



Fig. S3 Design of the GLM analysis to estimate *cis*- and *trans*-regulated expression variances. The flies at the top represent F₁ hybrids from the Mel6 and DGRP cross. The paired chromosome-shaped bars show the combinations of alleles from Mel6 (black) and DGRP (colored) strains. ASE is measured for each gene. The equation of the generalized linear model used in the type-II ANOVA is described. The numbers below chromosome images indicate categorical numbers for *cis*- and *trans*-regulatory effects. Category #* represents *trans*-regulatory effect from both Mel6 and DGRP *trans* alleles. Error terms were estimated using duplicate experiments. This design enables estimation of *cis*- and *trans*-regulatory effects, assuming that interaction between them is negligible. Note that the variance of Mel6 ASE across F₁ hybrids of 18 different genotypes is affected only by *trans*-regulatory mutations on paired DGRP chromosomes, because the same Mel6 *cis* genotype (*cis* category 0) is paired with Mel6 *trans* genotype and one of the 18 different DGRP *trans* genotypes (*trans* category 1*–18*). Inevitably, any *cis*-regulatory effects of Mel6 alleles are also included in the model as category 0.