Figures and figure supplements

Systems genomics of salinity stress response in rice

Sonal Gupta *et al.*

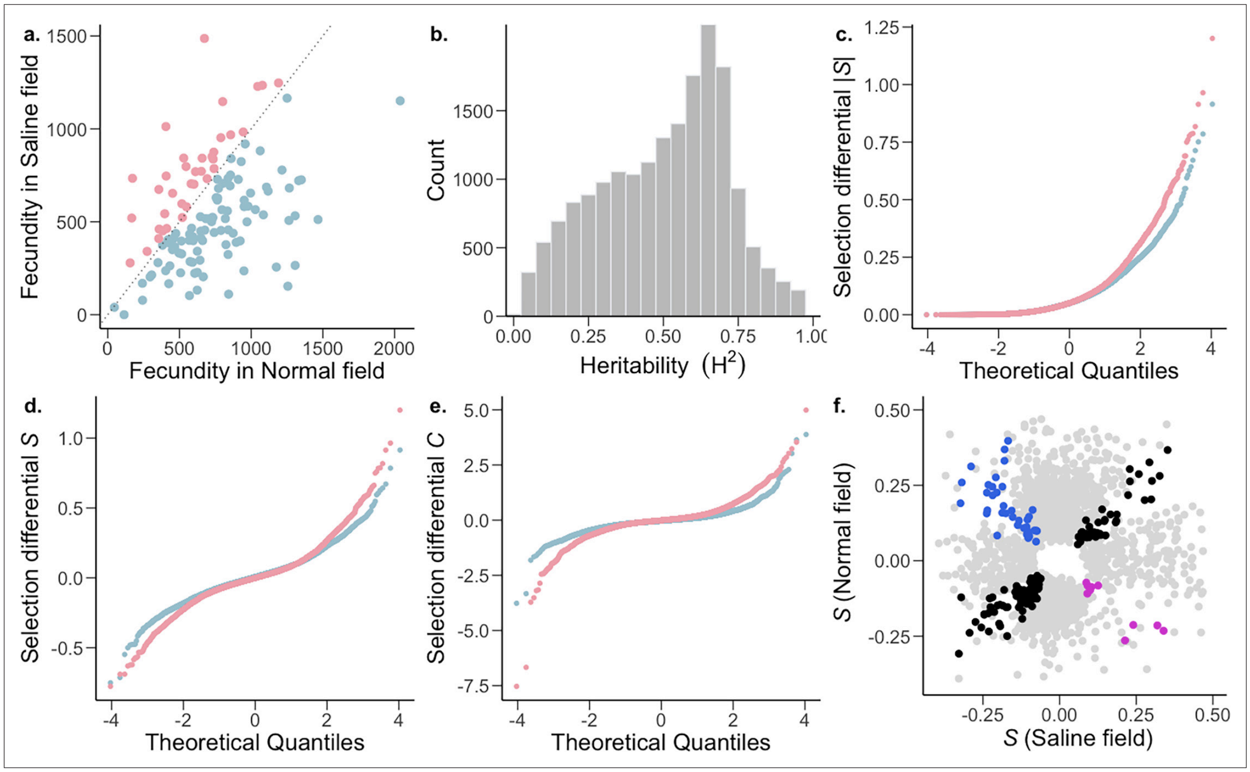


Figure 1. The strength and pattern of selection on heritable gene expression. (**a**) The *O. sativa* ssp. *indica* populations showed higher average fitness in the normal (blue) and saline (pink) field (two-tailed paired t-test $p=1.658 \times 10^{-8}$) and fitness further showed a significant effects of genotype (G) and environment (E); genotype ×environment (G×E) was not significant. Analysis of variance (ANOVA) [G and E ($p<0.001$), G×E ($p=0.49$)]; n=130 accessions. (**b**) Broad-sense heritability (H^2) distribution of *Oryza sativa* spp. *indica* transcripts. Two-way ANOVA, genotype FDR-adjusted $q<0.001$, n=130 accessions. (**c–e**) The strength of linear selection |*S*|, linear selection differentials (S), and quadratic selection differentials (C) for genome-wide gene expression in normal (blue) and saline (pink) conditions. X-axes represent a theoretical quantile for normal distribution with mean = 0 and standard deviation = 1. (**f**) Conditionally neutral (light gray), and antagonistically pleiotropic transcripts (blue and magenta represent beneficial expression in normal and saline conditions, respectively). Black represents transcripts experiencing selection in the same direction in both environments (expression is beneficial or detrimental in both environments).

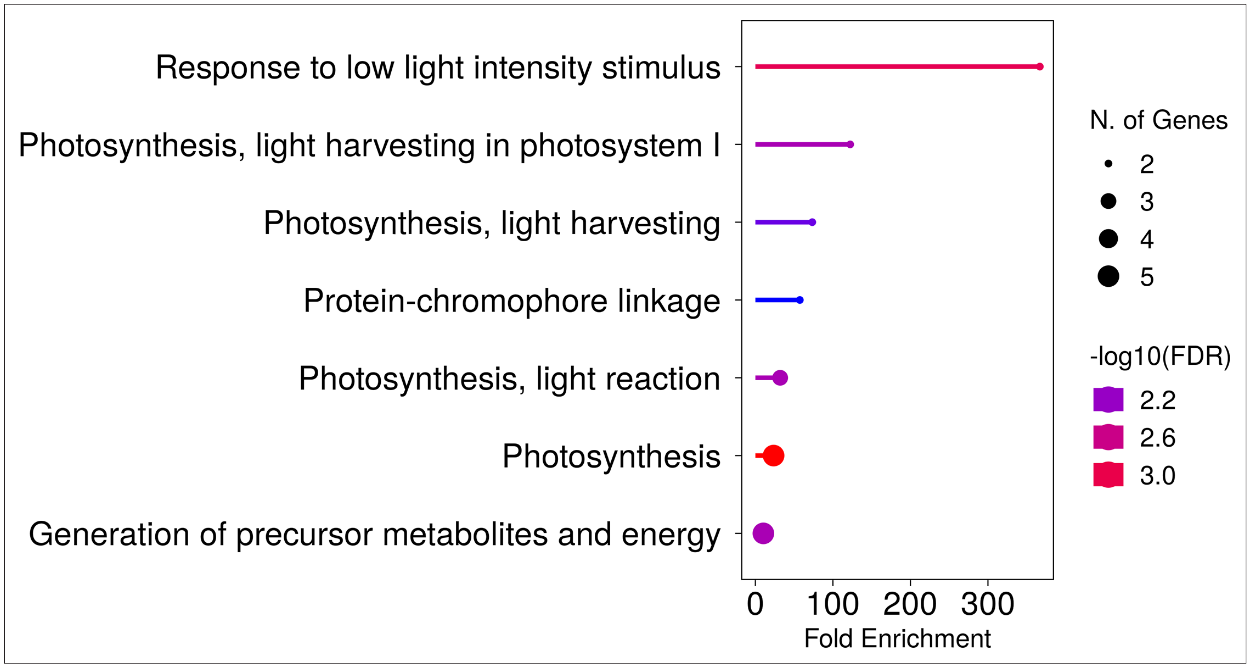


Figure 1—figure supplement 1. Pathway enrichment of the 51 antagonistically pleiotropic genes beneficial in normal conditions but detrimental in salinity stress conditions. Enrichment statistics provided in ***Supplementary file 4***.

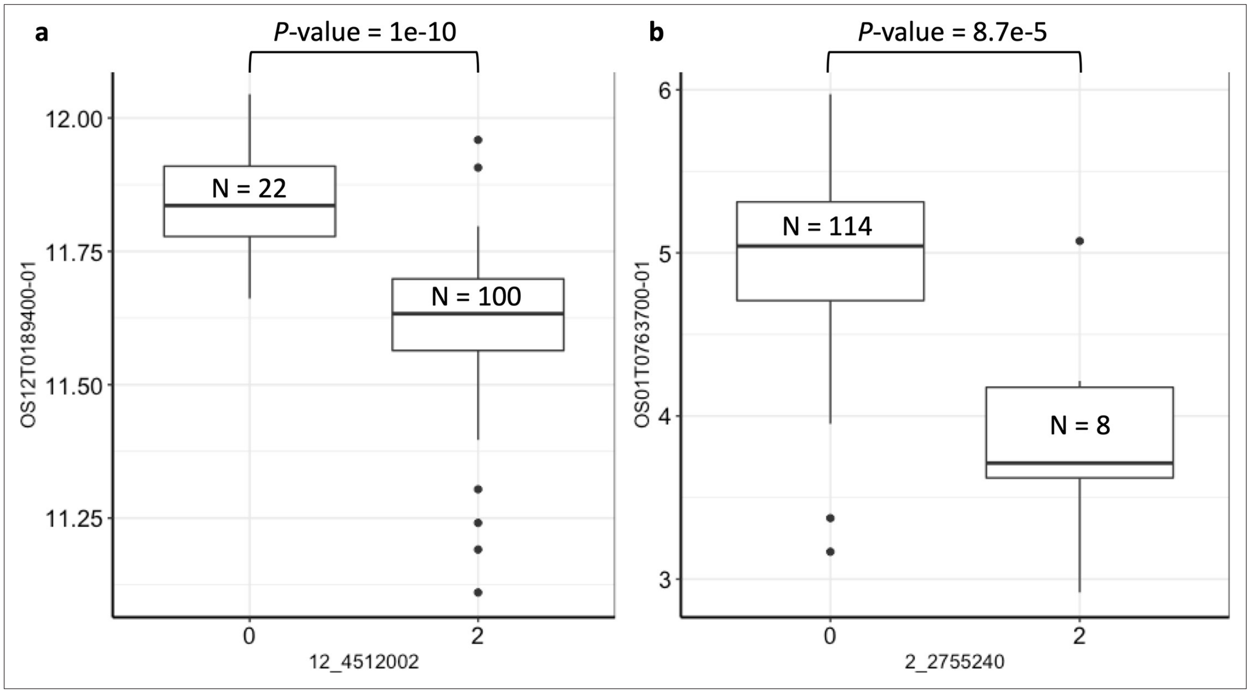


Figure 1—figure supplement 2. Boxplot representation of eQTLs for the two photosynthesis-related AP (antagonistically pleiotropic) genes beneficial in normal conditions. (**a**) *cis*-eQTL (SNP = Chr12:4512002) for *PSAN* (OS12T0189400-01). (**b**) *trans*-eQTL (SNP = Chr02:2755240) for *CRR7* (OS01T0763700-01). eQTL statistics provided in ***Supplementary file 10***. X-axis represents the eQTL SNP haplotypes, y-axis represents normalized transcript expression in normal conditions. Numbers inside box plots represent the number of accessions in each group. p-value indicates the one-sided Wilcoxon-test significance.

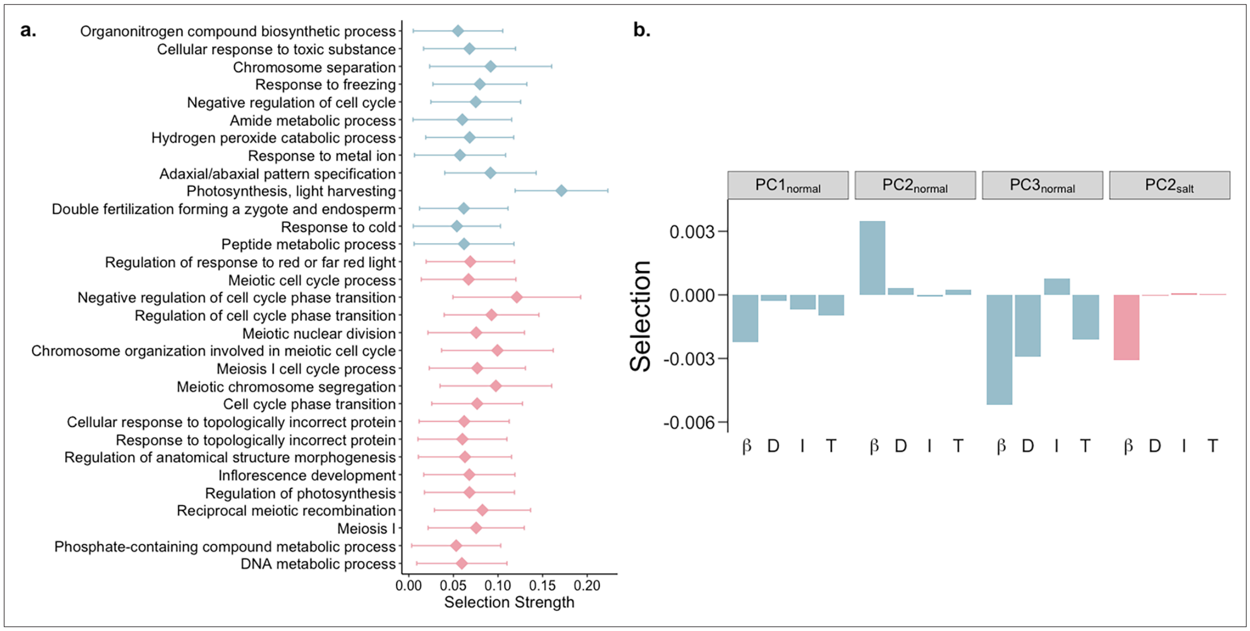


Figure 2. Biological processes and pathways with differential responses to selection under saline conditions. (**a**) GO biological processes under stronger selection in normal (blue) and saline conditions (pink). Error bars represent 95% confindence intervals. around the median. (**b**) Linear selection gradients (*β*), along with direct (D), indirect (I) and total (T) responses to selection on suites of transcripts in normal (blue) and saline conditions (pink).

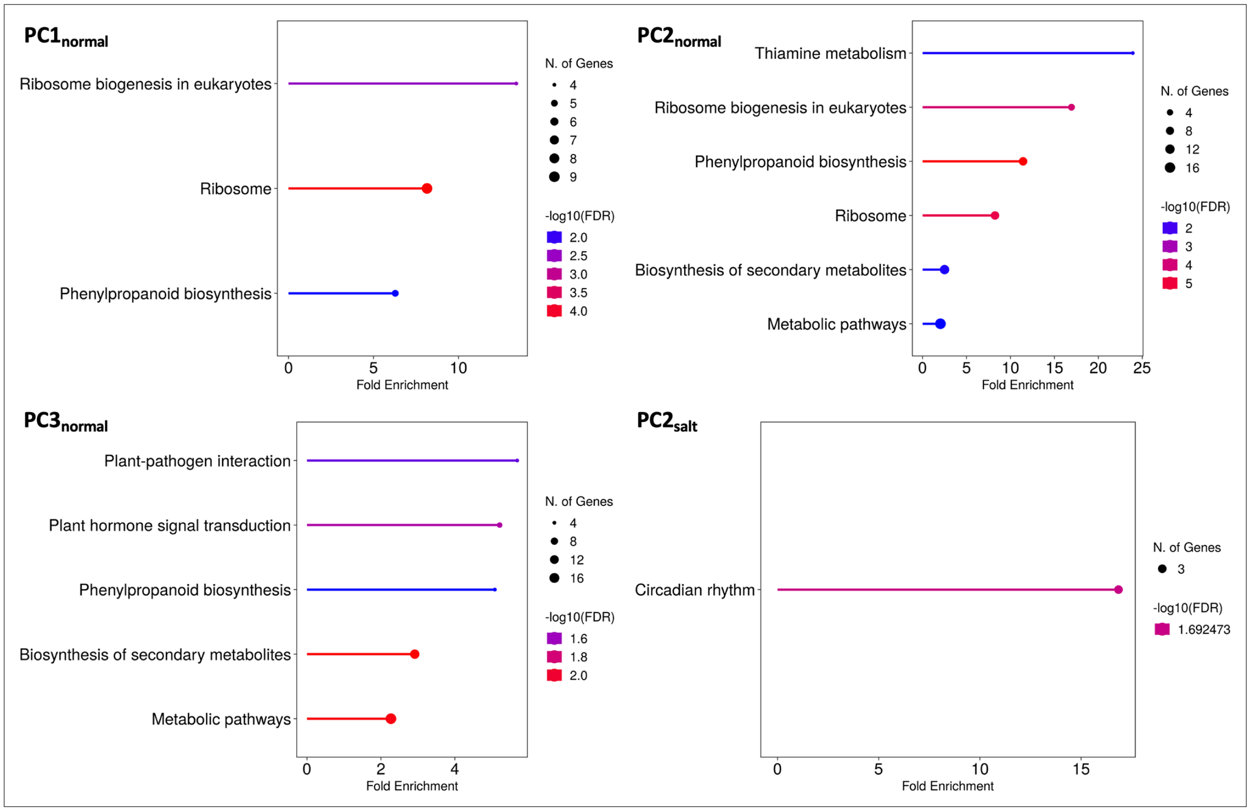


Figure 2—figure supplement 1. Enrichment of the suite of transcripts (*1% tails of the distributions of transcripts' loading values on principal components*) with significant selection gradients in both normal and salinity stress conditions.

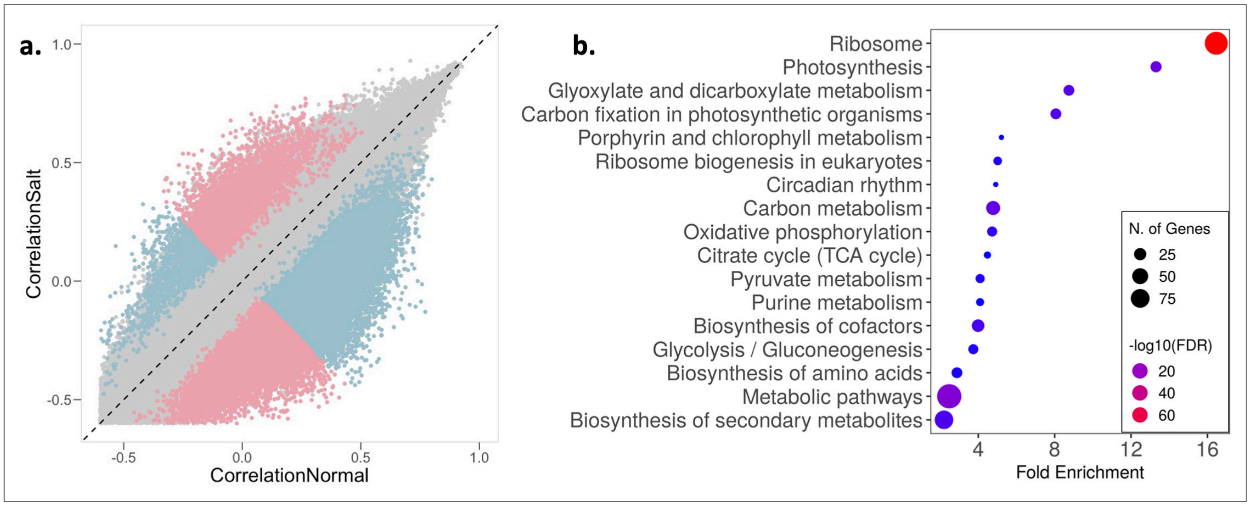


Figure 3. Salinity stress induces regulatory decoherence. (**a**) Pearson correlation coefficients between pairs of transcripts ($|S|>0.1$ and expression greater than 0 in at least 50% individuals) in normal (x-axis) and saline conditions (y-axis). Pink and blue represent pairs with correlation stronger in saline and normal conditions, respectively; gray represents correlation that is not significantly different between conditions. (**b**) Enrichment of transcripts with significant pairs greater than the median (median significant pair per transcript = 12, n=853) involved in regulatory decoherence post salt exposure.

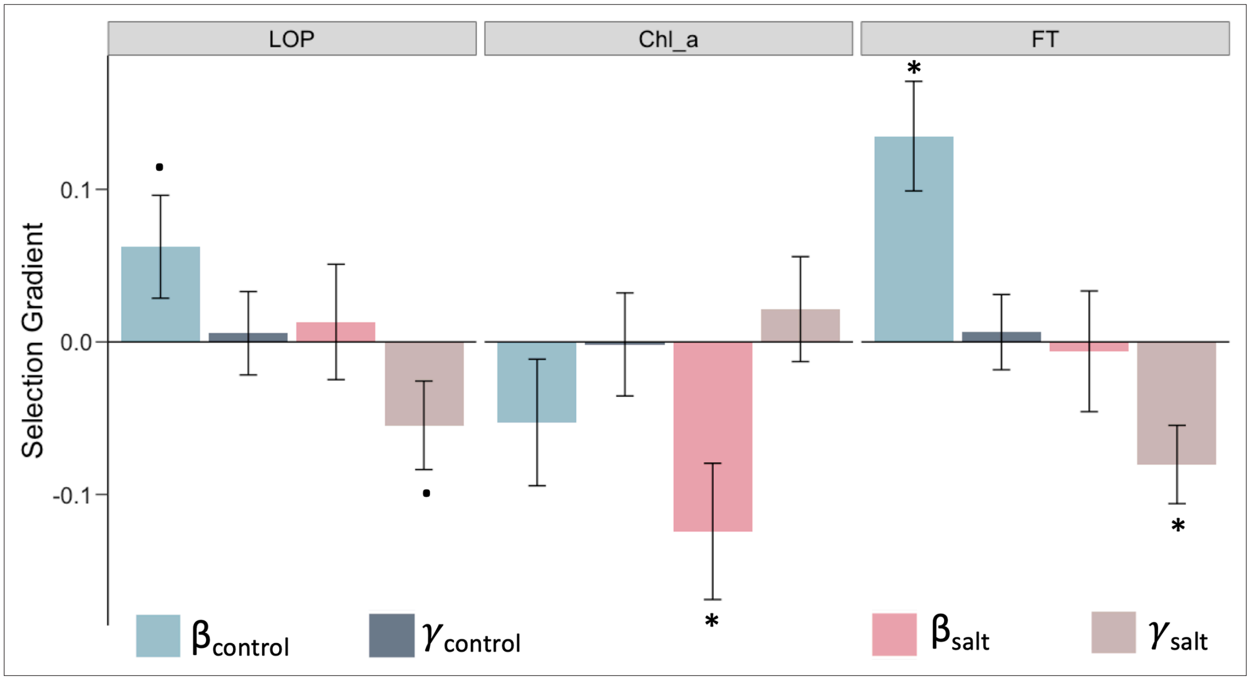
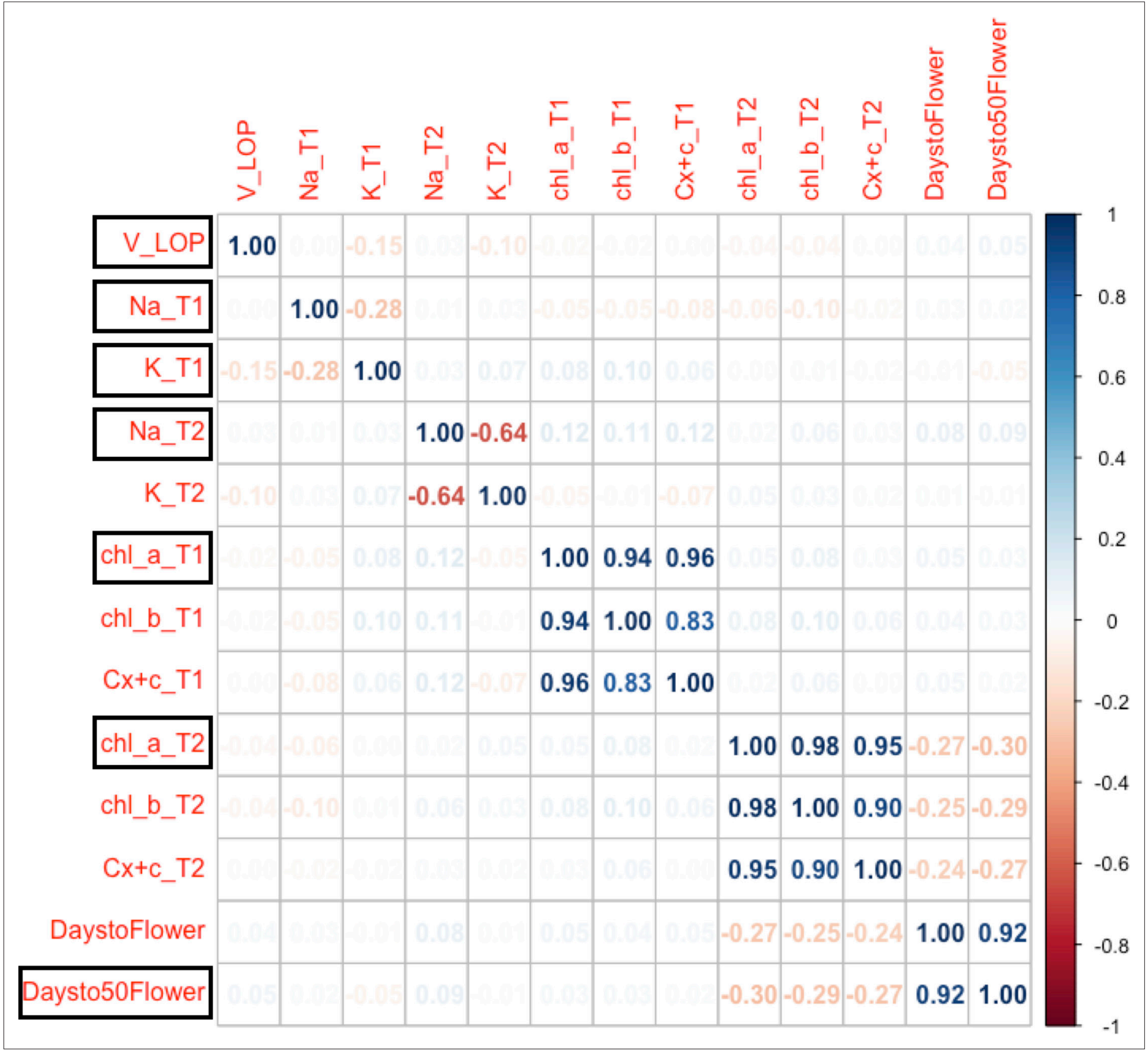


Figure 4. Traits with different selection profiles under salt stress. Linear (*β*) and quadratic (*γ*) selection gradients on the traits LOP (leaf osmotic potential), Chl_a (chlorophyll a content), and FT (flowering time). Error bars represent mean ± SE (n_{normal}: 384; n_{salt}:365); dots and asterisks indicate significance of selection-gradient at two-sided unadjusted $p<0.1$ and $p<0.05$.



	V_LOP	Na_T1	K_T1	Na_T2	K_T2	chl_a_T1	chl_b_T1	Cx+c_T1	chl_a_T2	chl_b_T2	Cx+c_T2	DaystoFlower	Daysto50Flower
V_LOP	1.00	0.00	-0.15	0.03	-0.10	-0.02	-0.02	0.00	-0.04	-0.04	0.00	0.04	0.05
Na_T1	0.00	1.00	-0.28	0.01	0.03	-0.05	-0.05	-0.08	-0.06	-0.10	-0.02	0.03	0.02
K_T1	-0.15	-0.28	1.00	0.03	0.07	0.08	0.10	0.06	0.00	0.01	-0.02	-0.01	-0.05
Na_T2	0.03	0.01	0.03	1.00	-0.64	0.12	0.11	0.12	0.02	0.06	0.03	0.08	0.09
K_T2	-0.10	0.03	0.07	-0.64	1.00	-0.05	-0.01	-0.07	0.05	0.03	0.02	0.01	-0.01
chl_a_T1	-0.02	-0.05	0.08	0.12	-0.05	1.00	0.94	0.96	0.05	0.08	0.03	0.05	0.03
chl_b_T1	-0.02	-0.05	0.10	0.11	-0.01	0.94	1.00	0.83	0.08	0.10	0.06	0.04	0.03
Cx+c_T1	0.00	-0.08	0.06	0.12	-0.07	0.96	0.83	1.00	0.02	0.06	0.00	0.05	0.02
chl_a_T2	-0.04	-0.06	0.00	0.02	0.05	0.05	0.08	0.02	1.00	0.98	0.95	-0.27	-0.30
chl_b_T2	-0.04	-0.10	0.01	0.06	0.03	0.08	0.10	0.06	0.98	1.00	0.90	-0.25	-0.29
Cx+c_T2	0.00	-0.02	-0.02	0.03	0.02	0.03	0.06	0.00	0.95	0.90	1.00	-0.24	-0.27
DaystoFlower	0.04	0.03	-0.01	0.08	0.01	0.05	0.04	0.05	-0.27	-0.25	-0.24	1.00	0.92
Daysto50Flower	0.05	0.02	-0.05	0.09	-0.01	0.03	0.03	0.02	-0.30	-0.29	-0.27	0.92	1.00

Figure 4—figure supplement 1. Correlation among functional traits in normal conditions. Highlighted black boxes indicate the uncorrelated traits chosen for selection analyses. Numbers inside the boxes and the heatmap represent Pearson correlation coefficients. V_LOP: Leaf Osmotic Potential; Na: Sodium content; K: Potassium content; chl_a and chl_b: Chlorophyll a and b content, respectively; Cx +c: Total carotenoid content; DaystoFlower: First day of flowering; Daysto50Flower: Day on which 50% of plants in a plot flowered (focal plant and its nine neighboring plants); T1 and T2 represent vegetative and reproductive timepoints, respectively.

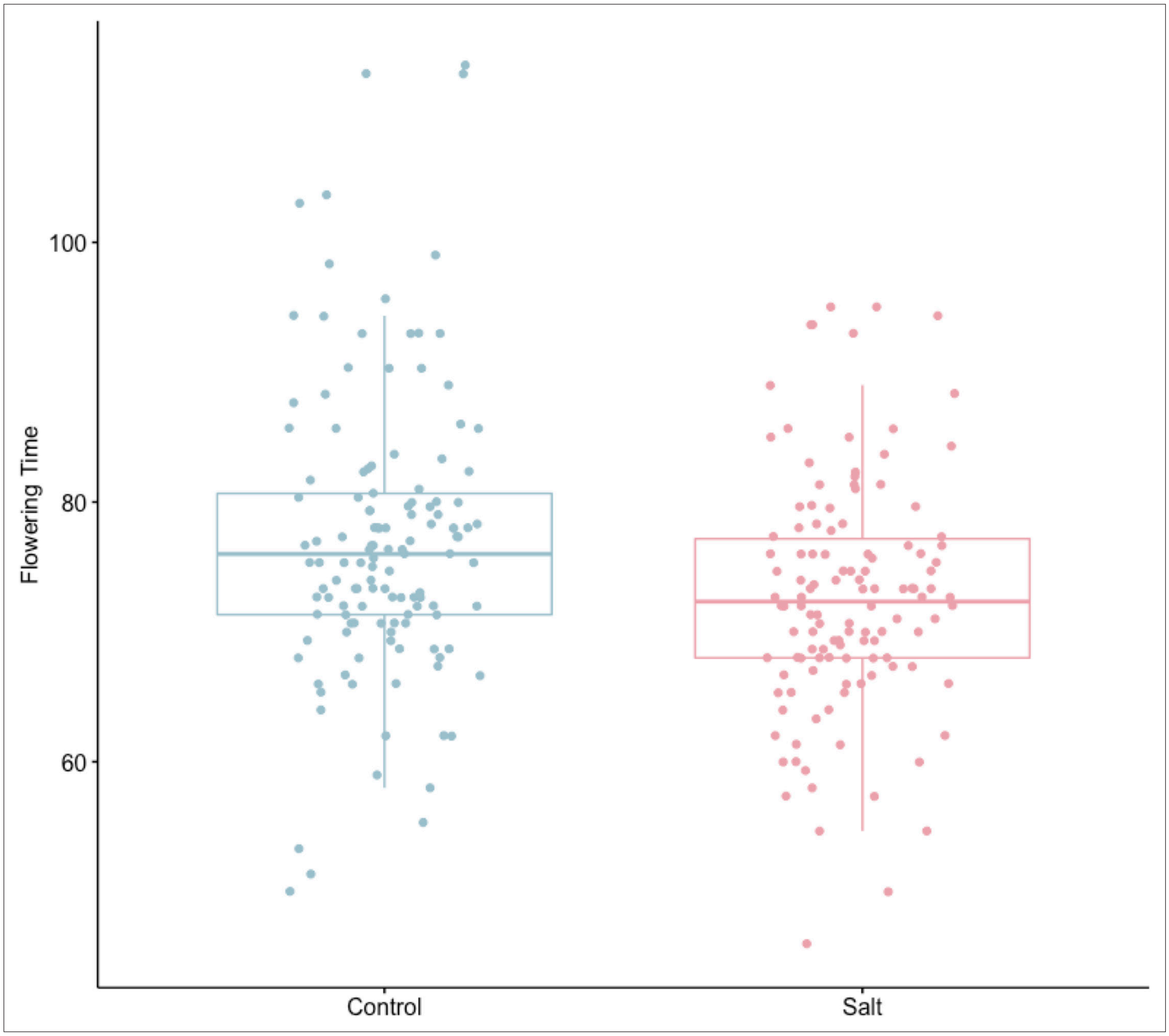


Figure 4—figure supplement 2. Distribution of flowering time (representing days to when 50% of plants in a plot flowered) in normal and salinity stress conditions. Paired t-test (two-sided paired t-test p=0.001) showed significant reduction in flowering times after salt treatment, indicating earlier flowering saline conditions.

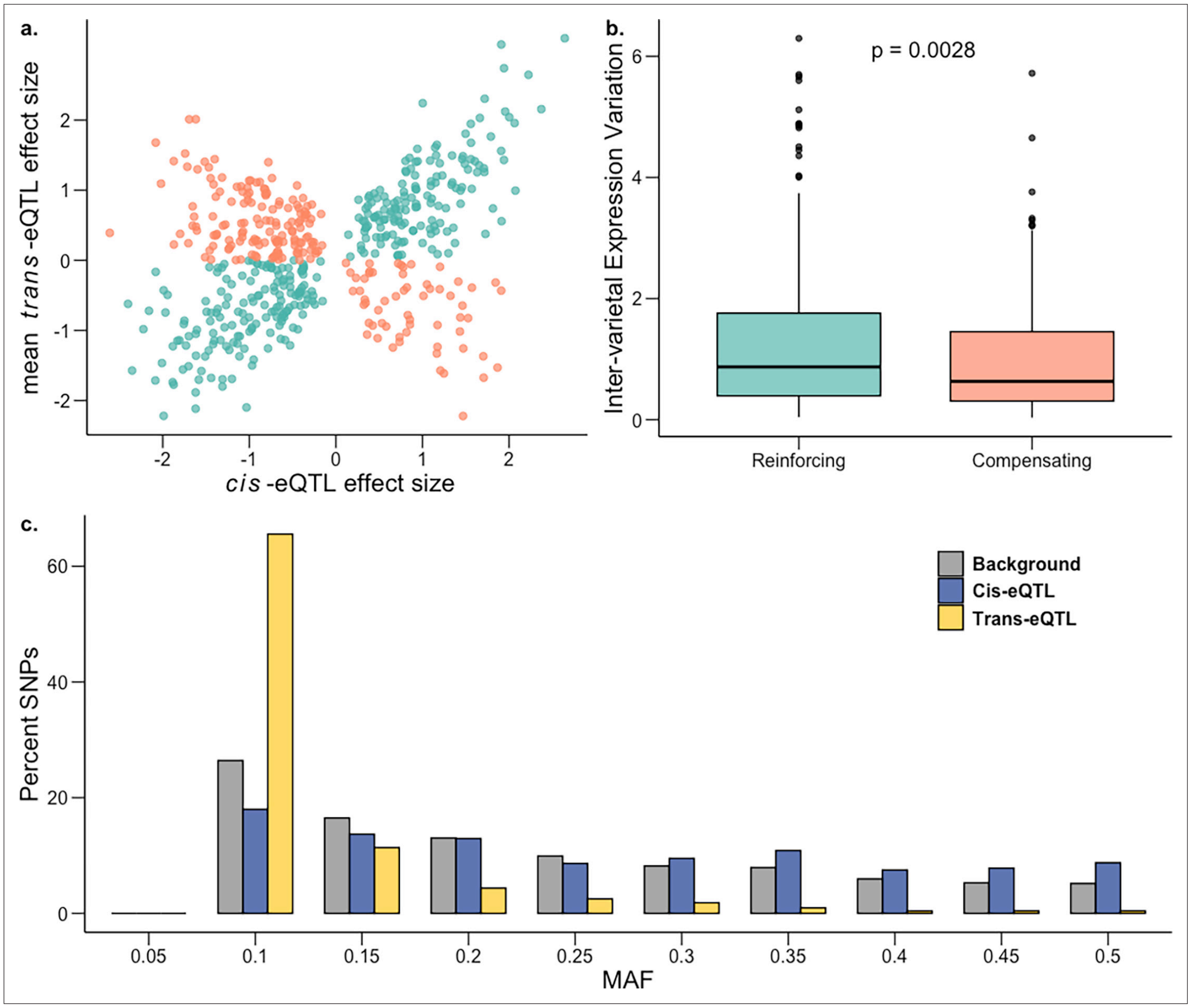


Figure 5. Genetic architecture of gene expression variation during salt stress. (**a**) Effect sizes of genes with both *cis* and *trans* factors under saline conditions showing excess of reinforcing cis-trans (teal) in comparison to compensating cis-trans (salmon). (**b**) Inter-varietal variation in gene expression for genes under compensating control is significantly lower than for those under reinforcing control; one-sided Mann-Whitney p=0.0028. c, Frequency distribution of MAF (minor allele frequency) for *cis*-eQTLs (blue) and *trans*-eQTLs (yellow) in saline conditions against the genome-wide background (gray).

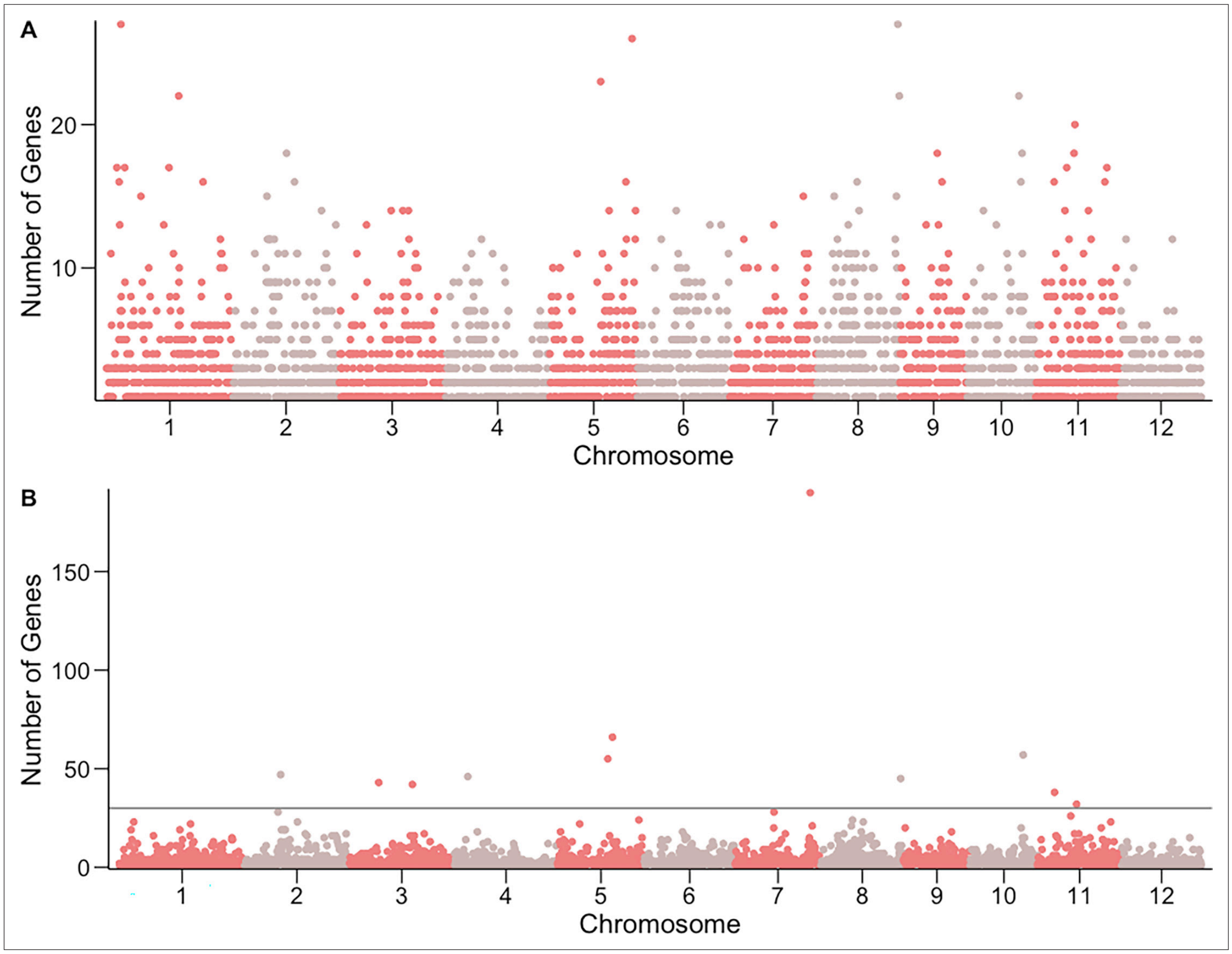


Figure 5—figure supplement 1. *Trans*-eQTL hotspots in normal (**a**) and salinity stress (**b**) conditions. X-axes indicate genomic locations of 100 kb nonoverlapping windows for which the total numbers of unique genes regulated were calculated (Y-axis). Hotspots were defined as windows regulating expression of over 30 genes; there were 0 hotspots in normal conditions (max number of genes = 28), and 11 hotspots in saline conditions. Gray horizontal line represents the cutoff of 30 genes.

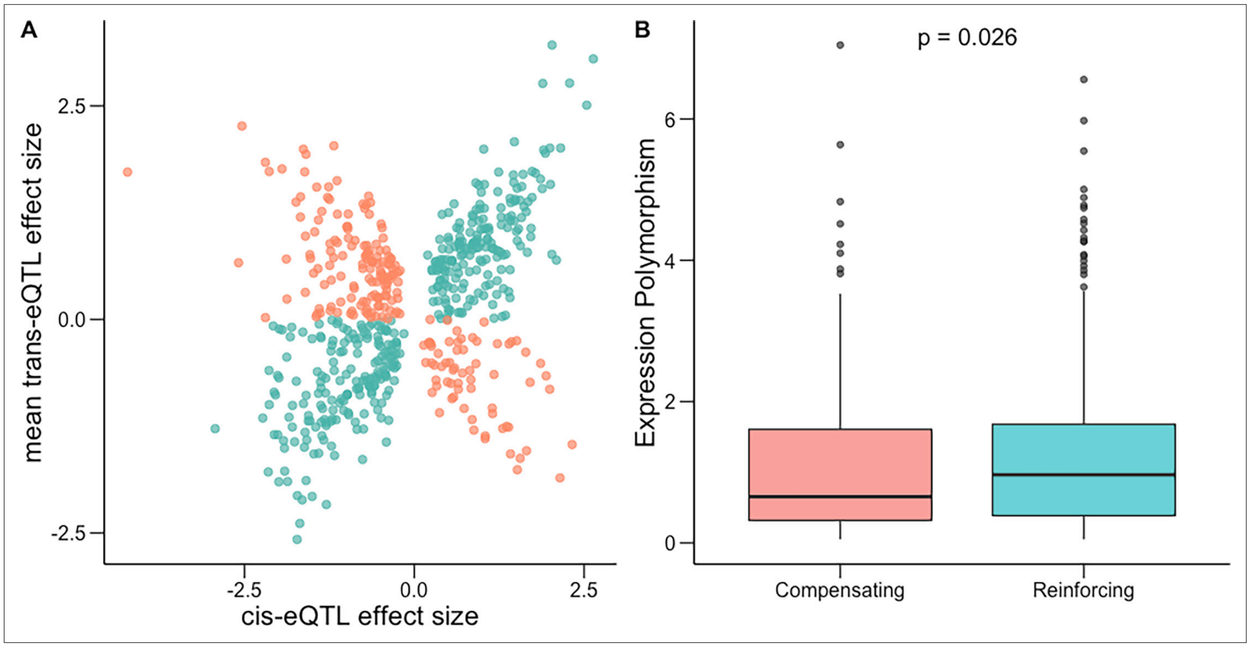


Figure 5—figure supplement 2. Compensating and reinforcing *cis-trans* effects in normal conditions. (**A**) Effect sizes of genes with both *cis* and *trans* factors showing excess of *cis-trans* reinforcement (teal) in comparison to *cis-trans* compensation (salmon). (**B**) Inter-varietal differences in gene expression for genes under compensating control is significantly lower than that for genes under reinforcing control; one-sided Mann-Whitney p=0.026.

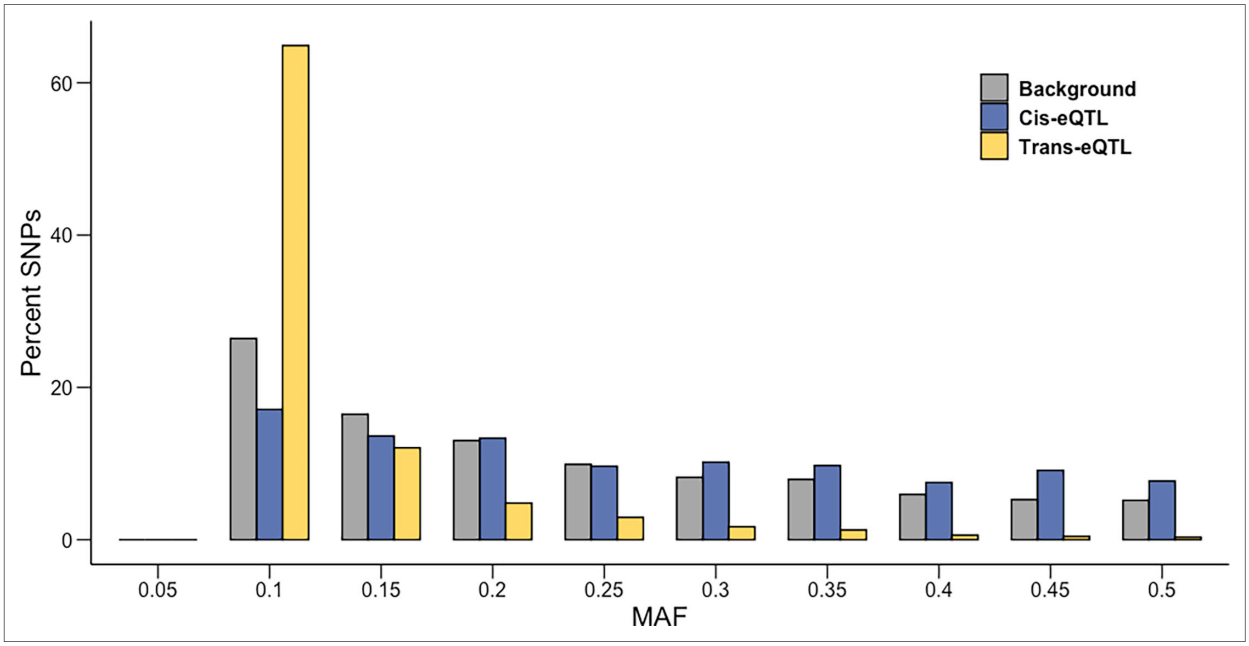


Figure 5—figure supplement 3. Frequency distribution of MAF (Minor Allele Frequency) for *cis*-eQTLs (blue) and *trans*-eQTLs (yellow) in normal conditions against the genome-wide background (gray).