Supporting Information

Zhou et al. 10.1073/pnas.1207367109

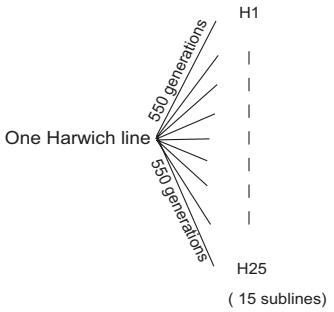

Fig. S1. Background of Harwich mutation accumulation lines. The diagram shows a brief history of the 15 Harwich lines that were split from a single line 550 generations ago.

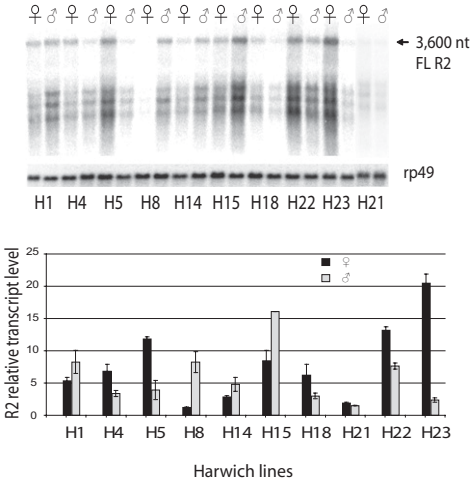

Fig. S2. R2 transcription in males and females of Harwich lines. A Northern blot probed with an R2 5′ antisense RNA probe shows R2 expression levels in males and females of Harwich lines. The same blot probed with antisense RNA generated from ribosomal protein gene rp49 was used as an RNA loading control. The bar graph at bottom shows quantitative results.

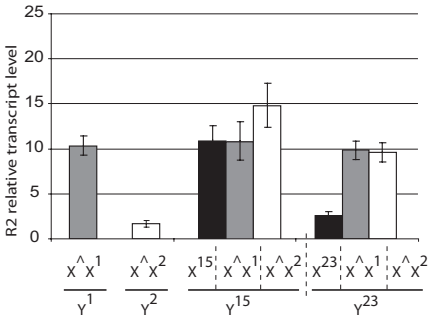

Fig. S3. R2 expression levels in X^X Y females. H15 and H23 males were crossed to females of two different attached X^X stocks. These chromosomes are designated $X^{\wedge}X^{1}$ and $X^{\wedge}X^{2}$, respectively. The first two columns represent the R2 transcript levels in the original females of these two attached X^X stocks. The remaining columns compare the R2 transcript levels in the female progeny of crosses to the parental males of H15 and H23.

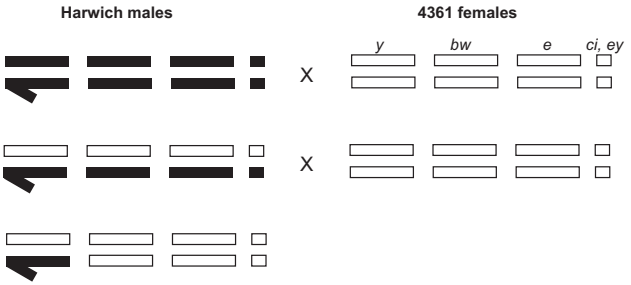

Fig. S4. Diagram of introgression of 15 Harwich Y chromosomes. Crossing scheme for introgressing Harwich Y chromosomes into the 4361 laboratory stock background. *y* (*yellow*), *bw* (*brown*), *e* (*ebony*), *ci* (*cubitus interruptus*), and *ey* (*eyeless*) were used as recessive genetic markers in the isogenic background line.

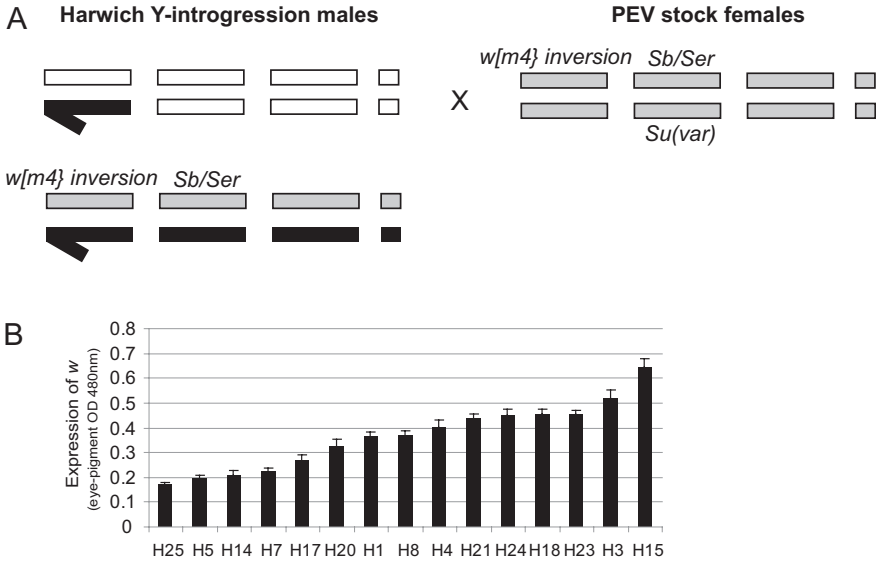

Fig. S5. (*A*) Cross-scheme for investigating the suppressive effects of 15 Harwich Y chromosomes on PEV phenotype. (*B*) Quantitative results of eye pigmentation measured by absorbance at 480 nm.

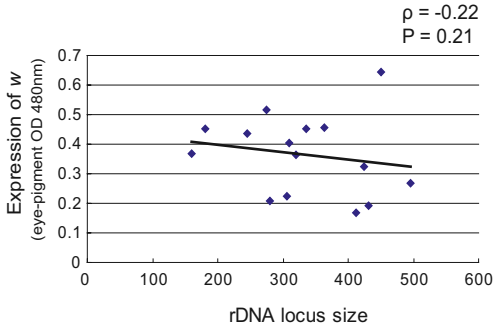

Fig. S6. Association between rDNA locus size and degree of suppression of the *w* gene expression level in 15 Harwich Y chromosome introgression lines.

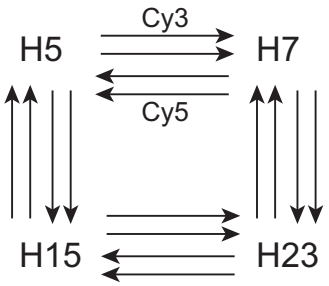

Fig. S7. Experimental design of microarrays for gene expression profiling. The lines with arrowheads represent hybridizations in which the Cy3 and Cy5 dyes were swapped and replicated.

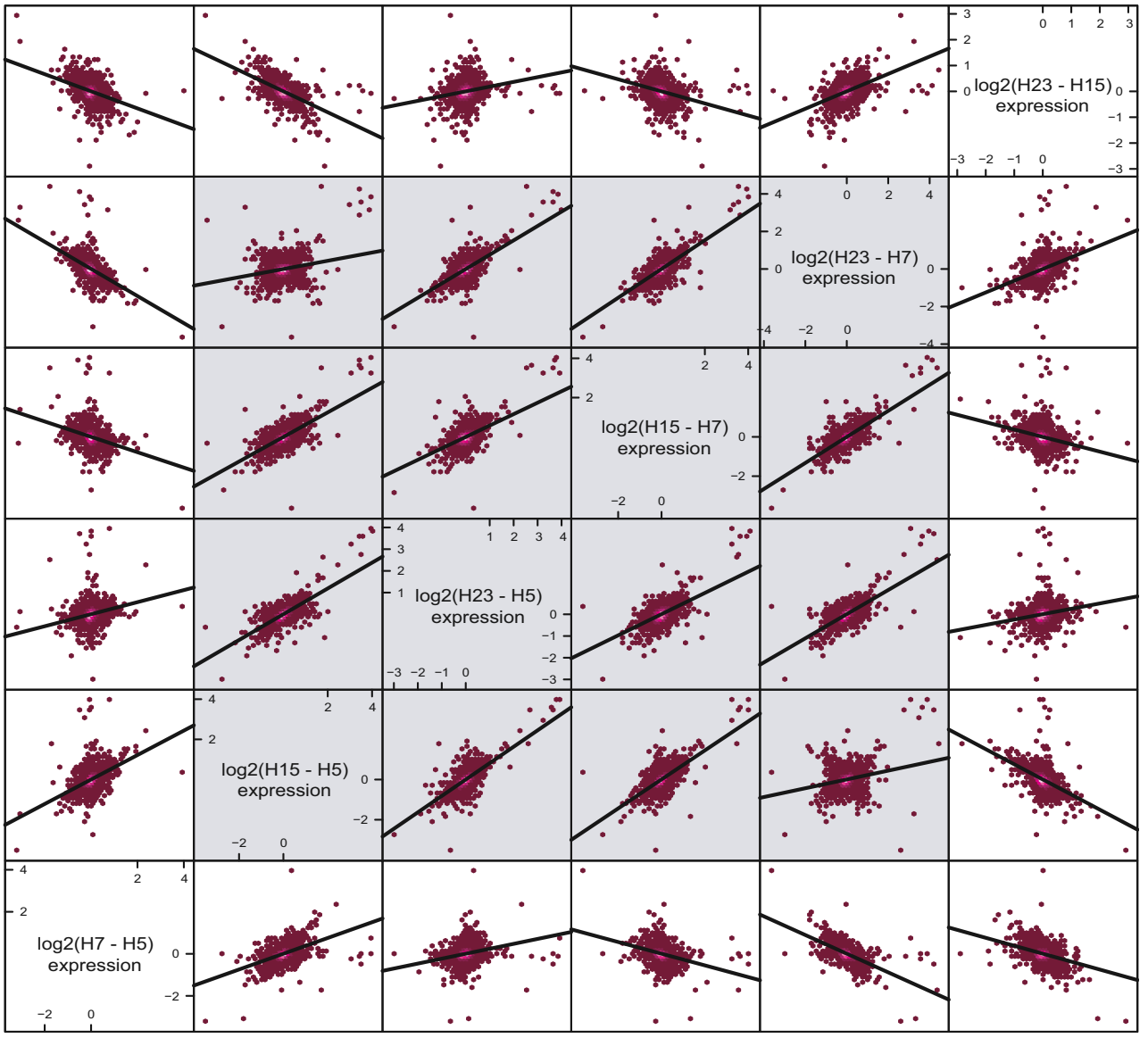

Fig. S8. Gene expression matrix plot for the four Harwich Y introgression lines. In addition to fitting a contrast to estimate expression differences between high-PEV and low-PEV lines, we fit six pairwise models comparing each individual Harwich line with all others: H15–H7, H15–H5, H23–H7, H23–H5, H7–H5, and H23–H15. We plotted the correlations among these pairwise comparisions using hexbin plots with the R function hexplom. Each cell of the plot shows the correlation between two pairwise comparisons specified by the row and column labels in the central diagonal. Correlations between the pairwise comparisons that represent comparisons between a high-PEV and a low-PEV line (H15–H7, H15–H5, H23–H7, and H23–H5; shaded gray) are stronger than correlations between pairwise comparisons that represent comparisons between either the two high-PEV lines or the two low-PEV lines (H7–H5 and H23–H15). That is, the genes that are differentially expressed between any high-PEV line and low-PEV line are similar, leading to positive correlations among the pairwise comparisons (shaded in gray).

Table S1. Gene Ontology enrichment in the comparison of low-PEV and high-PEV flies

Red vs. white	Category	Description	*n*	*P* value*
Up-regulated in red-eye (low-PEV) flies	BP			
	GO:0019236	Response to pheromone	6	8.5E-09
	GO:0007606	Sensory perception of chemical stimulus	5	5.2E-05
	GO:0042048	Olfactory behavior	4	5.6E-04
	GO:0006810	Transport	9	1.0E-03
	CC			
	GO:0005576	Extracellular region	11	7.6E-07
	MF			
	GO:0005549	Odorant binding	5	1.7E-05
	GO:0005550	Pheromone binding	3	2.3E-05
	GO:0004252	Serine-type endopeptidase activity	5	1.2E-02
Down-regulated in red-eye (low-PEV) flies	BP			
	GO:0006961	Antibacterial humoral response	4	2.7E-05
	GO:0050832	Defense response to fungus	3	3.3E-05
	CC			
	GO:0005575	Cellular component	5	8.6E-02
	MF			
	NA	NA	NA	NA

BP, biological processes; CC, cellular component; MF, molecular function; NA, not available (no enrichment identified).
**P* values were corrected for multiple hypothesis testing.