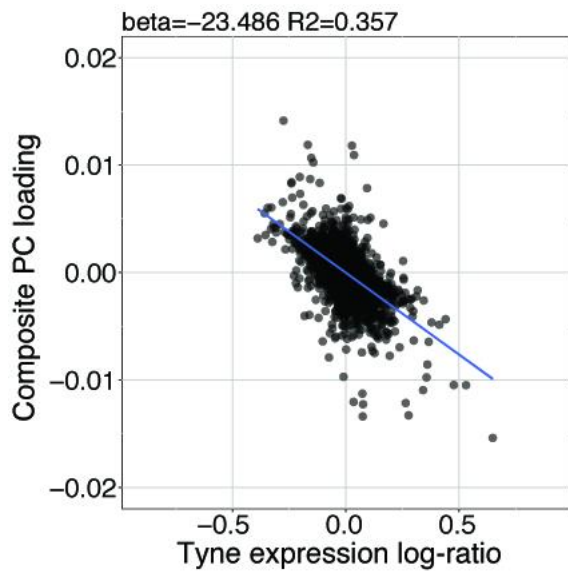


Fig S4. Marine-freshwater expression ratio versus composite PC loading. Tyne ecotypes (left panel) and Little Campbell ecotypes (right panel).

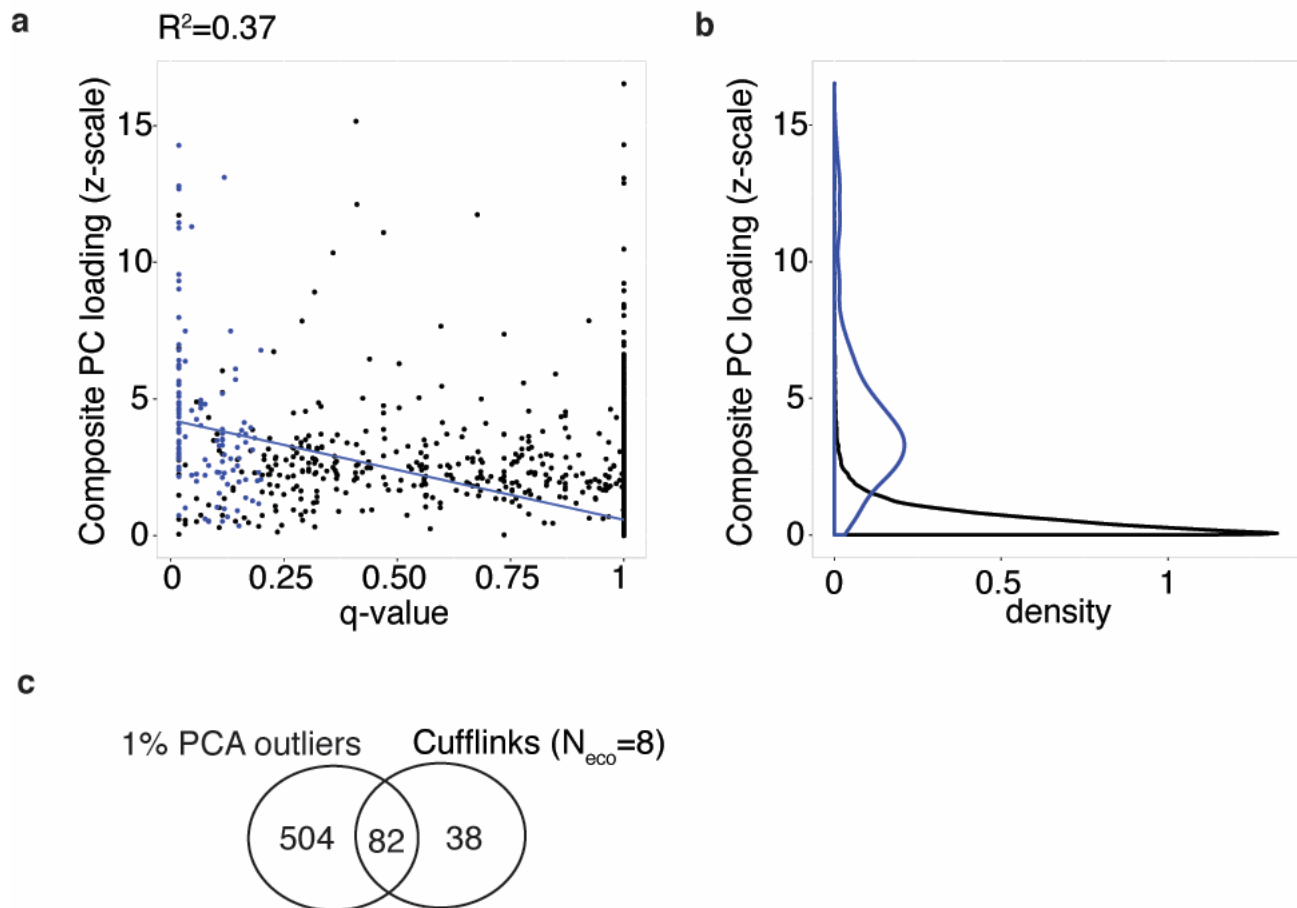


Fig S5. (A) Parallel freshwater-marine differentially expressed transcripts (FDR 20%, blue points and density estimation) versus composite PC loadings. (B) Sharing of parallel freshwater-marine differentially expressed transcripts versus transcripts that have the strongest contribution to sample separation on composite PC ("PC outliers", i.e. composite PC loading in the lowest or highest 1% of loading distribution and correlated freshwater-marine mean expression difference in Little Campbell and Tyne).

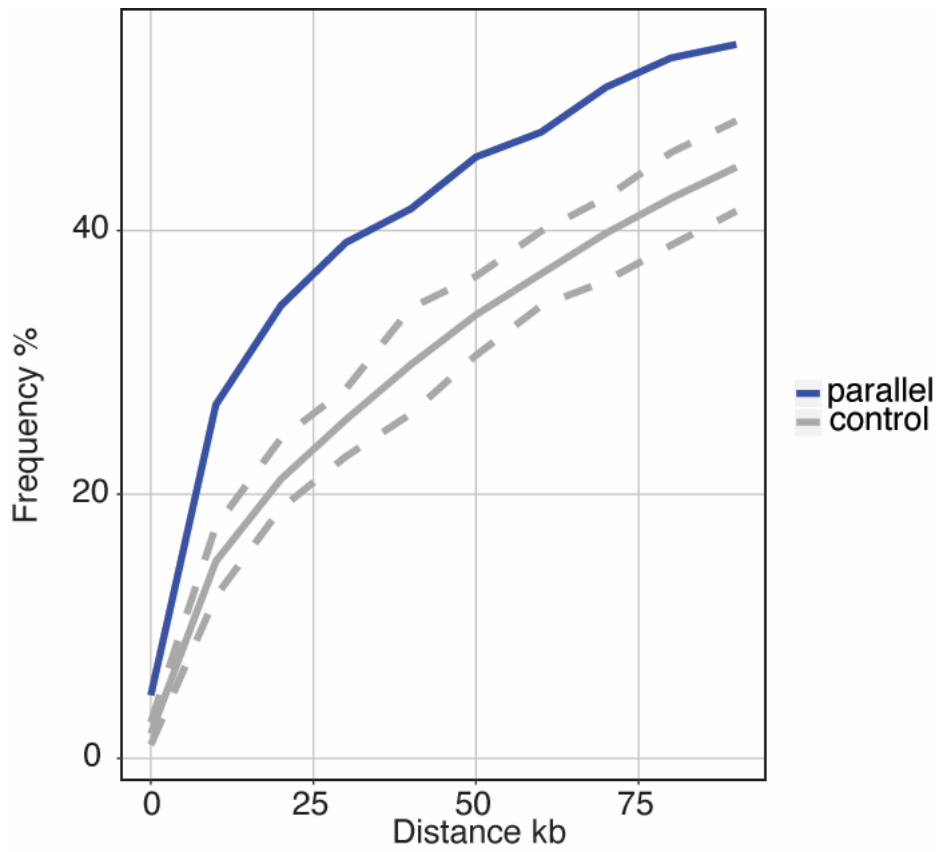


Fig S6. Association between parallel diverged transcripts and CSS outlier loci in Tyne and Little Campbell ecotype pairs sequenced in this study.

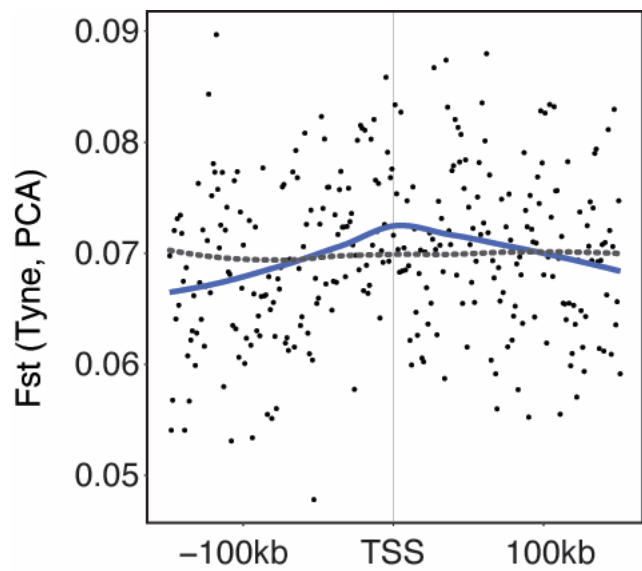


Fig S7. Genetic divergence (Fst) associated with parallel evolving loci in Tyne populations.

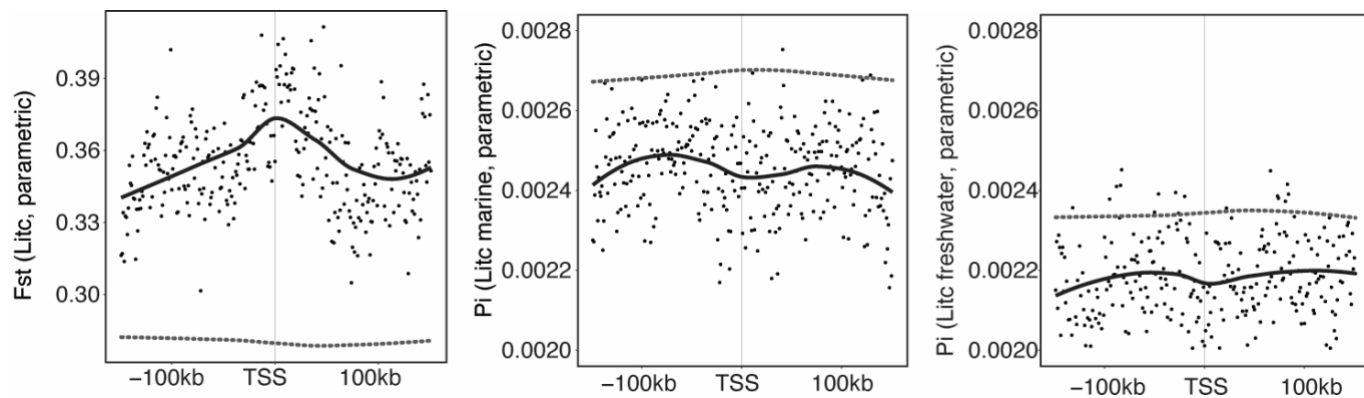


Fig S8. Genetic divergence ( $F_{st}$ ) and nucleotide diversity ( $\Pi$ ) associated with parallel evolving loci defined based on parametric test (Little Campbell). Results for Tyne were non-significant (not shown).



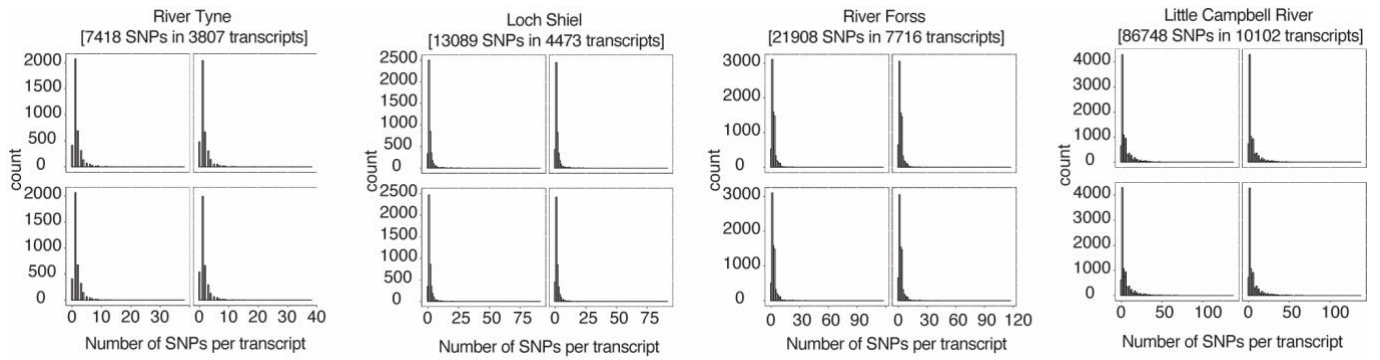


Fig S9. Number of informative (tested) SNPs per transcript per cross. Four panels per cross represent four analyzed F1's. Zero corresponds to positions where SNP position was not informative (expressed) in a given F1, but informative in other F1's of the same family.

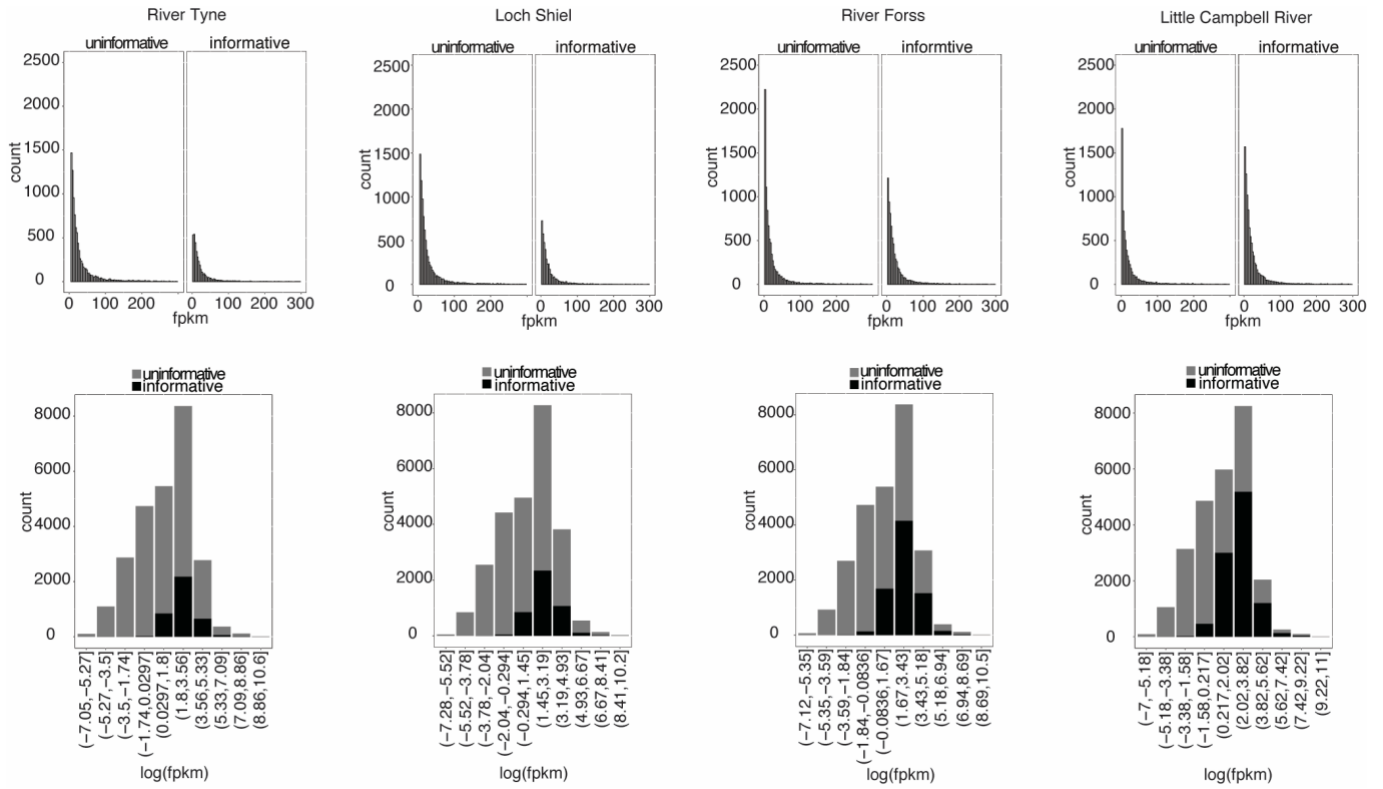


Fig S10. Expression levels of transcripts with informative SNPs. Average FPKM values in transcripts with informative SNPs for allele-specific expression test and those without informative SNPs (uninformative).

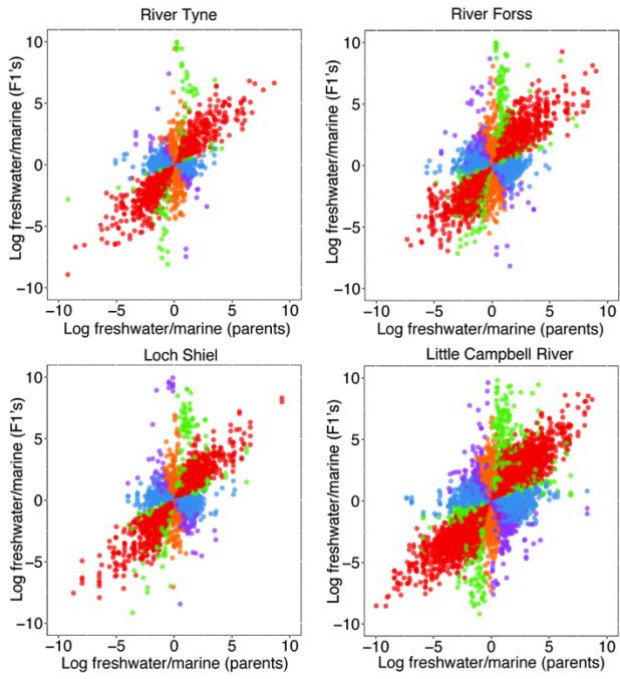


Fig S11. The genetic architecture of expression divergence between ecotype pairs in all investigated ecotype-pairs (as per Fig 3b).

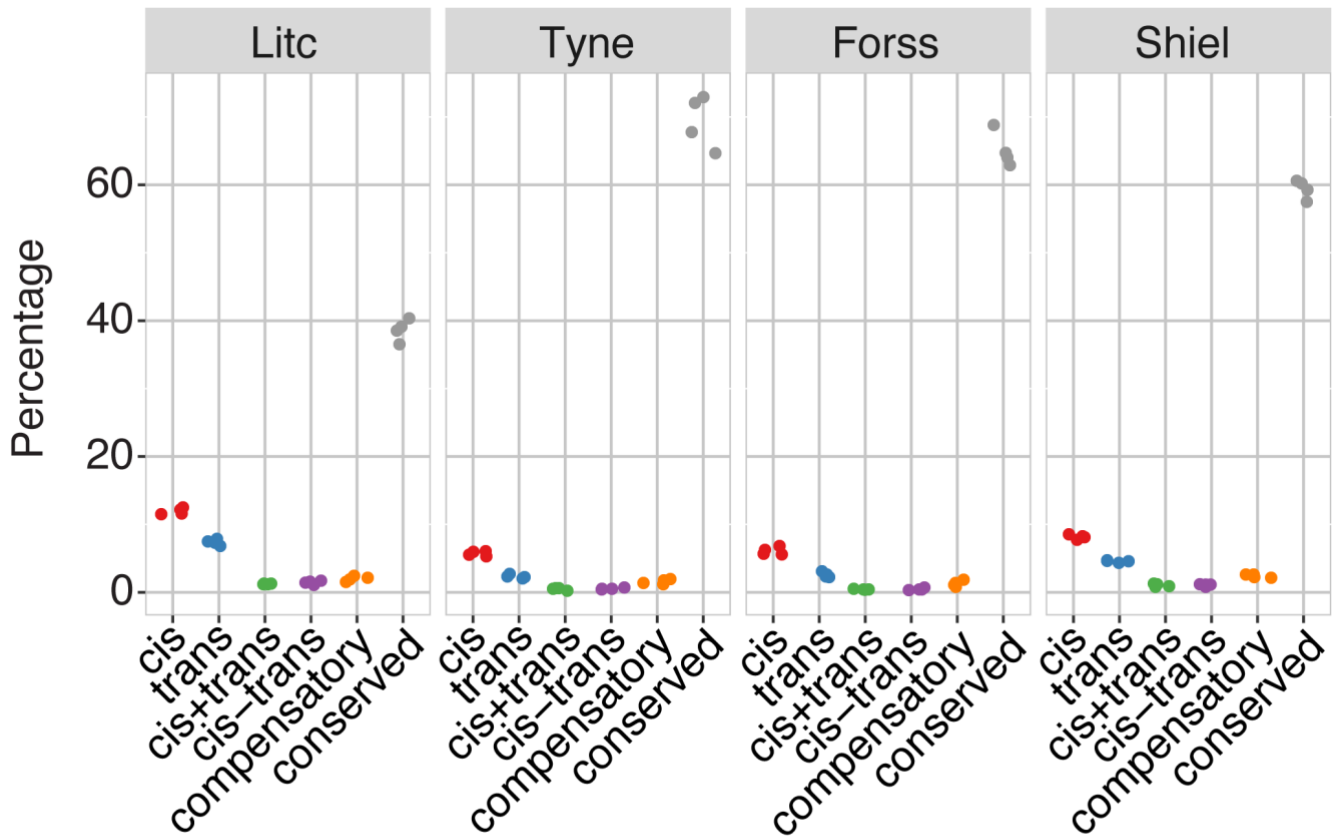


Fig S12. Frequencies of genetic architectures of expression divergence in down-sampled (30M uniquely mapping reads per sample) dataset.

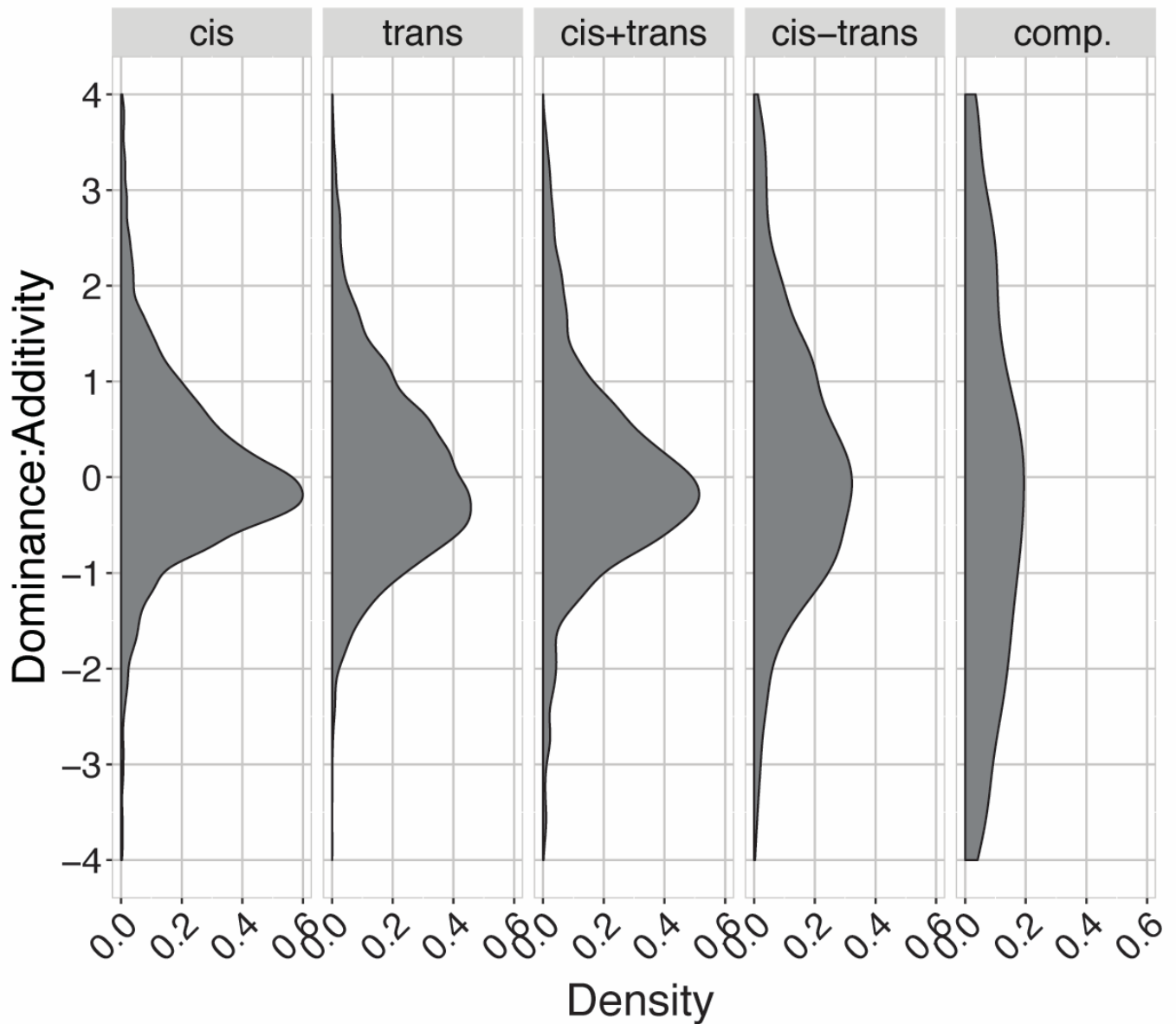


Fig S13. Density estimates of dominance-additivity ratio associated with different genetic architectures (Little Campbell). A ratio of zero indicates additivity,  $\pm 1$  indicates full dominance, and values  $>1$  or  $<-1$  imply over- or underdominance. Slight bias towards negative values indicates tendency of stronger dominance of low-expressed allele (irrespective of ecotype).

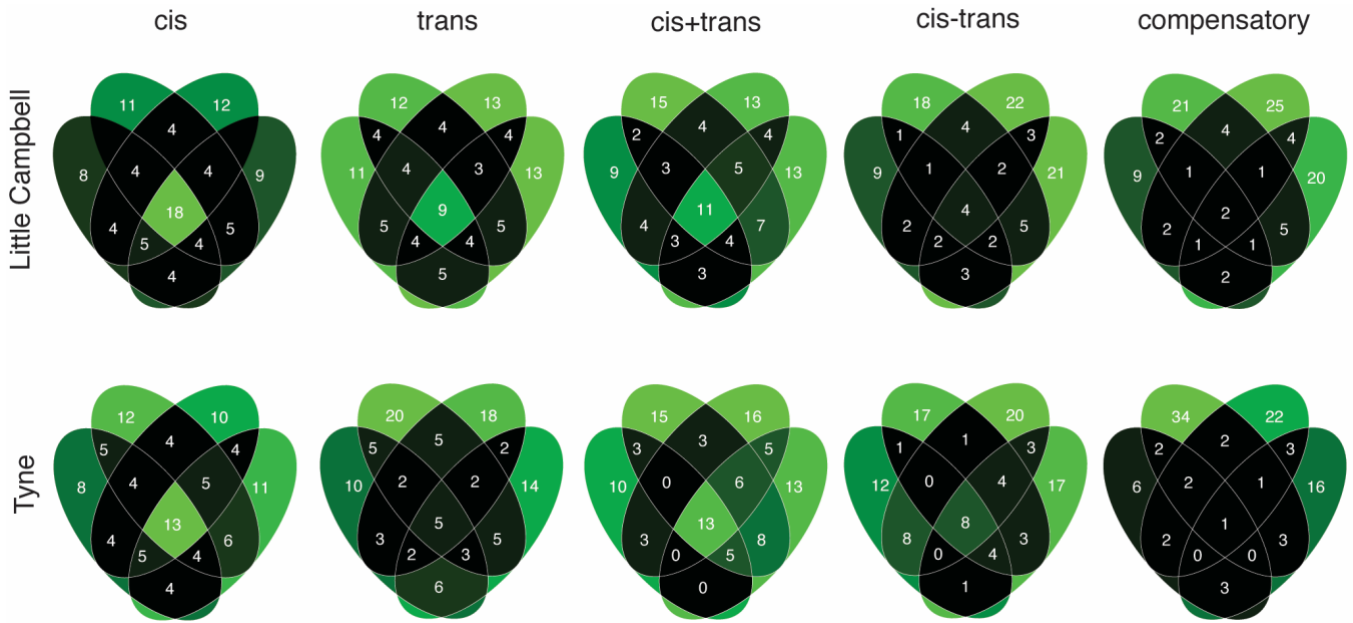


Fig S14. Sharing of regulatory divergence between siblings as a proxy for the level of epistasis associated with different genetic architectures in Little Campbell and Tyne. The type of regulation observed for each gene is compared among siblings (overlapping ovals) with numbers representing percent of loci shared between siblings (rounded to integer). Color scale ranges from black (low) to pale green (high). Cis-regulatory divergence tended to be most stable across genetic backgrounds indicating that cis-acting divergence is least influenced by epistasis.

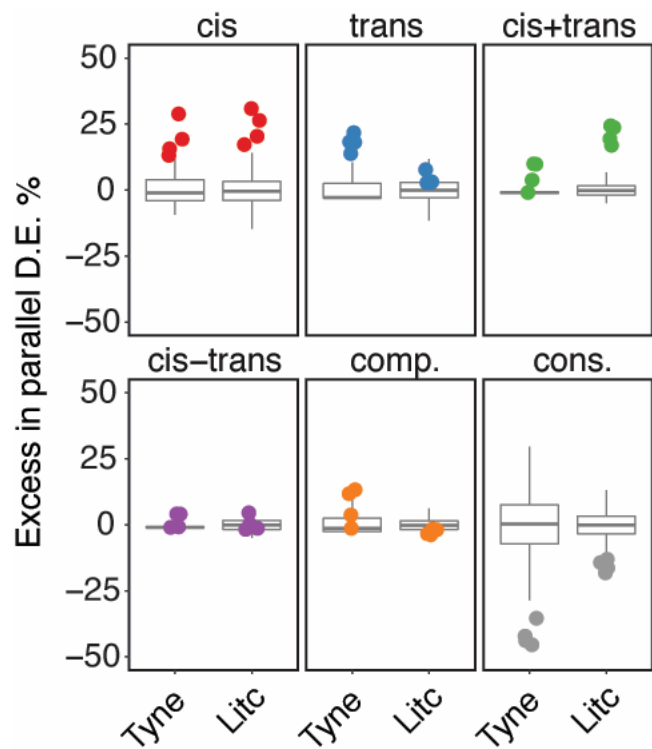


Fig S15. Cis/trans-overrepresentation (as per Fig 4) in parallel evolving genes as defined by parametric test.

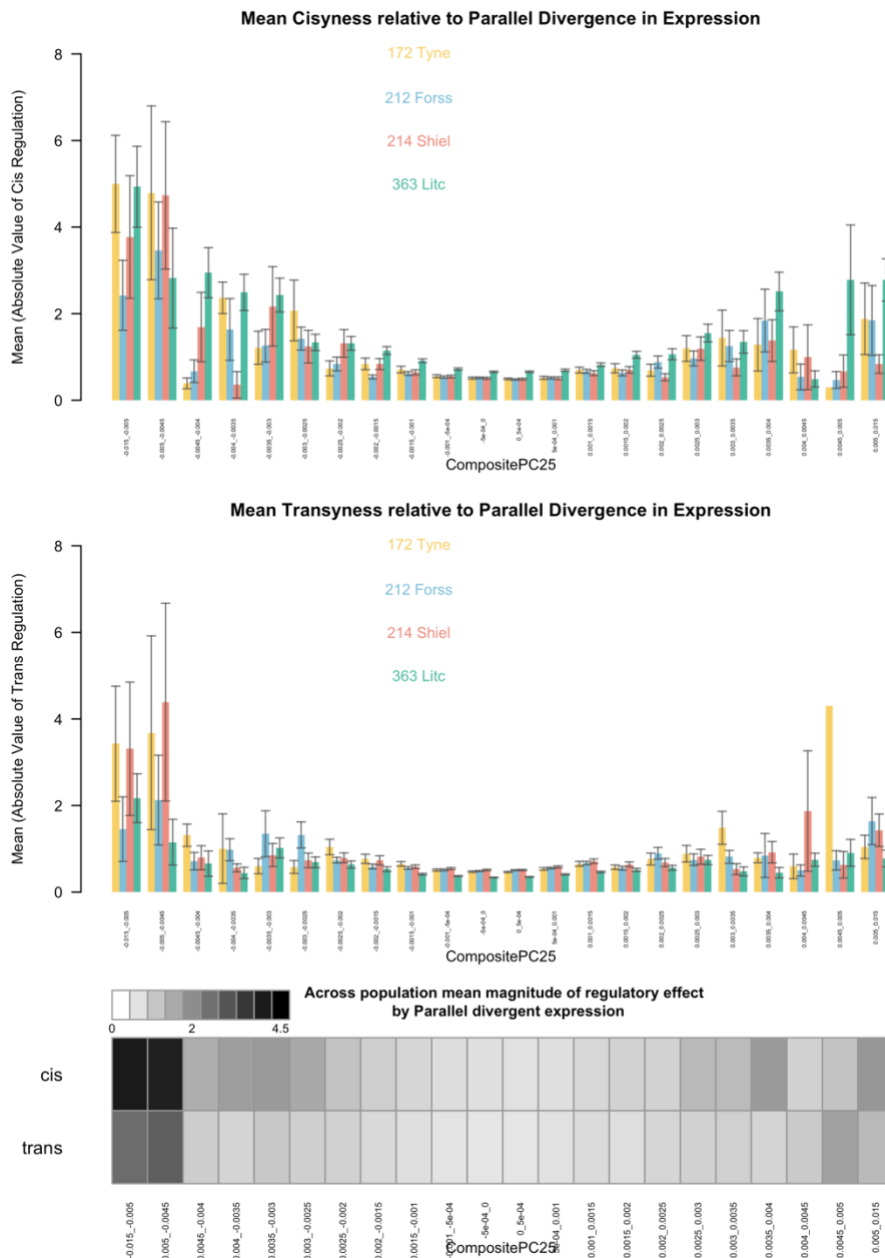


Figure S16. Mean magnitude of cis- (a) and trans- (b) regulation relative to parallel divergence in gene expression measured by composite PC. For each population studied for allele-specific expression, loci were binned according to their loadings on the composite PC describing parallel divergence in gene expression of pure strains. For each bin and each population the mean and standard error of the quantitative degree of cis-regulation and trans-regulation was calculated (see main text). (c) As per Fig5c in main text. For each bin, the mean across populations was calculated and plotted as a heat map with darker grey representing larger magnitudes of cis- and trans- effects.



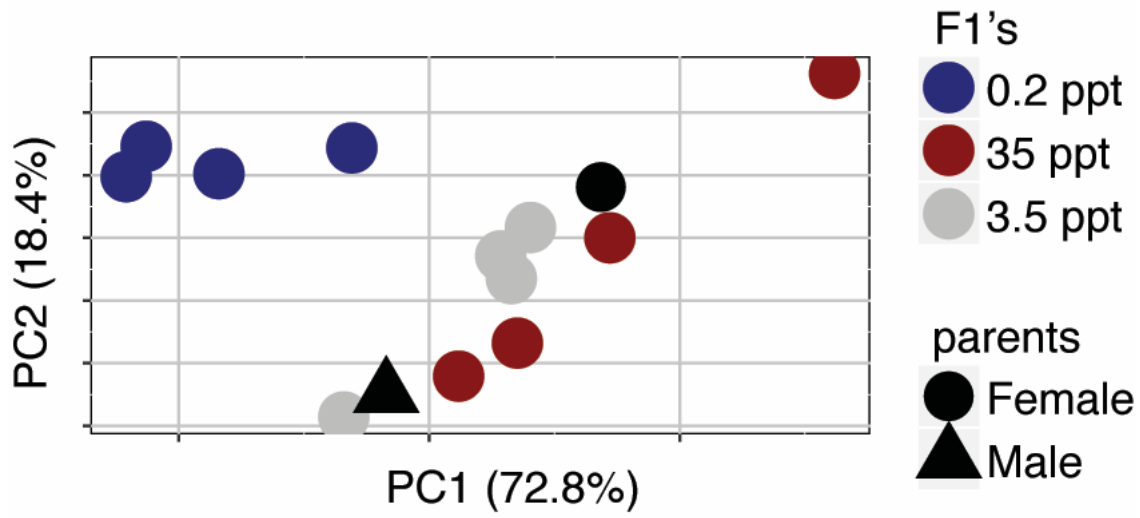


Fig S17. Principal Component Analysis of expression profiles in response to salinity acclimation.

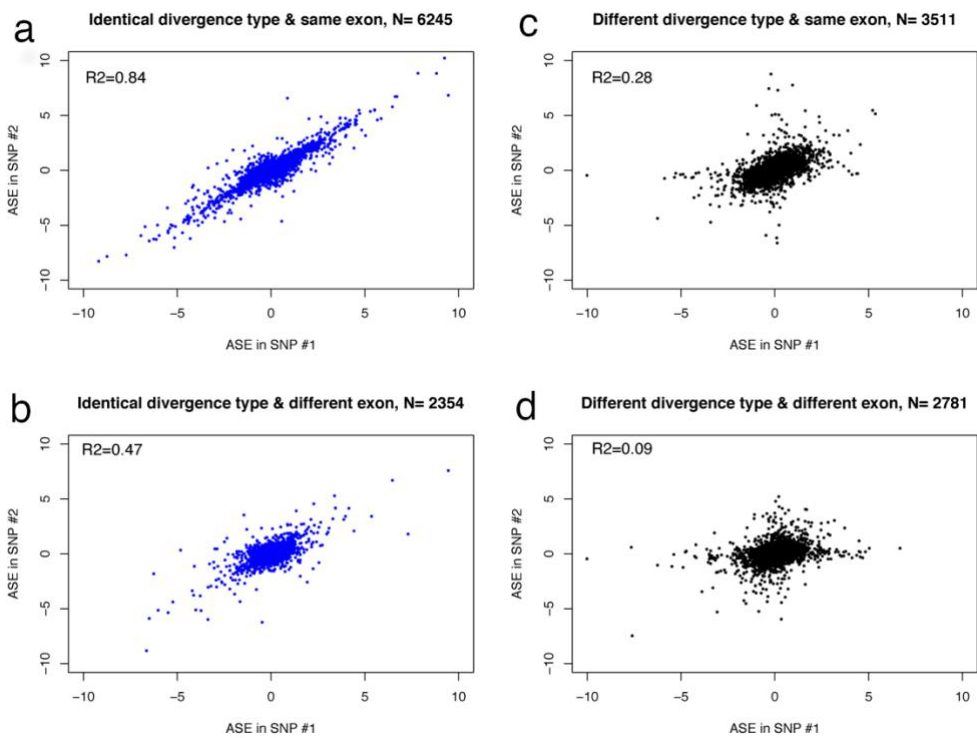


Fig S18. Correlation of ASE (log-fold change of RNA-seq counts of marine over freshwater allele) in different pairs of SNPs was used to perform concordance analysis. (a) SNPs assigned to same exons and showing identical type of genetic divergence (concordant SNPs). (b) SNPs assigned to same exons but showing different classes of genetic divergence (discordant SNPs). (c) SNPs assigned to different exons of the same transcript and showing identical type of genetic divergence. (d) SNPs assigned to different exons of the same transcript and showing different type of genetic divergence.

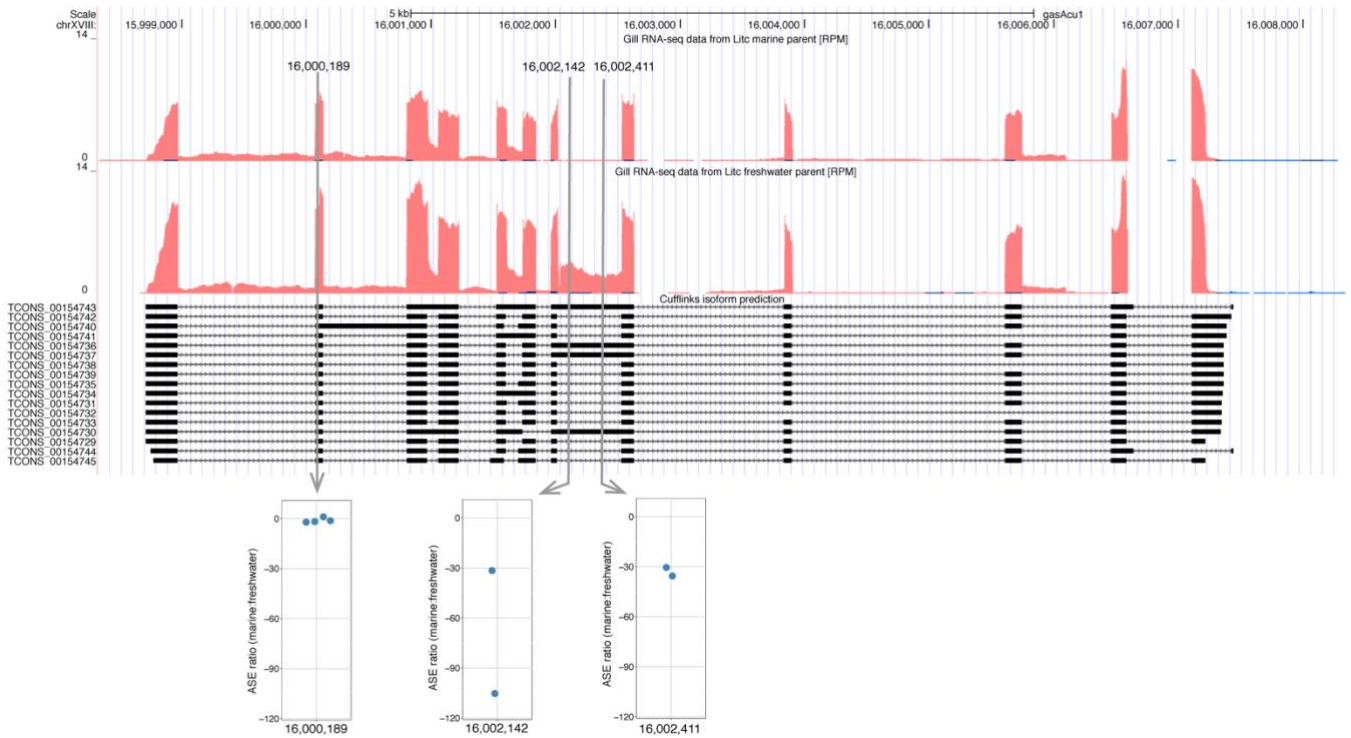


Figure S19. Candidate transcript for alternative isoform expression. This example illustrates that SNPs in different regions of the same transcript can show varying levels of ASE associated with alternative splice forms. Expression level is represented by RPM (Reads [mapping to position] Per Million [reads mapped overall]) and corresponds to red track in Little Campbell marine (upper) and freshwater (lower) parents. CuffLinks isoform predictions are represented below expression tracks. The marine and freshwater alleles are distinguished by three SNPs mapping to two exons. SNP at position chrXVIII:16000189 shows similar expression level of both alleles and hence no ASE. SNPs at positions 16002142 and 16002411 map to an alternatively spliced region of the transcript where expression is observed in freshwater allele but not in marine allele, and hence the SNPs show ASE towards freshwater.