SUPPLEMENTAL

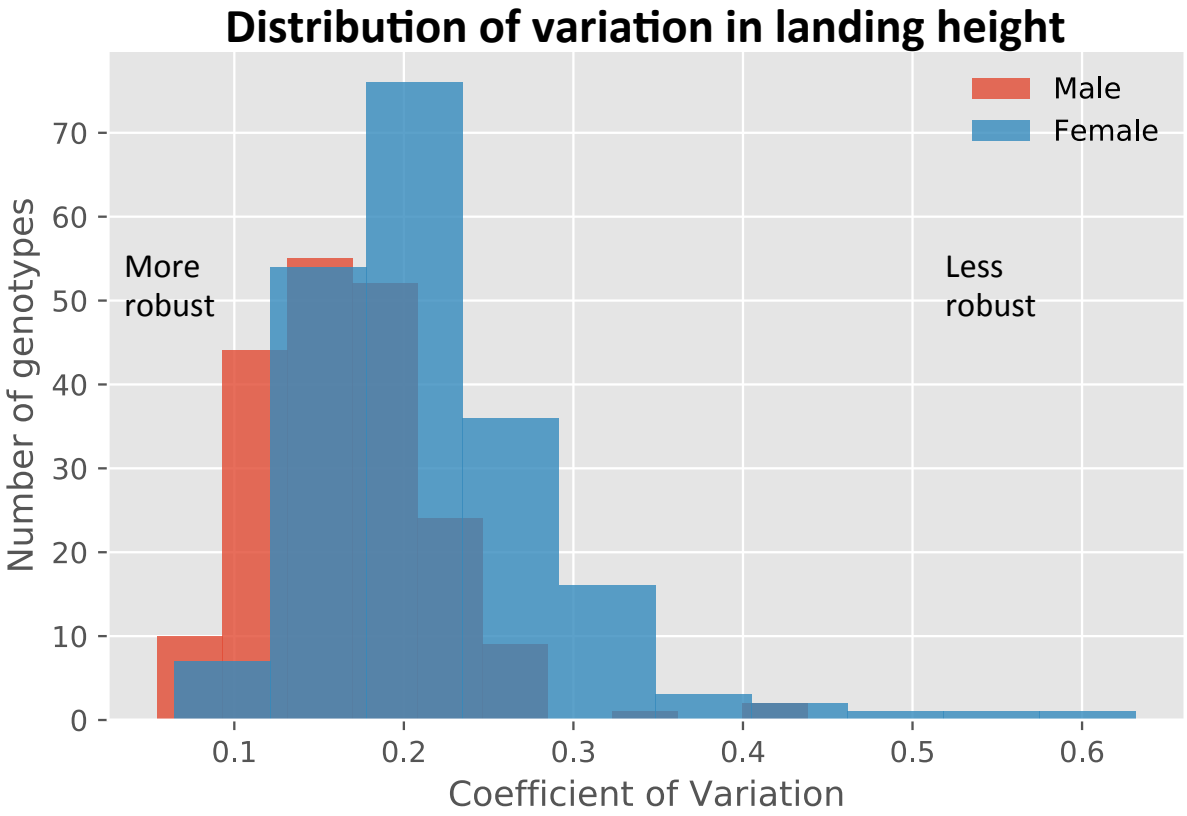

Figure S1. Coefficient of variation is near-normally distributed across sexes. Genotypes' coefficient of variation is a measure of the standard deviation divided by the mean, representing a normalized measure of variation across genotypes. The distribution for each sex (males more so) was near-normally distributed, though there was a tail to the distribution favoring greater coefficients of variations. Lower coefficients of variation correspond with a greater degree of robustness for flight performance.

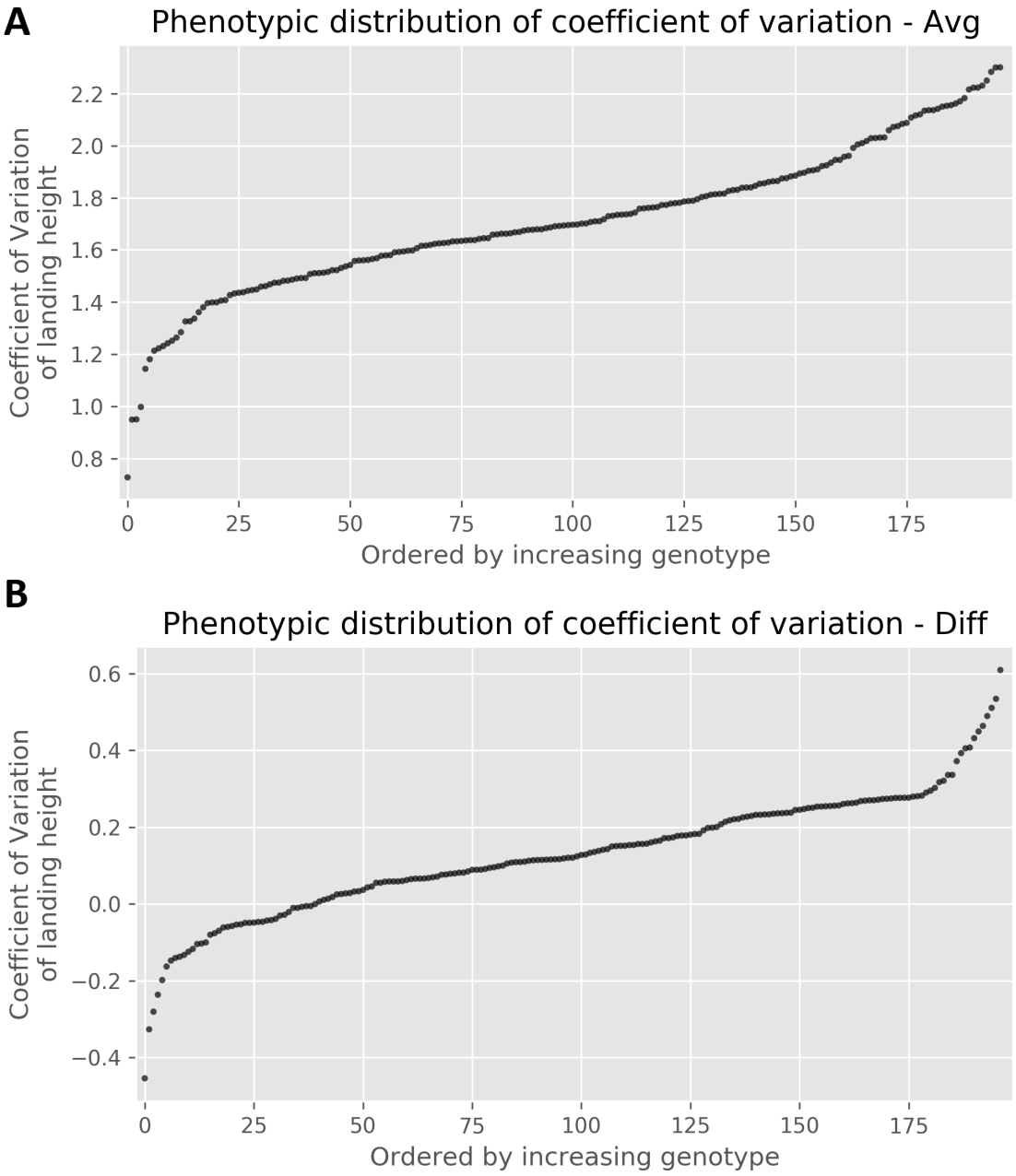

Figure S2. Phenotypic distributions for sex-average and sex-difference phenotypes. (A) Sex-average and (B) sex-difference phenotypes exhibit phenotypic variation for the coefficient of variation in flight performance. Each distribution is independently arranged by increasing phenotype score. The sex-difference scores represent females – males.

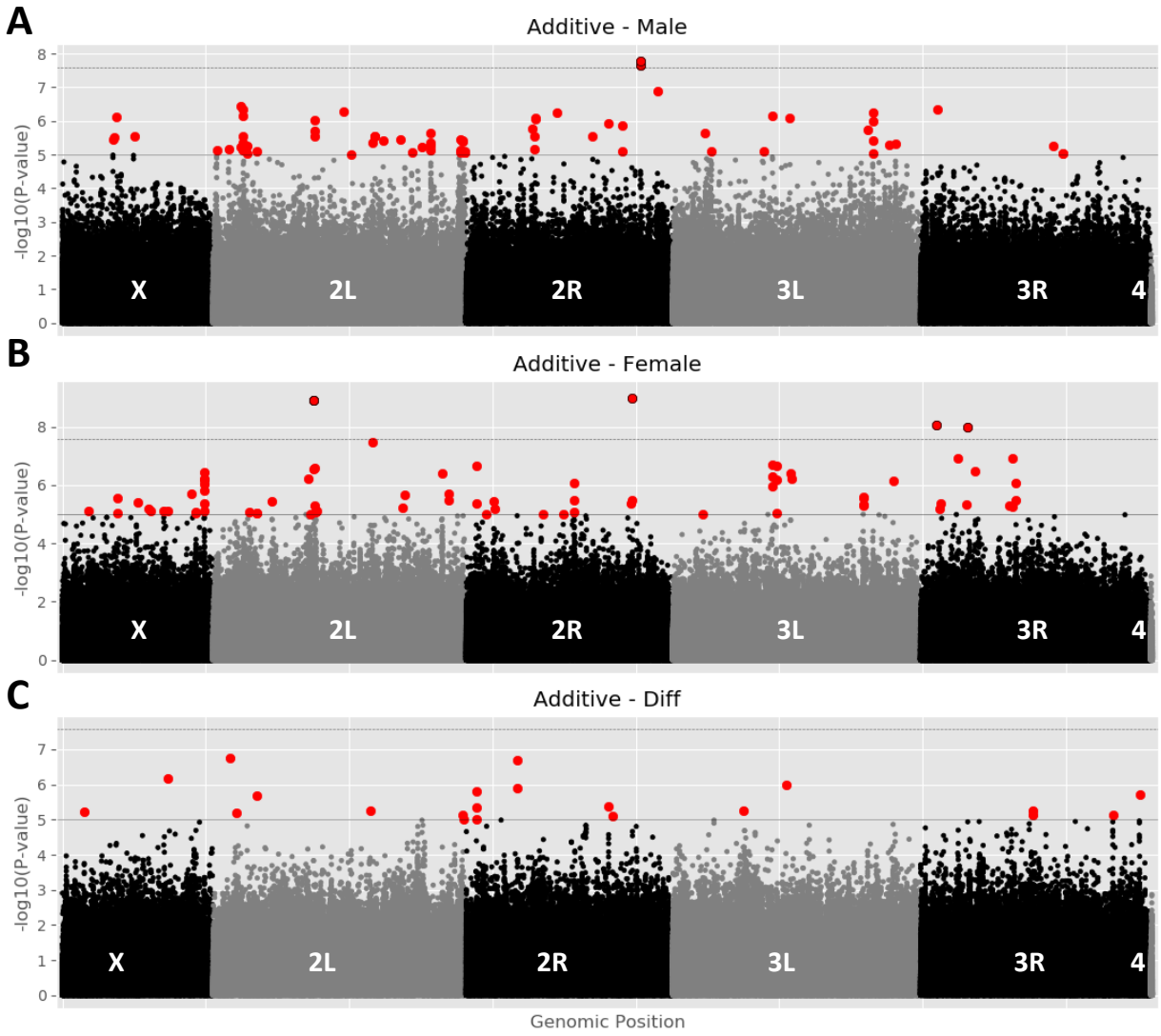

Figure S3. Significant variants in additive analysis by sex-based phenotype. Several additive variants were identified across the (A) male, (B) female, and (C) sex-difference phenotypes. Variants that passed a traditional DGRP significance threshold ($P \leq$ 1E-5; gray solid line) are in red, while those that passed a Bonferroni threshold ($P \leq$ 2.63E-8; gray dashed line) are red with black outline. Points ordered by their relative position across each chromosome (labeled) and plotted against the –log10 of their significance score.

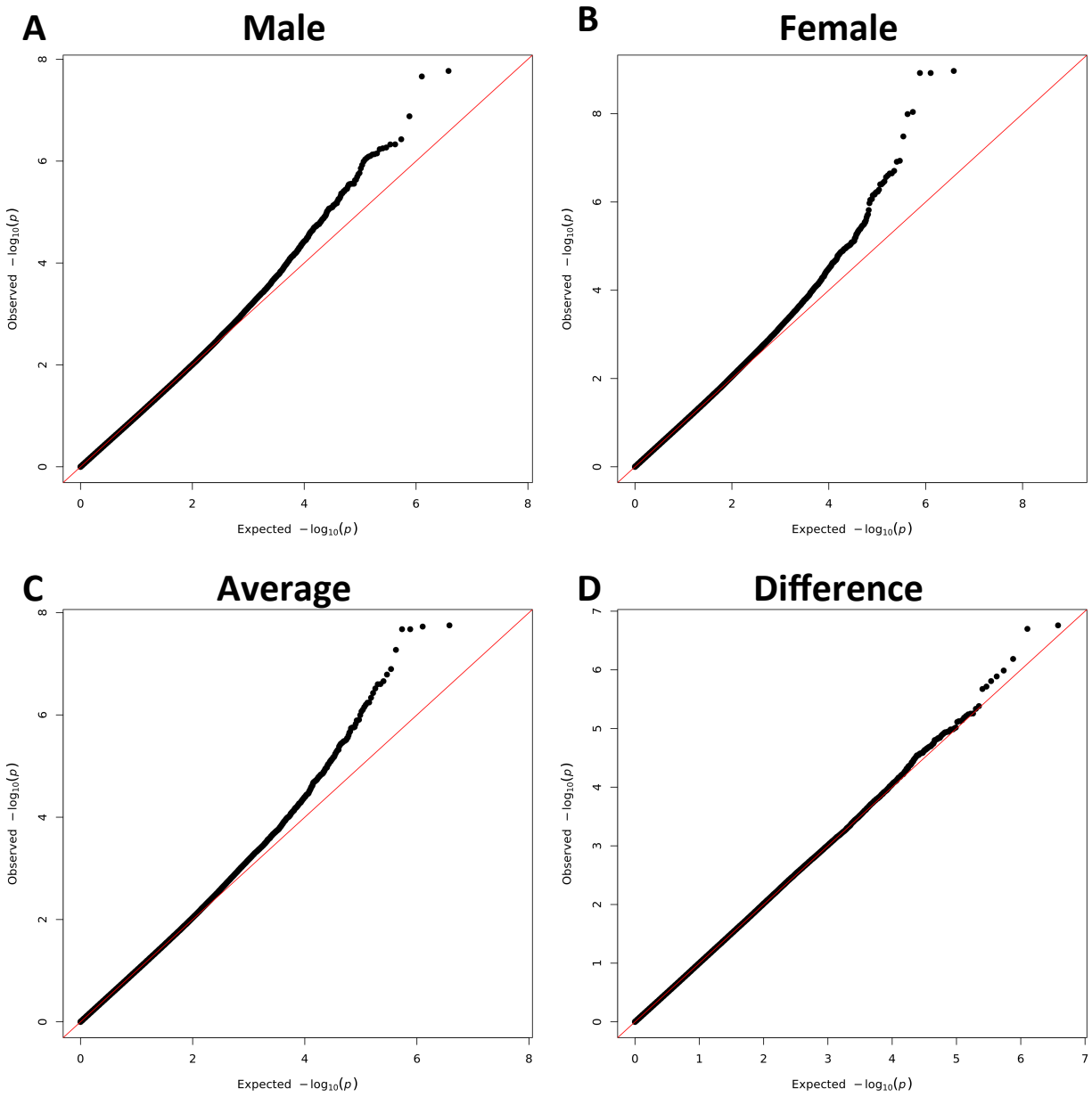

Figure S4. Quantile-quantile (QQ) plots suggest several additive variants associate with robustness in flight performance. QQ-plots illustrating the distribution in observed vs. expected *P*-values for the (A) male, (B) female, and (C) sex-average phenotypes, and (D) sex-difference phenotypes. These plots suggest each sex-based phenotype has several significant variants based on the deviation from the red line representing a 1:1 (expected:observed) relationship.

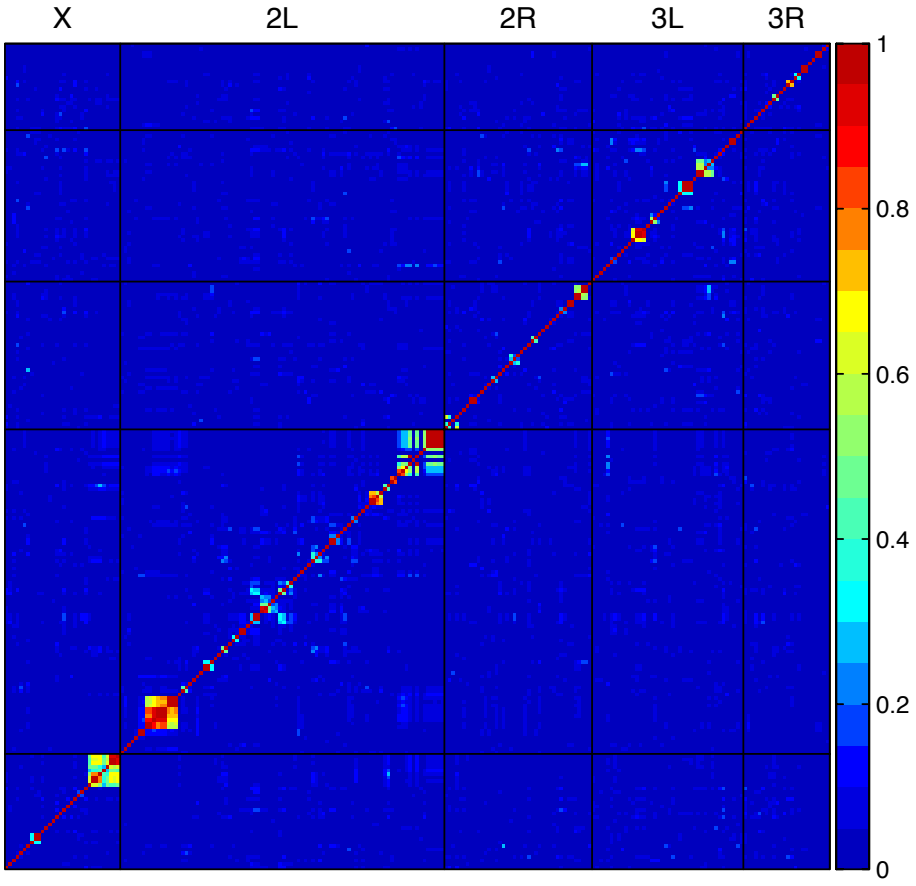

Figure S5. Significant additive variants are broadly distributed across the genome. Heat map illustrating the chromosomal location of each of the DGRP2 webserver's putative 'top hits' (returned from DGRP2 webserver) colored from decreasing (blue, 0) to increasing (red, 1) linkage score. Most variants were distributed throughout all but chromosome 4, with some variants in linkage blocks (multicolored squares).

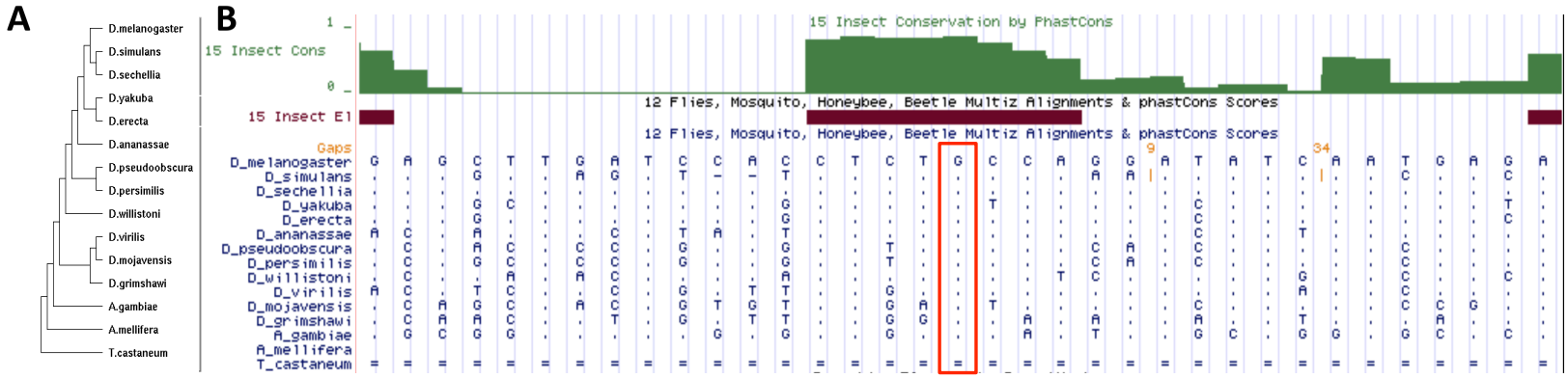

Figure S6. Non-synonymous variant (3R_4379159_SNP) in *Odorant receptor 85d* lies in a strongly conserved region across several insect species. (A) Phylogeny of 15 insect taxa includes several members of the *Drosophilidae* family, as well as members of the order *Diptera*. (B) This variant (red box) encodes a moderate missense mutation (tGc/tAc; C277Y) of unknown function. However, the high level of conservation among the 15 lineages hints at the site' s importance, despite a putatively neutral (-2.312, -2.5 is deleterious) PROVEAN score (CHOI AND CHAN 2015). Images were acquired from UCSC Genome Browser (http://genome.ucsc.edu/).

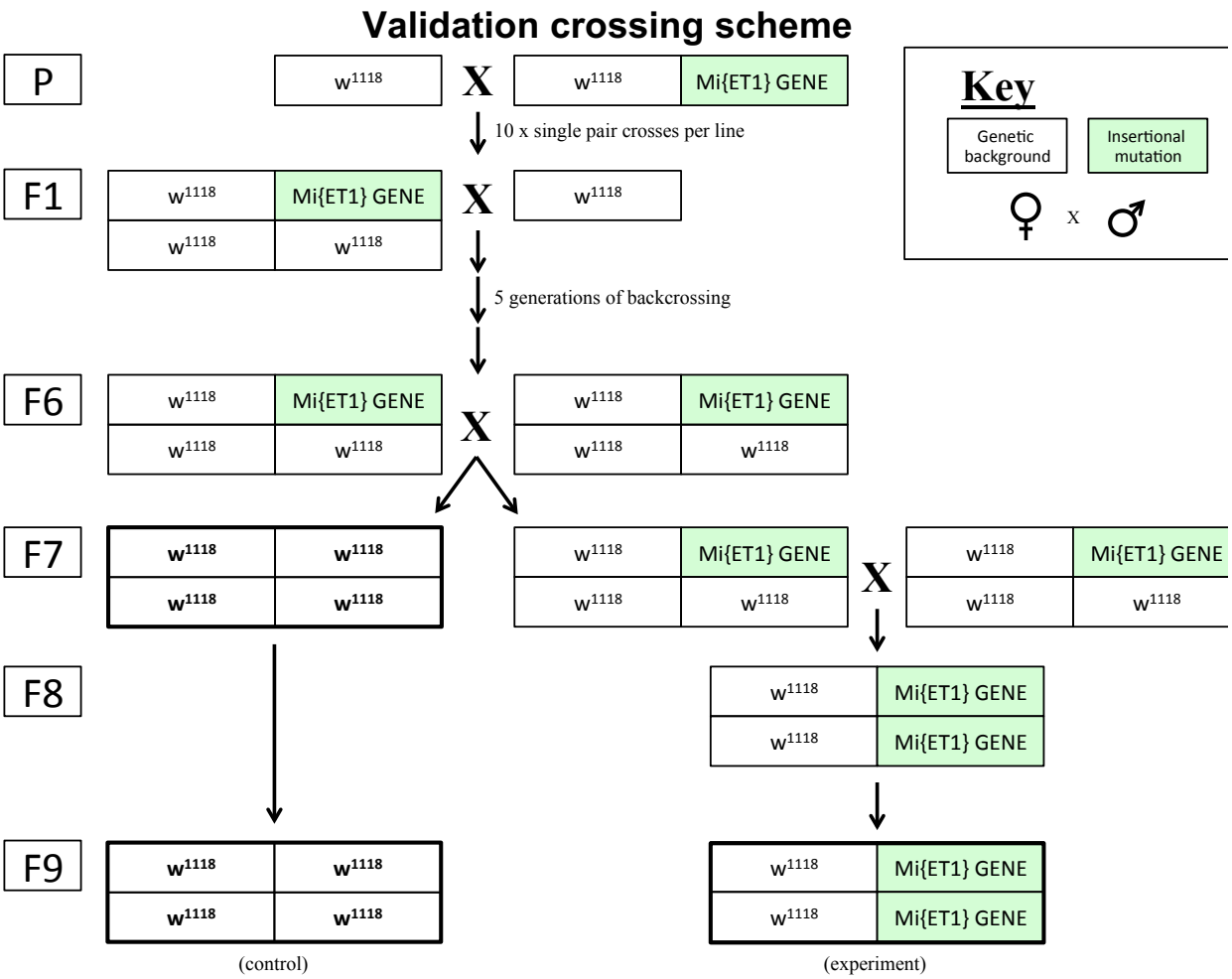

Figure S7. *Drosophila* crossing scheme used to generate control and experimental lines for candidate gene validation. All crosses take place between females on the left and males on the right. White boxes represent the background control line, either w^{1118} (or $y^{1w67c23}$), while green boxes represent the insertional mutation construct. The first generation cross generated females heterozygous for the construct, which were then backcrossed for five consecutive generations to the respective background control line. Isoparental crosses between heterozygotes for the construct were screen for flies without the construct (control) or heterozygous/homozygous for the construct. The latter group was self-crossed within the same vial and the resulting crosses that contained no flies without the reporter were deemed homozygous for the construct (experiment). Both control and experiment lines were maintained for 2 generations to confirm their genotype before testing. Figure reproduced with permission (SPIERER *et al.* 2020).

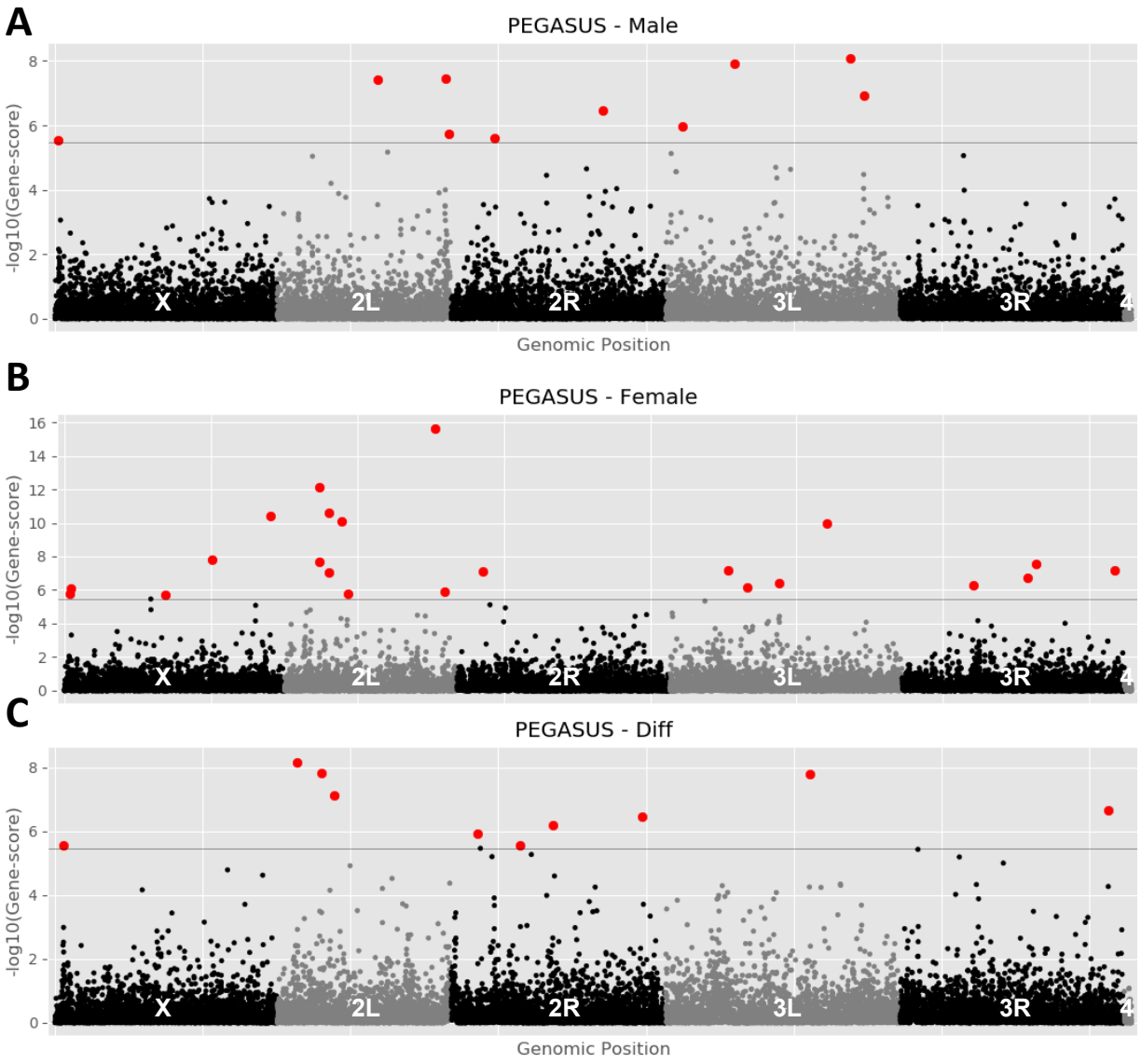

Figure S8. Several whole genes were identified across each sex-based phenotype using `PEGASUS_flies`. In total, 45 unique genes were found in (A) males, (B) females, (C) and sex-difference. Significant genes (red points) passed the Bonferroni threshold ($P \leq$ 3.43E-6; gray line), while the remaining did not (black and gray). Points are arranged in order of relative position on each chromosome (labeled), and plotted against the –log10 of their significance score.

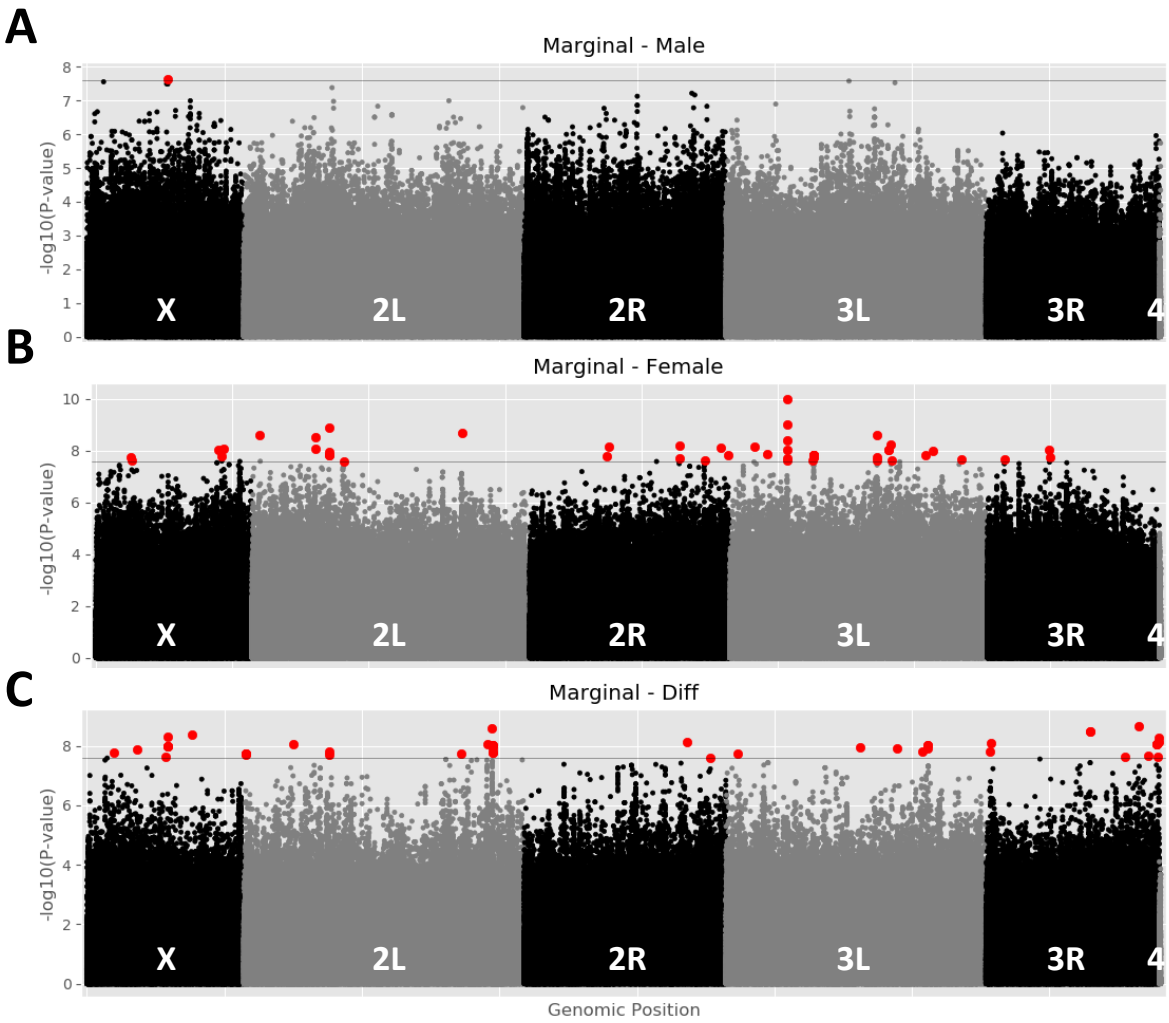

Figure S9. Significant marginal variants were identified across each sex-based phenotype. Several marginal variants were identified across the (A) male, (B) female, and (C) sex-difference phenotypes. Significant variants (red) passed a Bonferroni threshold ($P \leq 2.56E\text{-}8$; gray solid line), while those that did not are colored in black or gray. Points are ordered by their relative genomic position and their significance score –log10 transformed.