Supplementary Materials

A novel analytical framework to quantify co-gradient and counter-gradient variation

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Supplemental Methods. Variance partitioning

In the main manuscript, we focus on estimating the effect size of Cov_{GE} and $\bar{\Delta}_{GxE}$ and their significance. The effect size provides information about the strength of the pattern, which is a distinct type of information from the percent of variation in the phenotype explained by the following components: (i) genetic effects on phenotype, (ii) environmental effects on phenotypes, (iii) genetic x environment interactions, (iv) covariance between genetic and environmental effects on phenotypes, and (iv) residual error. Both Falconer (1989) and Conover and Schultz (1995) have previously discussed Cov_{GE} in this more traditional sense as the percent of variation in the phenotype explained by different variance components:

$$V_P = V_G + V_E + V_{GxE} + xCov_{GE} \tag{1}$$

In a 2x2 reciprocal transplant or common garden design with 2 genotypes in 2 environments, $x = 2$. This factor of $x = 2$ does not extend to more complex designs, as explained below.

Here, we show how to extend sums of squares (SS) calculations from a traditional analysis of variance to incorporate $SS_{Cov_{GE}}$, which can in turn be used to understand the percent of variation in phenotypes explained by different components. These calculations assume a fully factorial reciprocal transplant design. We do not advocate that these SS be used to test the significance of the variance components with a traditional F-test, because the presence of Cov_{GE} likely violates the assumption of independence among samples and complicates calculations of degrees of freedom. It is, however, useful to compare the percent of variation explained by different components to their effect sizes, because it furthers understanding of the relative influence of genetic differentiation and plasticity on the evolved patterns in the population.

In a reciprocal transplant experiment, there are g genotypes transplanted into e environmental patches, for a total of $g * e = n_{ge}$ genotype-environment combinations. In a fully factorial reciprocal transplant experiment, $g = e$, g is the number of levels of genotypes from $i = 1, 2...g$, and e is the number of levels of environments from $j = 1, 2...e$.

Assuming the equal sample sizes r (k $= 1, 2, ...r$) within each genotype-environment combination, the following sums of squares can be estimated as:

$$V_G = SS_G = re\sum_{i=1}^{g}(\bar{y_i} - \bar{y})^2 \tag{2}$$

$$V_E = SS_E = rg\sum_{j=1}^{e}(\bar{y_j} - \bar{y})^2 \tag{3}$$

$$V_{GxE} = SS_{GE} = r\sum_{i=1}^{g}\sum_{j=1}^{e}(\bar{y_{ij}} - \bar{y_i} - \bar{y_j} + \bar{y})^2 \tag{4}$$

$$V_{Cov_{GE}} = SS_{Cov_{GE}} = |xr\sum_{i=1}^{g}\sum_{j=1}^{e}(\bar{y_i} - \bar{y})(\bar{y_j} - \bar{y})I| \tag{5}$$

$$V_{error} = SS_{Error} = \sum_{i=1}^{g}\sum_{j=1}^{e}\sum_{k=1}^{r}(y_{ijk} - \bar{y}_{ij})^2 \tag{6}$$

where $x = \frac{ge}{(\sum_{i=1}^{g}\sum_{j=1}^{e} I_{ij})}$, and I is an indicator variable that is 1 when the genotype i originated from environment j and 0 otherwise. In a 2x2 reciprocal transplant design, $g = 2$, $e = 2$, and $\sum_{i=1}^{g}\sum_{j=1}^{e} I_{ij} = 2$, so $x = 2$ as is assumed in Eq. S1. However in a 4x4 reciprocal transplant design, $g = 4$, $e = 4$, and $\sum_{i=1}^{g}\sum_{j=1}^{e} I_{ij} = 4$, so $x = 4$. The factor x ensures that the $SS_{Cov_{GE}}$ scales appropriately with the SS of the other components with the size of the experiment. Finally, since Cov_{GE} can be negative under countergradient scenarios, we take the absolute value for partitioning variance.

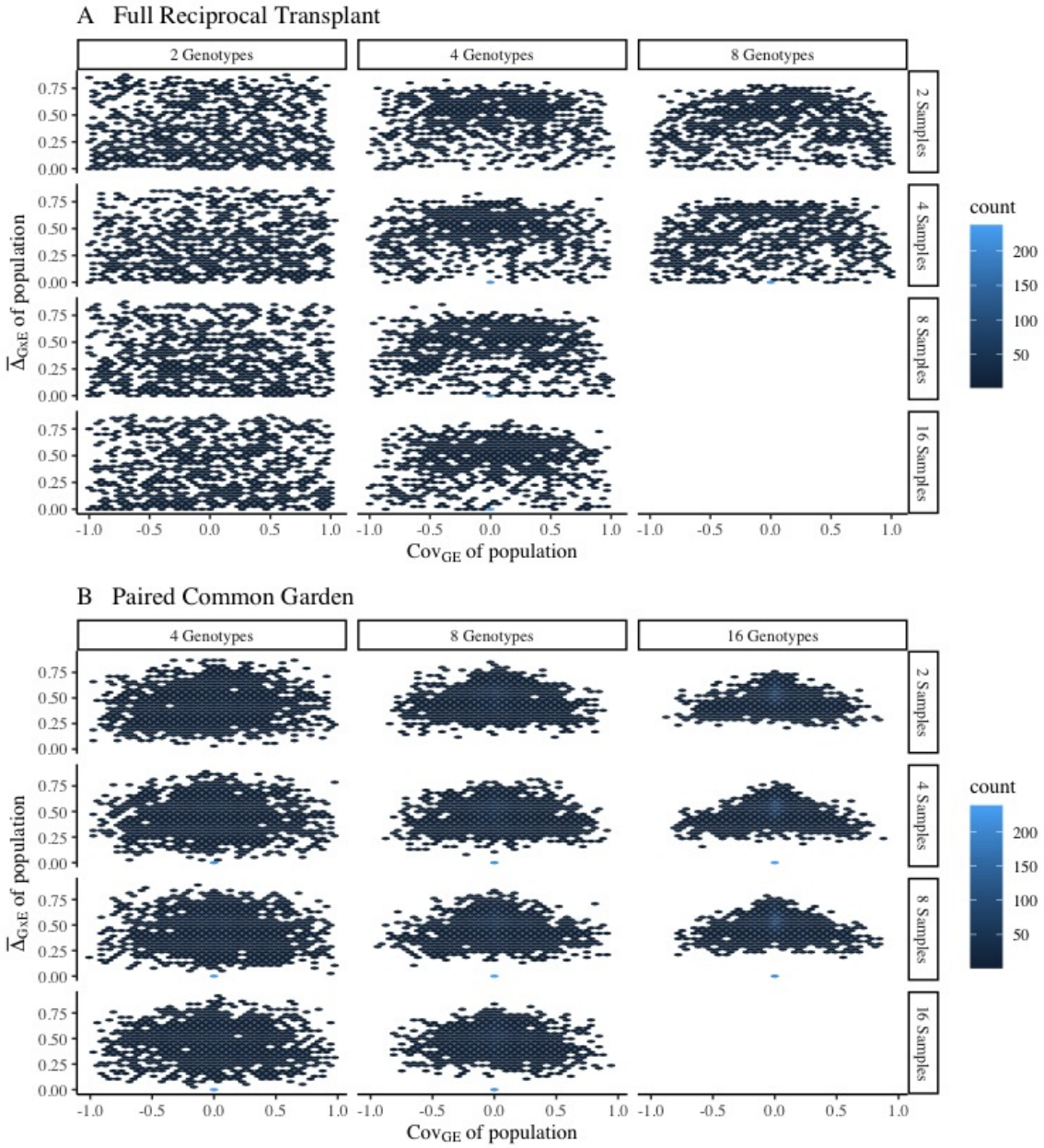
The percent of variation explained by each component ($Comp$) can then be estimated as

$$\eta^2_{Comp} = \frac{SS_{Comp}}{SS_G + SS_E + SS_{GxE} + SS_{CovGE} + SS_{Error}} \tag{7}$$

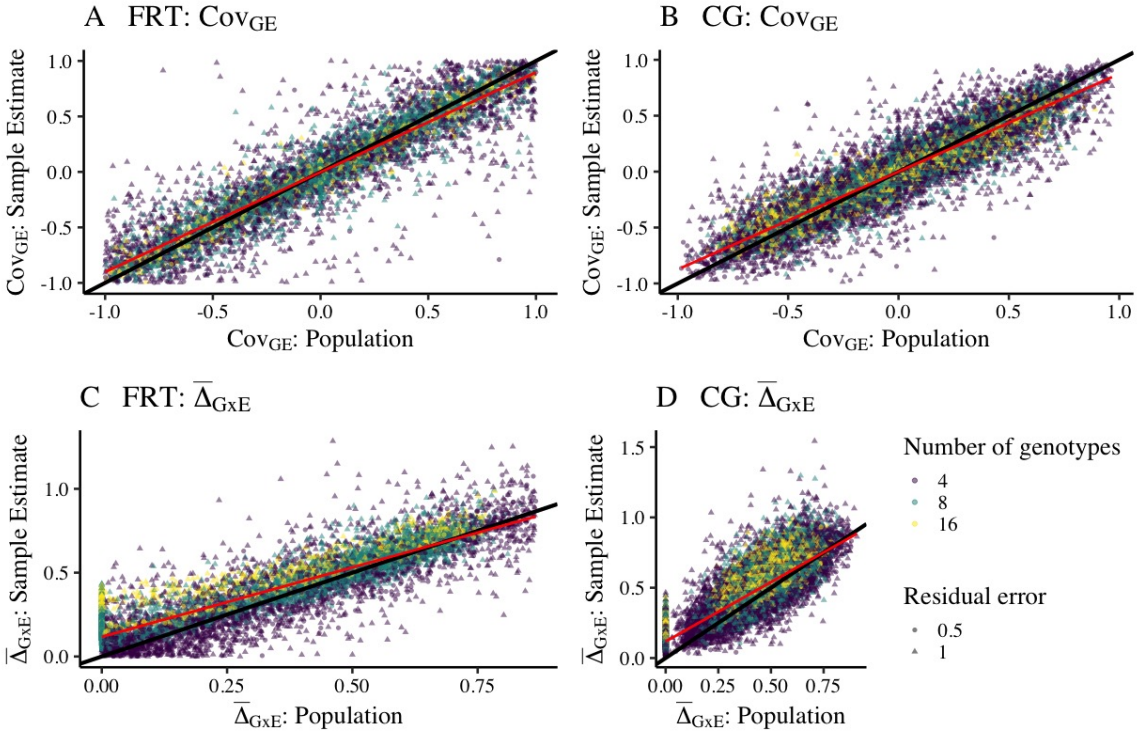
We recognize that this is a crude approach. However, it provides a reasonable way to compare to the percent of variation explained by different components to their effect size estimates in the main text. For example, when $|Cov_{GE}|$ is maximized, equal amounts of non-residual variance are explained by V_G, V_E, and $V_{Cov_{GE}}$ for the fully factorial reciprocal transplant experiment with an arbitrary number of populations (Supp. Fig 4a). For this reason, when residual variation is minimized the maximum percent of phenotypic variance explained by $V_{Cov_{GE}}$ is rarely greater than 1/3 of the total phenotypic variance (Supp. Fig 3, Supp. Fig. 4a).

The comparison of effect sizes and variance components is also useful for understanding the relative influences of V_G and V_E on a particular value of $|Cov_{GE}|$; for instance, similarly intermediate values of $|Cov_{GE}|$ can be driven by higher V_E and lower V_G (Supp. Fig. 4B), or lower V_E and higher V_G (Supp. Fig. 4C).

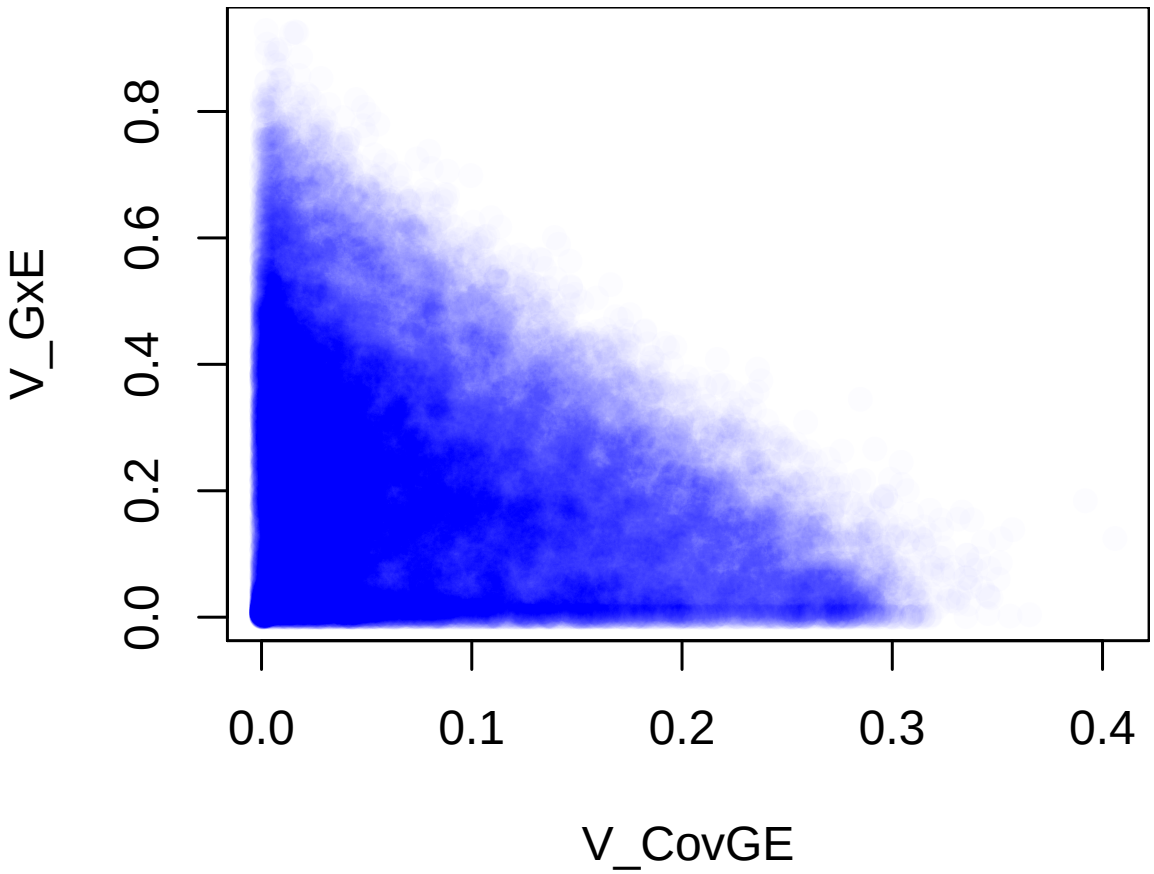
Supplemental Figures. FIGURES



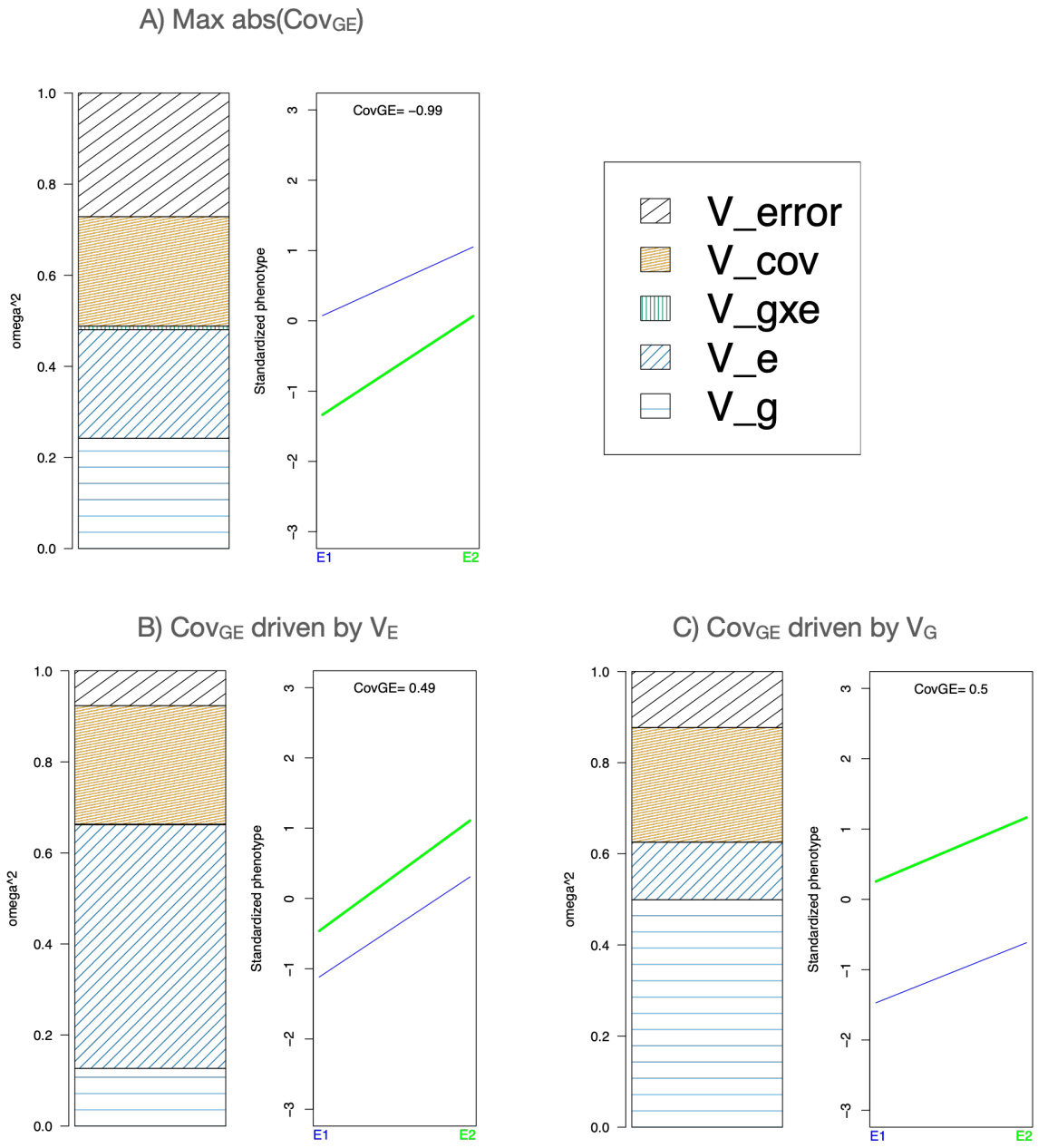
SUPPLEMENTARY FIGURE S1. Coverage of parameter space of Cov_{GE} and $\bar{\Delta}_{GxE}$ for full reciprocal transplant (A) and paired common garden designs (B). Hexagons are colored according to the density of observations in each bin.



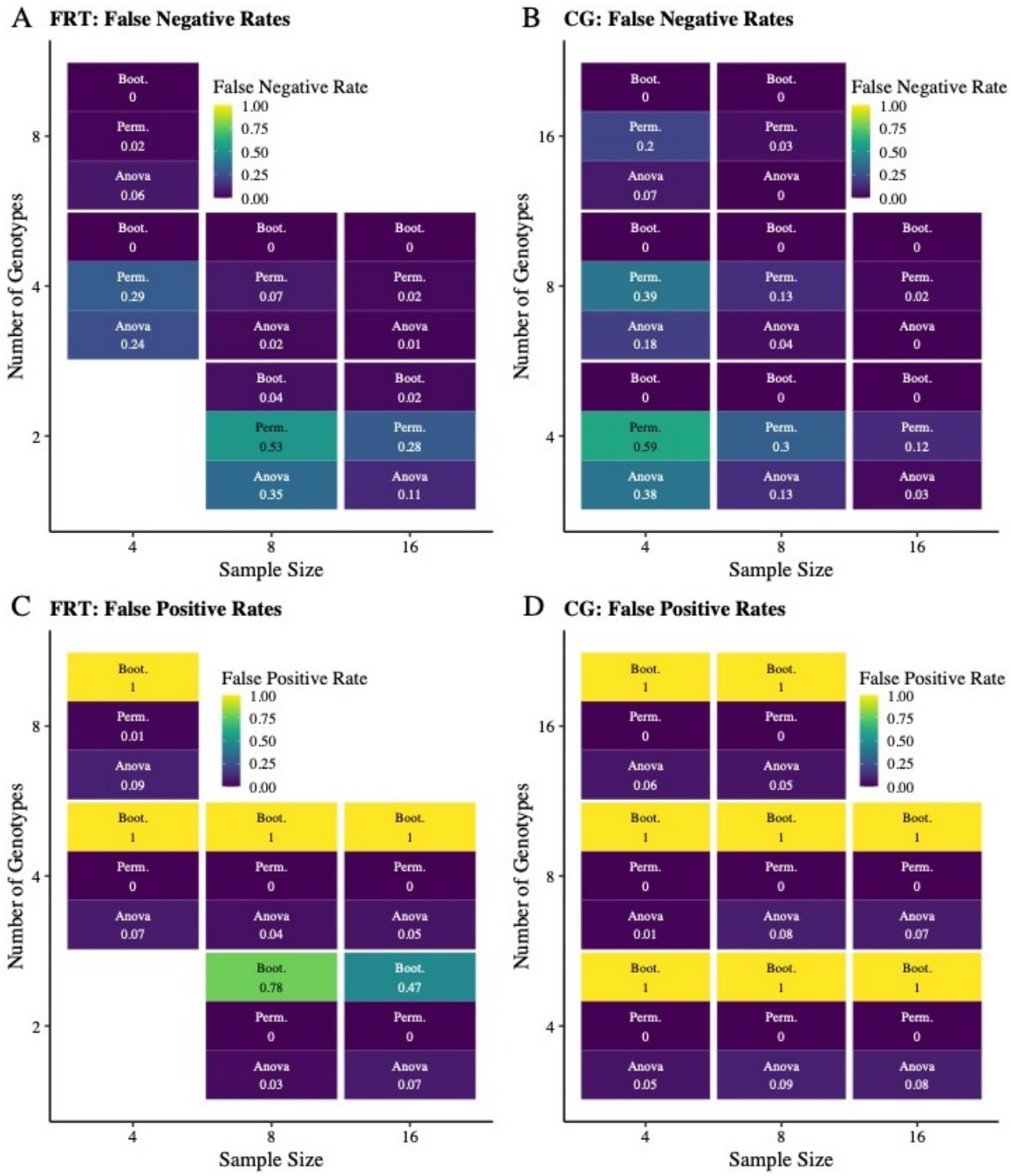
Supplementary Figure S2. Agreement between population measures and sample estimates of Cov_{GE} and $\bar{\Delta}_{GxE}$ for Full Reciprocal Transplant (A, C) and paired Common Garden designs (B, D). The black line falls along a 1:1 line while the red line reflects the pattern of the data. Point colors indicate the number of genotypes, while point shapes indicate the level of residual variation. As expected, sample estimates deviate more from the population measure in situations with low sample sizes and higher residual variation.



Supplementary Figure S3. Comparison of the percent of variance explained by V_{GxE} and $V_{Cov_{GE}}$ across all simulations.



Supplementary Figure S4. Comparison of the percent of variance explained by $V_{Cov_{GE}}$ with the effect size of Cov_{GE} for three example simulations from a 2 genotype x 2 environment reciprocal transplant design. In each subpanel, the stacked barplot on the left of each panel shows the proportion of variance explained by each component corresponding to the legend (Supp. Eq. 7), while the plot on the right shows the mean standardized reaction norms for each genotype (the effect size of Cov_{GE} is shown at the top). A) When $|Cov_{GE}| = 1$ (i.e., maximal Cov_{GE}), equal proportions of phenotypic variance is explained by V_G, V_E, and $V_{Cov_{GE}}$. B) An intermediate effect size of Cov_{GE} 0.5 arising from larger V_E compared to V_G. C) An intermediate effect size of Cov_{GE} 0.5 arising from larger V_G compared to V_E.



Supplementary Figure S5. Heat map showing false negative rates for $\bar{\Delta}_{GxE}$ for Full Reciprocal Transplant (A) and paired Common Garden designs (B) and false positive rates for full reciprocal transplant (C) and paired Common Garden designs (D). Tiles are split to show rates for bootstrapping (top), permutation (middle) and ANOVA (bottom) approaches. This figure complements Fig. 4 in the main text and is meant to demonstrate that bootstrapping produces unreliable confidence intervals for $\bar{\Delta}_{GxE}$.