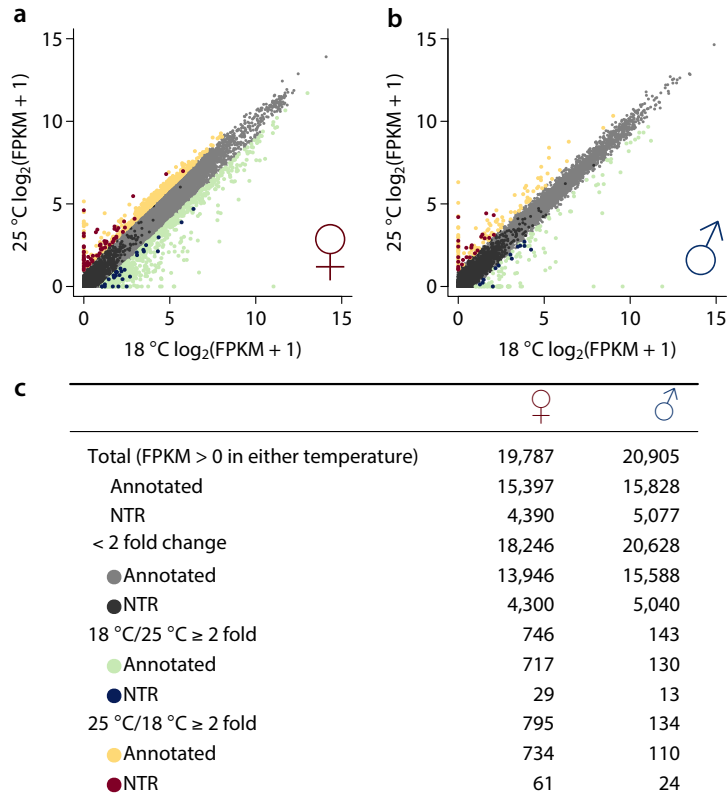
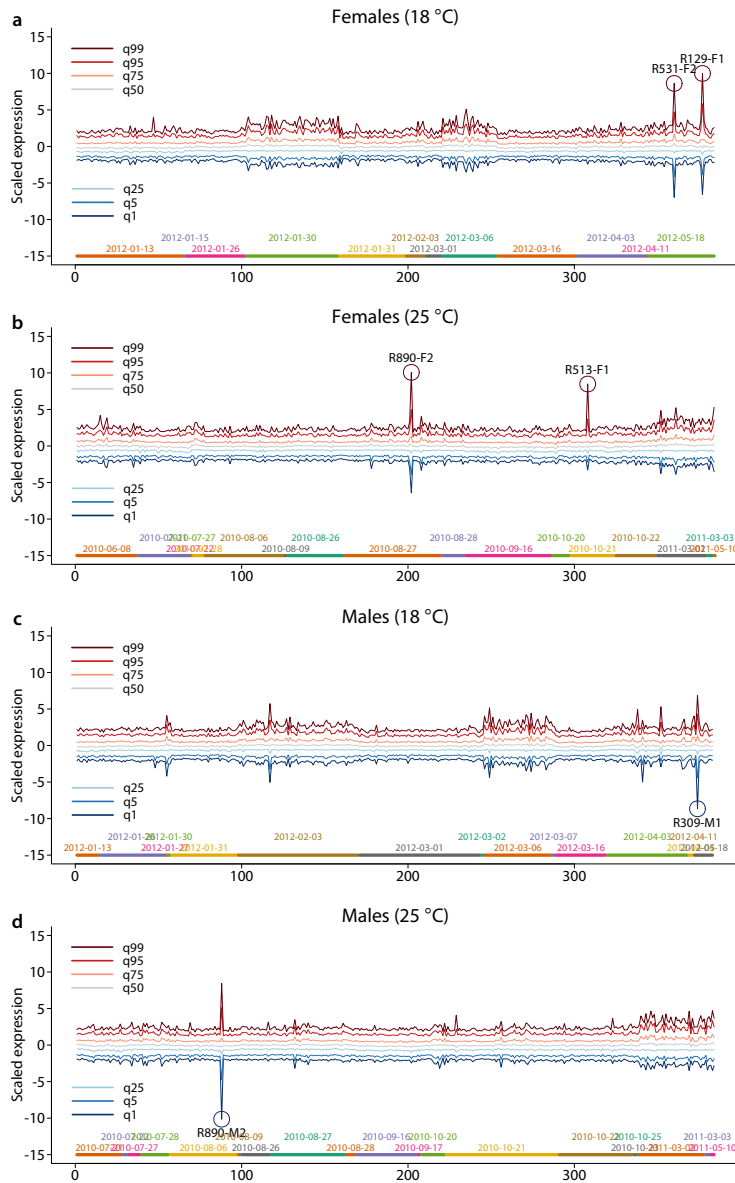


Supplementary Information



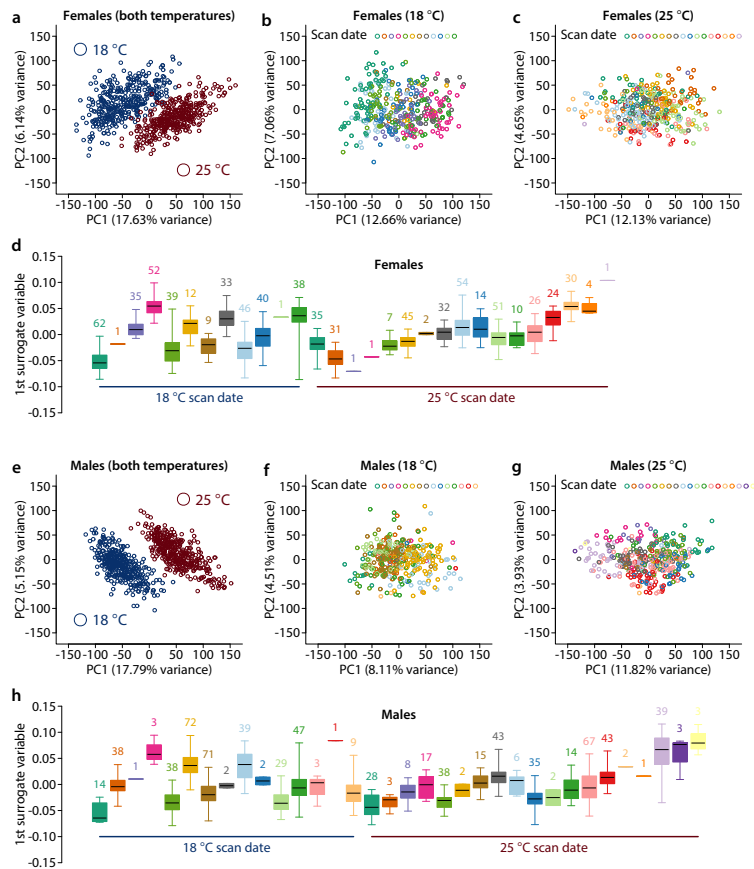
Supplementary Fig. 1 | RNA-Seq annotation of the pooled RNA from DGRP lines

(a) Comparison of RNA-Seq expression estimates at 18 °C and 25 °C in females, colors of points indicate different classes of genes as indicated in (c). (b) Comparison of RNA-Seq expression estimates at 18 °C and 25 °C in males, colors of points indicate different classes of genes as indicated in (c). (c) Numbers of the different classes of genes based on expression comparison at 18 °C and 25 °C in females and males.

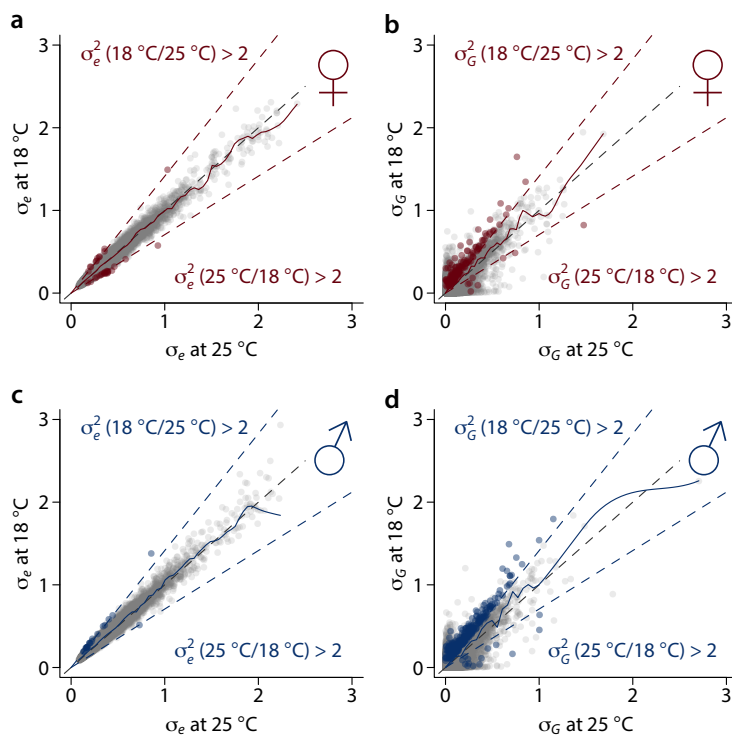


Supplementary Fig. 2 | Distribution of scaled gene expression across samples

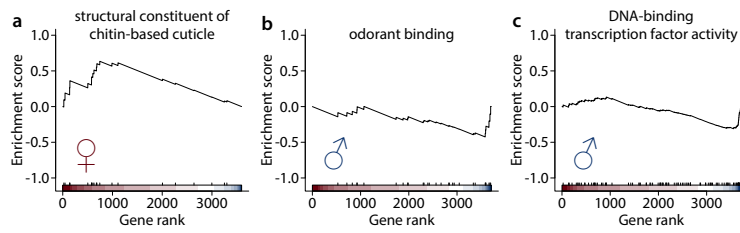
Within each sex and temperature, expression of genes was scaled across samples and the quantiles (1% [q1], 5% [q5], 25% [q25], 50% [q50], 75% [q75], 95% [q95], 99% [q99]) are visualized within each batch. Samples are arranged according to their array scan date, outliers are circled and annotated. **(a)** Females at 18 °C, **(b)** Females at 25 °C, **(c)** Males at 18 °C, **(d)** Males at 25 °C.



Supplementary Fig. 3 | Principal component analysis (PCA) and surrogate variable analysis (SVA) of gene expression (a) Principal component analysis in females combining samples from both 18 and 25 °C. (b) Principal component analysis in females at 18 °C. (c) Principal component analysis in females at 25 °C. (d) Variation in the first surrogate variable within and between the different array scan dates in females. (e - h) Same analyses but in males. Sample sizes are indicated above box plots in (d) and (h). For each box plot, the whiskers represent maximum and minimum values, box represents inter-quartile range and the middle line median.



Supplementary Fig. 4 | Decanalization and canalization of genetic variance of gene expression at 18 °C without adjusting for *Wolbachia*. Same analyses as in Fig. 2 except that *Wolbachia* was not adjusted.



Supplementary Fig. 5 | Significant GO terms for the GSEA of decanalization/canalization. Trace plots for the three Gene Ontology (GO) terms **(a)** structural constituent of chitin-based cuticle enriched for genes of higher decanalization in females, **(b)** odorant binding enriched for genes of lower decanalization in males, and **(c)** DNA-binding transcription factor activity enriched for genes of lower decanalization in males.