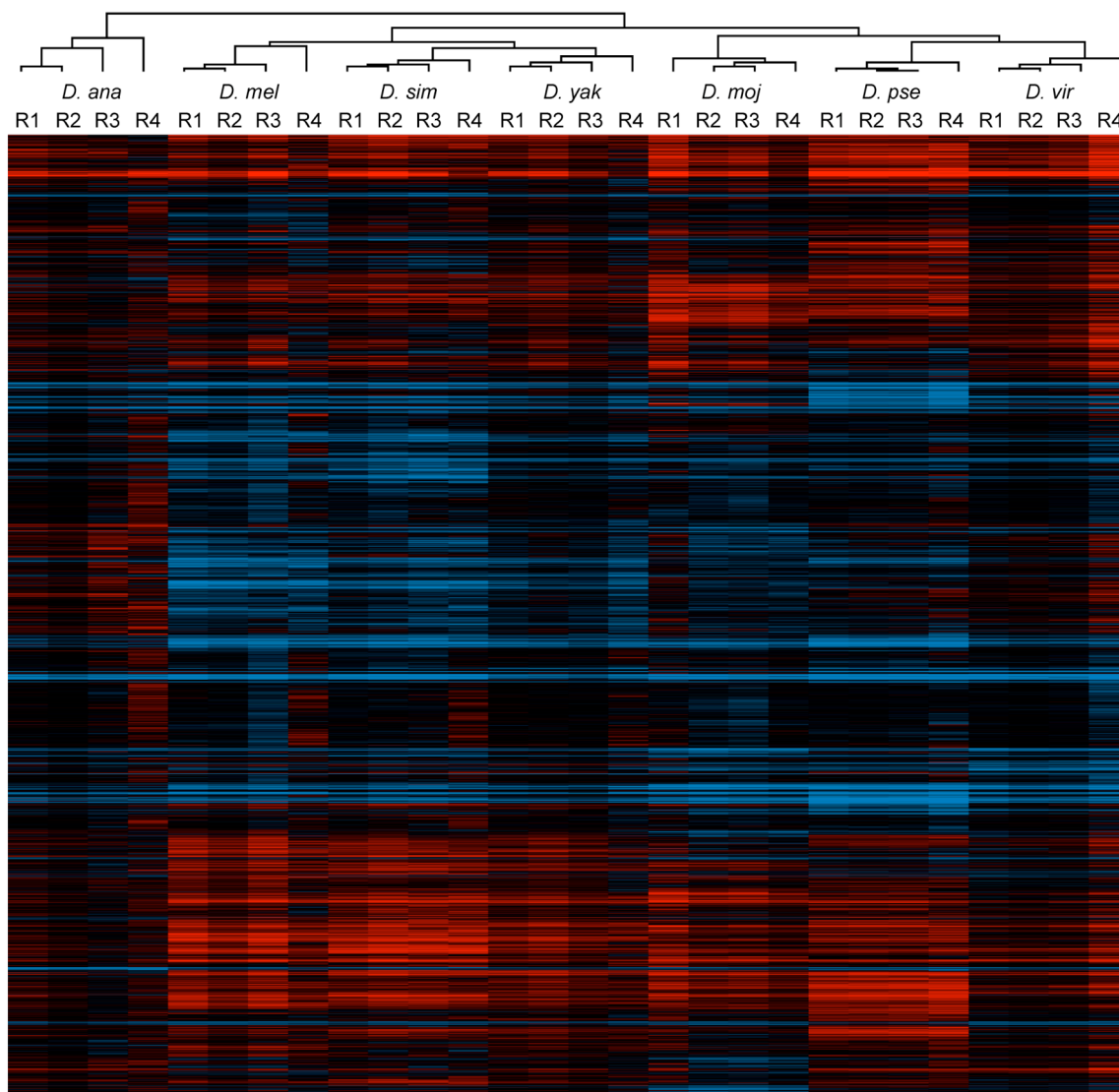
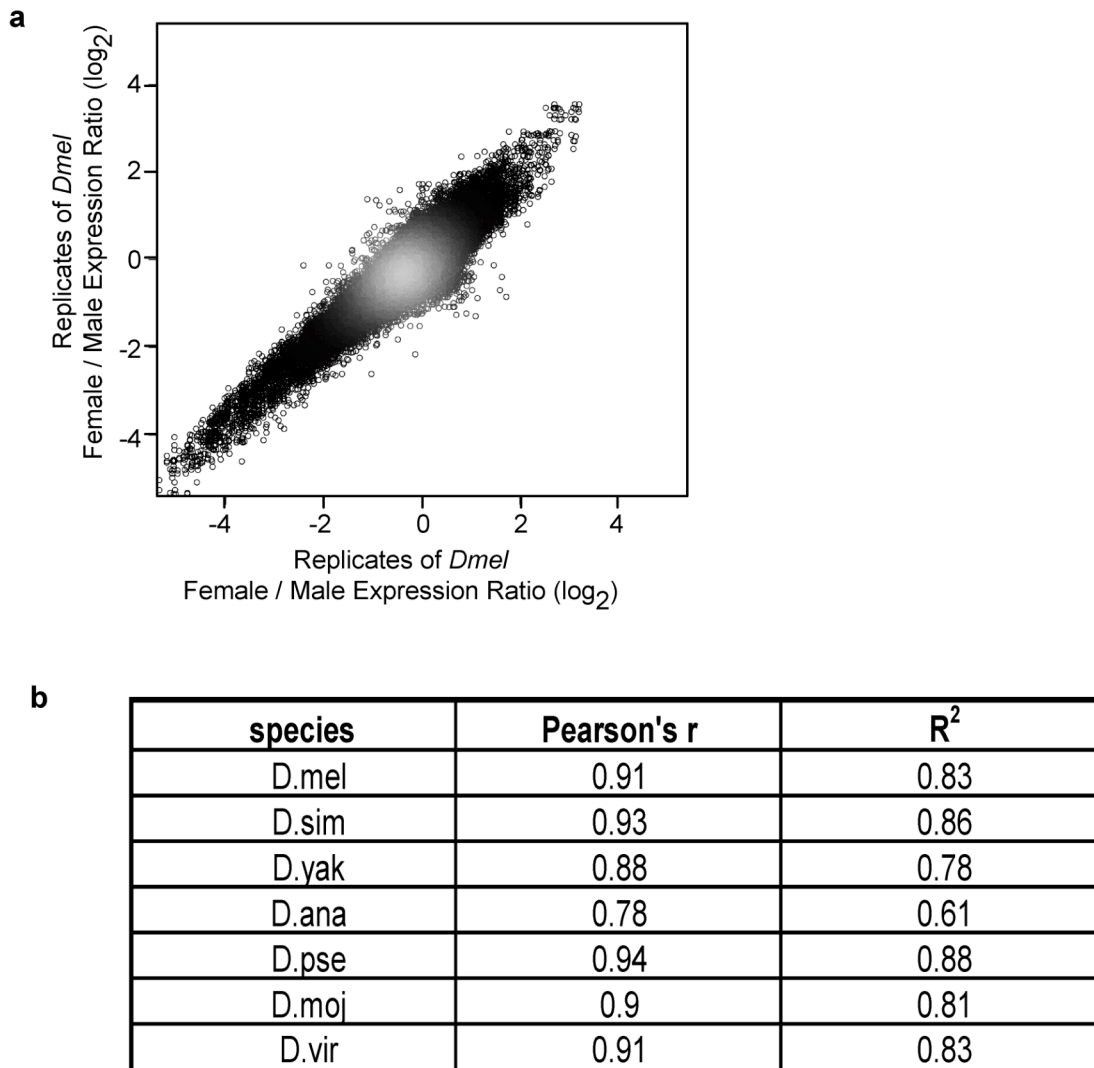


SUPPLEMENTARY INFORMATION

**Supplementary Figure 1.**

Hierarchical clustering of female/male expression ratios for all common orthologs of quadruplicate replicates with all 7 species, where columns indicate the replicates of each species and rows indicate genes. Clustering was carried out on both genes and species using a Pearson correlation metric and complete linkage. Female-biased (red) and male-biased (blue) expression are indicated. Data were clustered and visualized using Cluster3.0/Tree view.

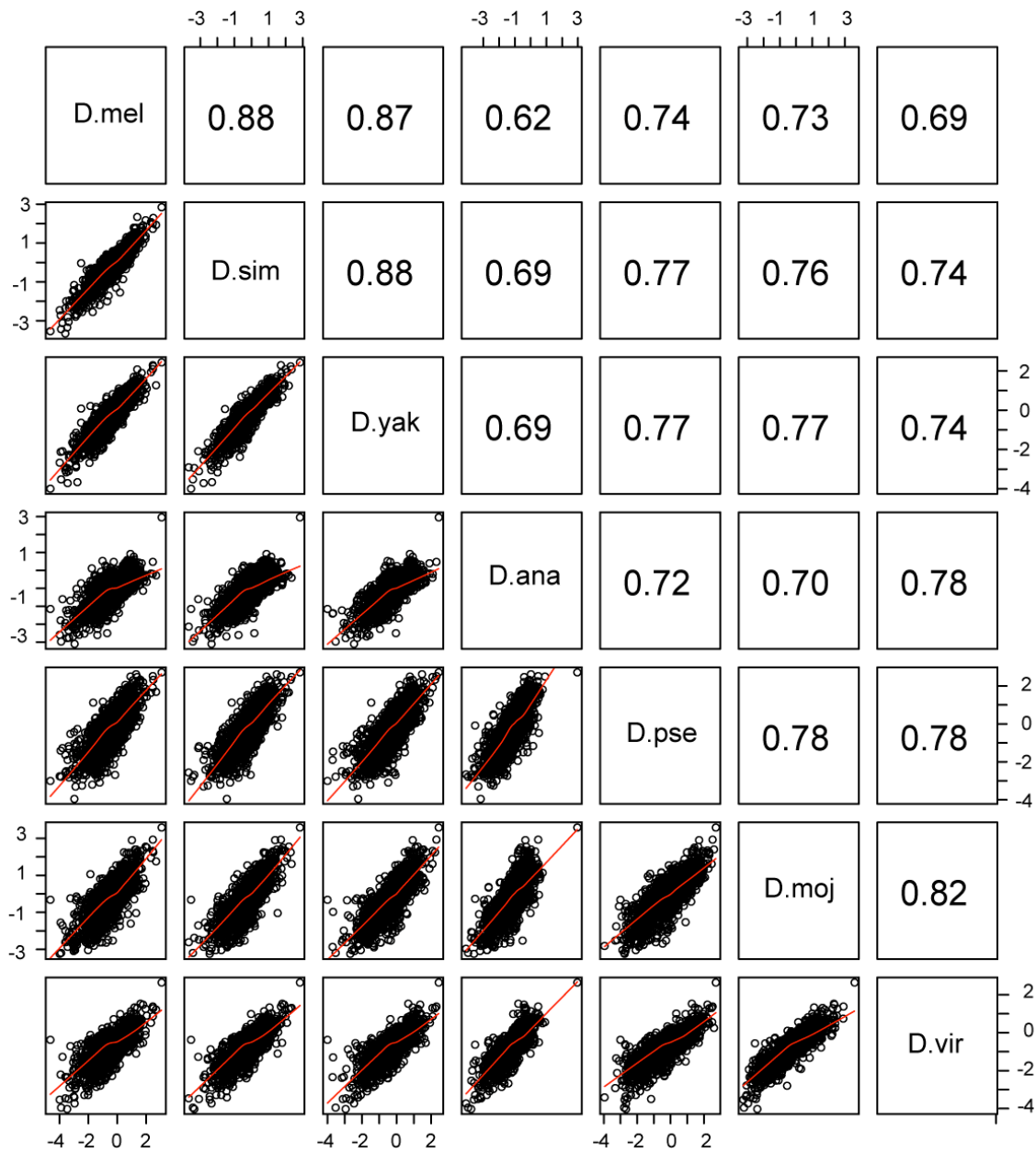


Supplementary Figure 2.

a, Female/male expression ratio (\log_2) for each possible pairs, plotted against each other, within the quadruplicate replicates of *D.melanogaster*. The colors of the spots represent the local densities at each point in the scatterplot. Grey shows the high local density and black shows low density. **b**, The Pearson's r value for the expression ratio of replicates in all 7 species. The *D. ananassae* outlier is omitted from Fig. 2d and Fig. 2e.

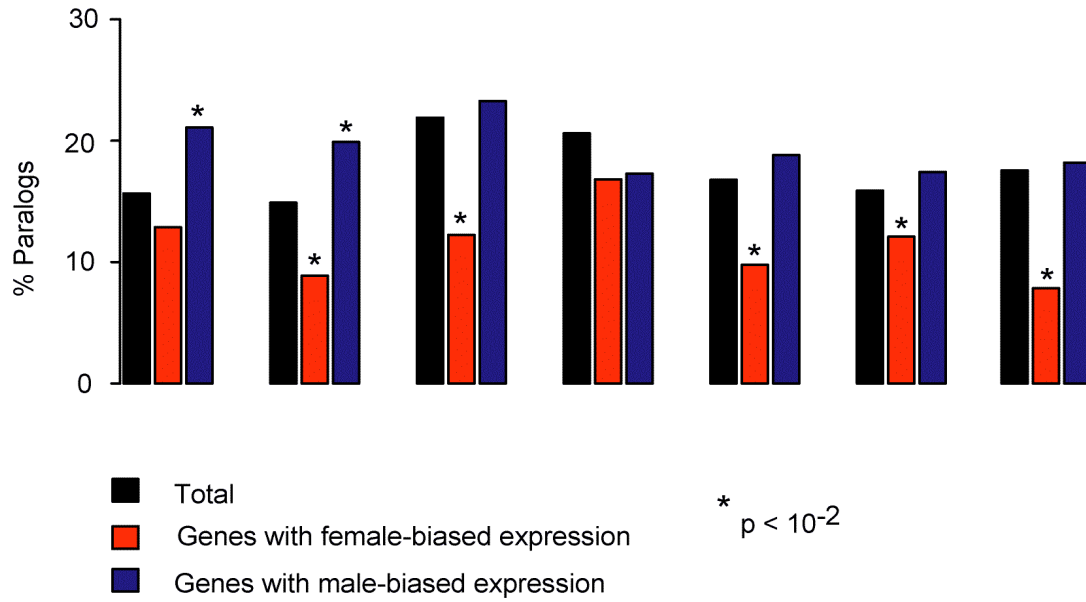
Developmental abnormalities, such as defective bilateral symmetry, are hallmarks of environmental stress and are pronounced in laboratory cultures of *D. ananassae*¹. Additionally, there is developmental plasticity in *D. ananassae*². Factors such as these could account for the comparatively greater expression divergence in this species.

1. Vishalakshi, C. & Singh, B.N. Fluctuating asymmetry in certain morphological traits in laboratory populations of *Drosophila ananassae*. *Genome* **49**, 777-85 (2006).
2. Singh, S.R. & Singh, B.N. Behavioral genetics of *Drosophila ananassae*. *Genet Mol Res.* **2**, 394-409 (2003).



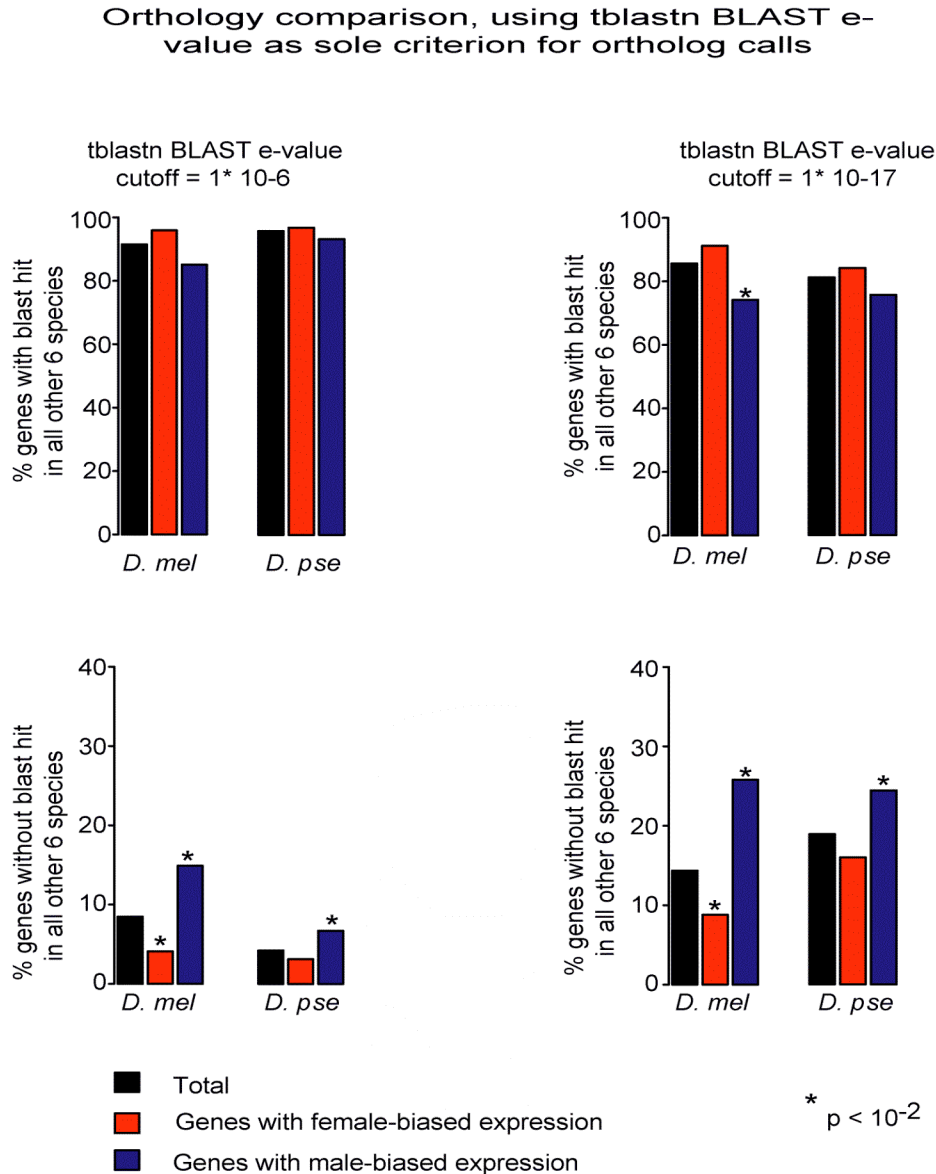
Supplementary Figure 3.

Expression divergence of common orthologs between all pairs of species female/male expression ratio (\log_2) for each ortholog pair between any possible pair of species, plotted against each other. The corresponding Pearson's r values are shown in the opposite block.



Supplementary Figure 4.

Gene content and expression of paralogs in each species. The percentage of genes in the genome (black) and with female- (red) or male-biased (blue) expression that are paralogs are shown. Significant differences ($p < 10^{-2}$, Chi-square test) between sex-biased classes and total genes are indicated (asterisks).



Supplementary Figure 5.

Gene content of *D. melanogaster* and *D. pseudoobscura* manually curated genes in other 6 species separately identified by TBLASTN and their sex-biased expression pattern. Determining if a gene is absent or evolves at a sufficiently high rate to obscure orthology is difficult. TBLASTN searches were done using the *D. melanogaster* and *D. pseudoobscura* manually curated annotations as query sequences against the other six assembled genome sequence to avoid any bias introduced by annotation models. The percentage of genes in the genome (black) and with female- (red) or male-biased (blue) expression that can be identified in all other 6 species and can be just identified in one or several species are shown. Significant differences ($p < 10^{-2}$, Chi-square test) between sex-biased classes and total genes are indicated (asterisks). Two different TBLASTN e-value cutoff have similar patterns. Genes with male-biased expression were less often identified at an e-value cut-off of $< 10^{-6}$ (which finds a homolog for 90% of genes between *D. melanogaster* and *D. pseudoobscura*) and cut-off of $< 10^{-17}$.

Supplemental Table 1: Summary of array platform probes after annotation re-mapping

species	Total Probes	Unique Probes after remapping	Unique Genes after remapping	Median Unique Probes / Gene	Putative unannotated genes*
mel	148763	125558	13449	9	na
sim	186701	99992	12937	7	72
yak	190097	111822	14611	8	87
ana	173102	102027	14690	6	222
pse	190109	143832	12703	11	26
vir	187292	134007	12661	10	178
moj	190904	131982	12477	10	101

* Estimated as the number of original OLIV gene predictions that have probes that do not map to GLEAN, with a probeset that does not overlap with GLEAN predictions and a count > 1 probe.

Supplementary Table 2: Summary of alternative ab initio annotation for each species

(the same annotation for original array design)

Species	<i>D. sim</i>	<i>D. yak</i>	<i>D. ana</i>	<i>D. pse</i>	<i>D. vir</i>	<i>D. moj</i>
EIS model based on <i>D.mel</i> ortholog	13,561	12,754	12,377	12,395	11,316	9,818
GenelD prediction	1,919	5,977	9,263	2,550	5,593	7,546
Genbank mRNA	843	1,273	4	150	92	0
Flybase gene	0	0	0	651	0	0
Total OLIV annotation	16,323	20,004	21,644	15,746	17,001	17,364
Total probes	186,701	190,097	173,102	190,109	187,292	190,904

* For *D.mel*, the annotation is based on based Flybase annotation V4.2.