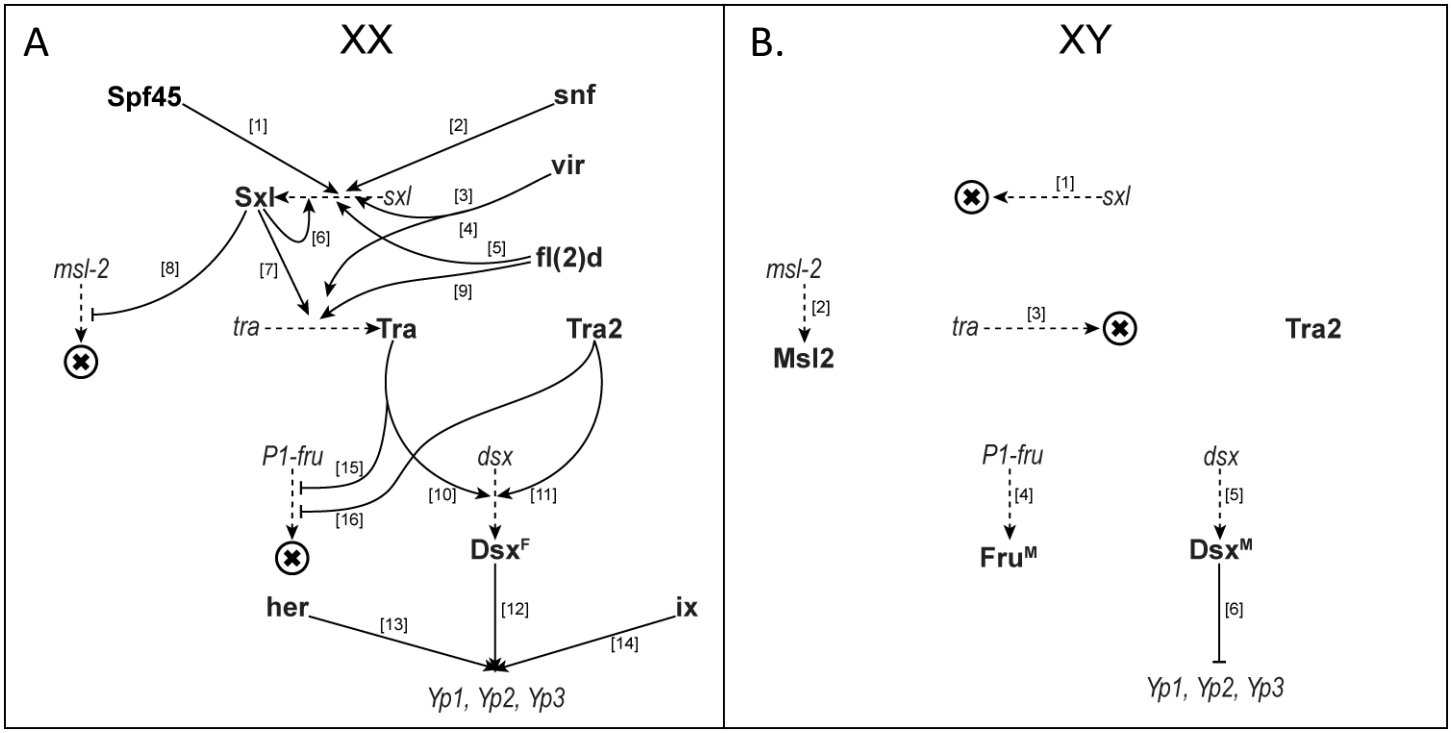
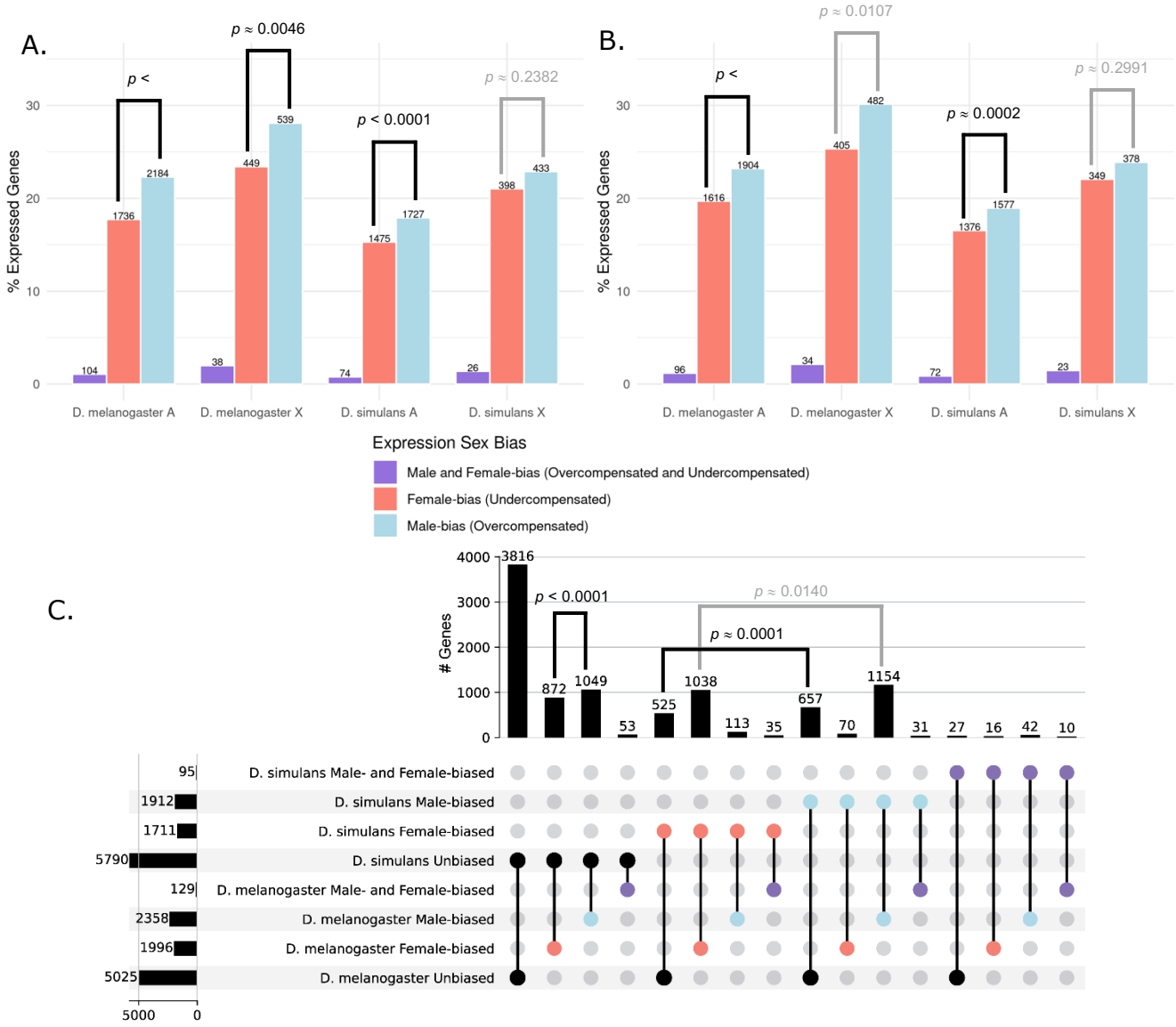
Supplementary Figures: pages 1-10

Supplementary Tables: pages 11-16

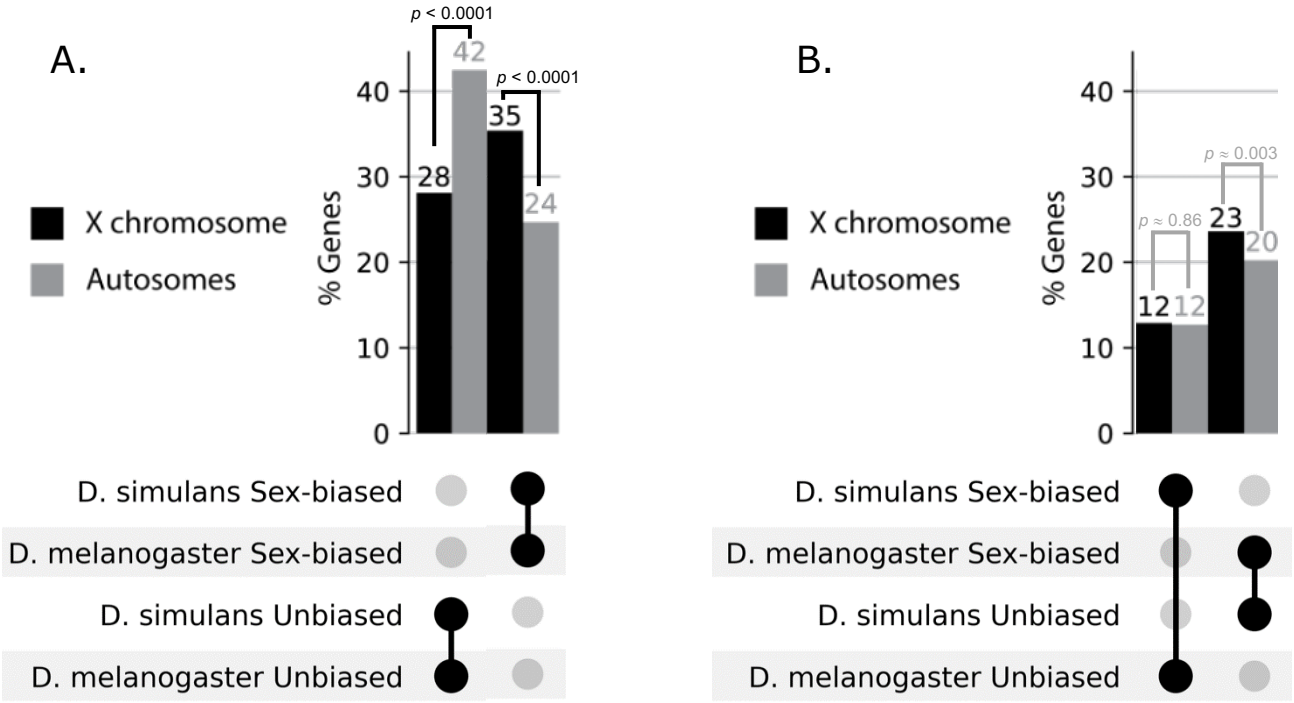
Supplementary Files: page 17



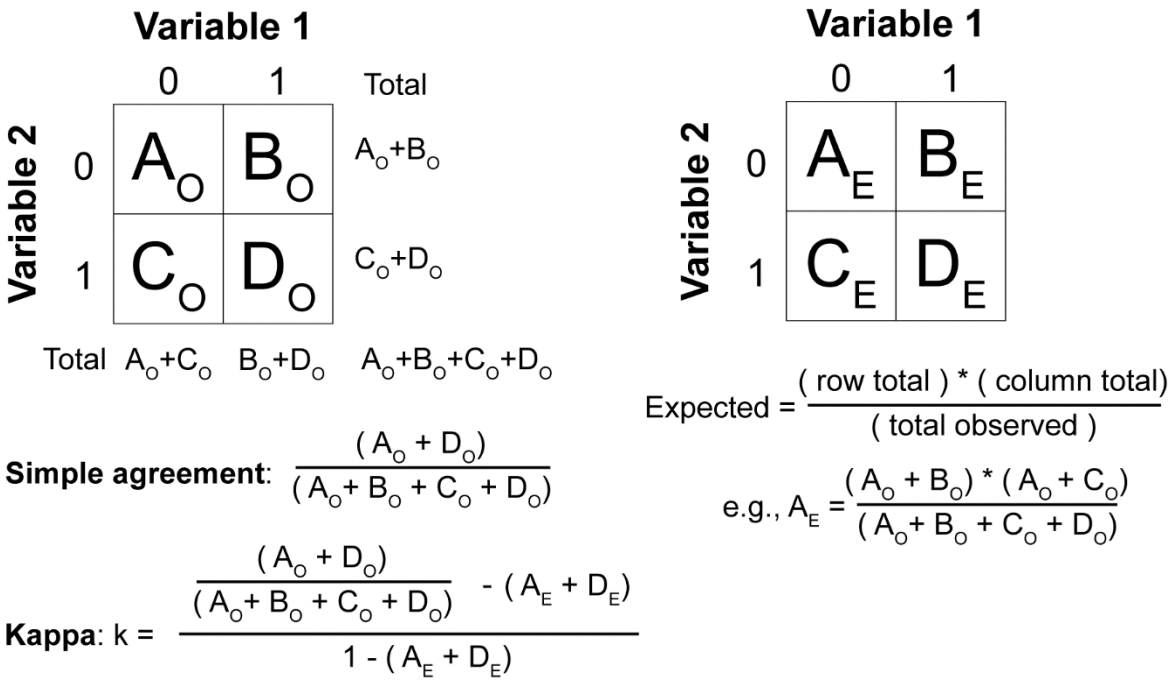
Supplementary Figure 1 – Drosophila sex determination hierarchy, XX females (A) and XY males (B) adapted from Figure 1 in Fear, et al. 2015. Transcripts are italicized and proteins are bold. Solid arrows are genetic interactions (e.g., splicing, transcription) and dashed arrows are protein translation. The X within a circle represents no productive protein product. (A1) Spf45 → Sxl (Lallena, et al. 2002), (A2) Snf → Sxl (Flickinger and Salz 1994), (A3) vir → Sxl (Hilfiker, et al. 1995), (A4) vir → tra (Hilfiker, et al. 1995), (A5) fl(2)d → Sxl (Granadino, et al. 1990), (A6) Sxl → Sxl (Cline 1978; Bell, et al. 1988; Lallena, et al. 2002), (A7) Sxl → Tra (Sosnowski, et al. 1989; Inoue, et al. 1990), (A8) Sxl ⊣ Msl-2 (Bashaw and Baker 1997; Kelley, et al. 1997; Gebauer, et al. 1998), (9A) fl(2)d → Tra (Granadino, et al. 1996), (A10) Tra → Dsx^F (Inoue, et al. 1992), (A11) Tra2 → Dsx^F (Inoue, et al. 1992), (A12) Dsx^F → Yps (Burtis, et al. 1991; Coschigano and Wensink 1993; An and Wensink 1995; Erdman, et al. 1996), (A13) Her → Yps (Li and Baker 1998), (A14) ix → Yps (Garrett-Engele, et al. 2002), (A15) Tra ⊣ Fru^M (Ryner, et al. 1996; Heinrichs, et al. 1998), (A16) Tra2 ⊣ FruM (Ryner, et al. 1996; Heinrichs, et al. 1998), (B1) default splicing of *sxl* transcripts results in no functional protein (Bell, et al. 1988), (B2) Msl-2 protein produced (Bashaw and Baker 1995; Kelley, et al. 1995; Zhou, et al. 1995), (B3) default splicing of *tra* transcripts results in no functional protein (Boggs, et al. 1987), (B4) Fru^M protein produced (Ryner, et al. 1996; Heinrichs, et al. 1998), (B5) default splicing of *dsx* transcripts in XY individuals results in Dsx^M protein (Burtis and Baker 1989), (B5) Dsx^M represses expression of Yps (Coschigano and Wensink 1993).



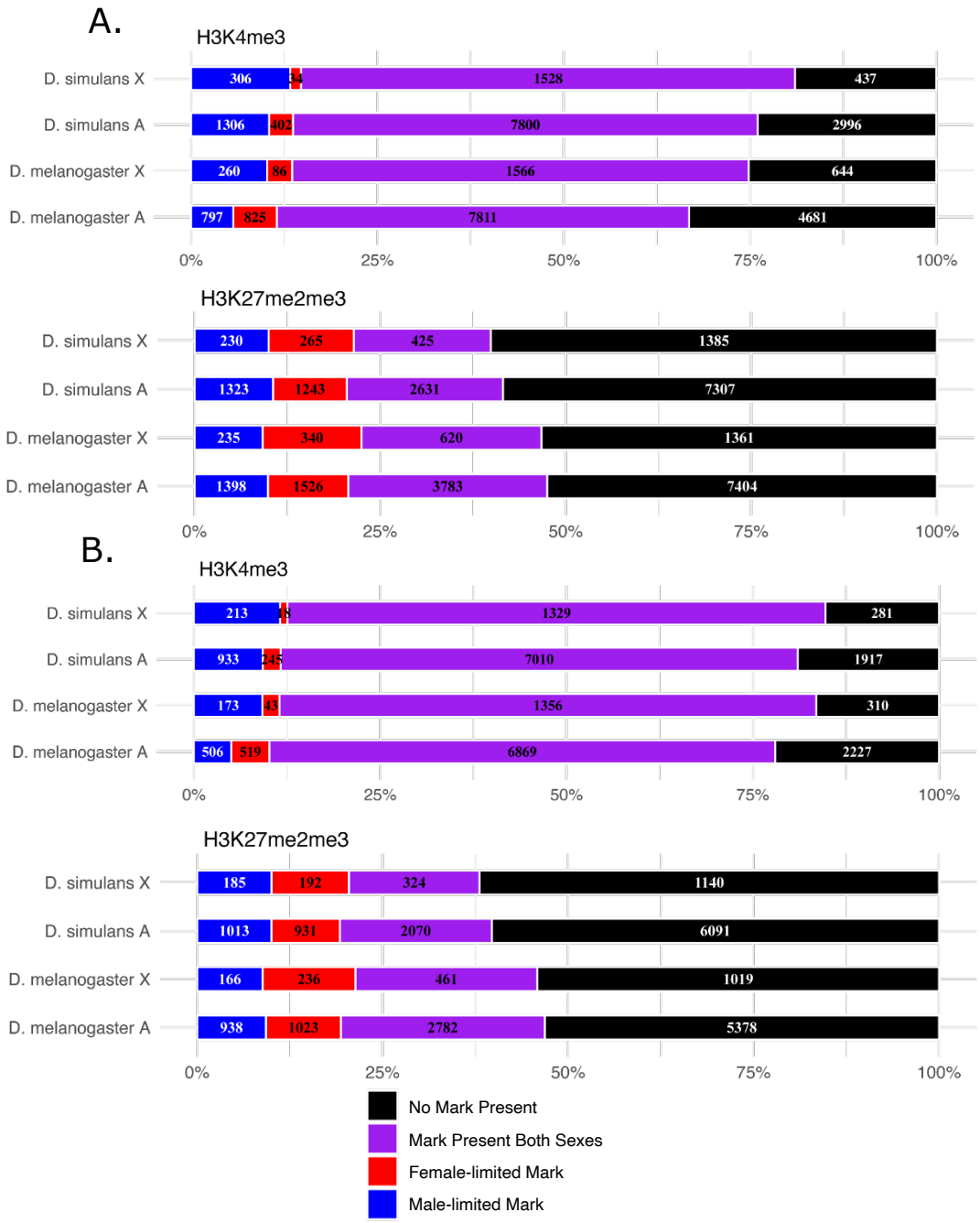
Supplementary Figure 2 – Excess of male-bias in *D. melanogaster* and *D. simulans* is due to divergent male-biased expression. (Panel A) Relative percent of expressed genes for *D. melanogaster* on the X (X, 1,919) and autosomes (A, 9,797) and for *D. simulans* on the X (X, 1,893) and autosomes (A, 9,650). (Panel B) Relative percent of orthologous genes expressed in *D. melanogaster* X (1,599) and autosomes (8,206) and *D. simulans* X (1,583) and autosomes (8,327). Chromosome 4 is excluded from the autosomes. Note that total numbers of orthologous genes differ between the species due to differences in chromosomal assignments. The number of genes in each category is printed over the box with male-bias (blue), female-bias (red), and both male- and female-biased (purple). Sex-limited genes (expressed in only one sex) are excluded. P-values for differences in male-biased and female-biased expression are reported. (Panel C) Conservation and divergence of sex-biased expression for expressed orthologous genes (n=9,508, with a consistent X/autosome chromosomal assignment between the species). Dots below the histogram are solid for the combination of factors reported as the number of genes in the bar plot.



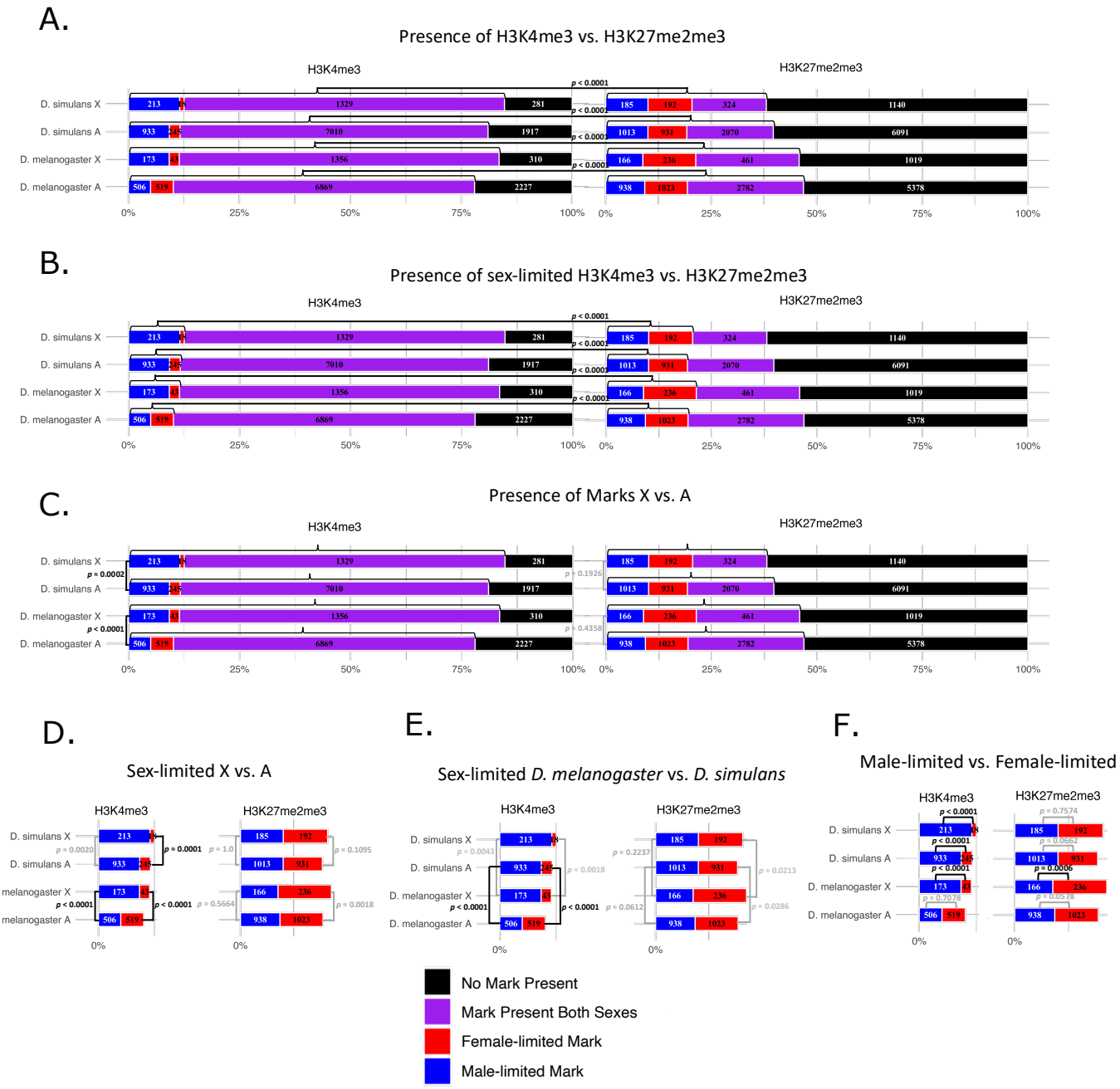
Supplementary Figure 3 – X vs. autosomes of orthologs with conserved and divergent sex-biased expression. Expression of orthologous genes in the head for both sexes and both species on the X (n_x=1,529 genes) and autosomes (n_a= 7,979). (Panel A) Genes that are conserved in their sex bias (n_x=541, n_a= 1,968) are more likely to be on the X (35% on X vs. 24% on autosomes; χ^2: $p < 0.0001$), while those that are unbiased are more likely to be on the autosomes (28% on X vs. 42% on autosomes; χ^2: $p < 0.0001$). (Panel B) Genes divergent in sex bias (n_x=558, n_a= 2,625) have no significant chromosomal bias for either *D. simulans*-specific sex-biased genes (12% on X vs. 12% on autosomes; χ^2: $p = 0.86$) or *D. melanogaster*-specific sex-biased genes (23% on X vs. 20% on autosomes; χ^2: $p = 0.003$). Connected black dots indicate the category plotted in the two bars above. The Y-axis is of the percentage of the total number of genes on the X or autosomes in each of the four categories. Chromosome 4 is excluded from the autosomes. X vs. autosome tests were performed using Pearson's Chi-square (χ^2) test (Pearson 1900) with a significance threshold of $p < 0.001$.



Supplementary Figure 4 – Measurements of Agreement. Given the table of observed values A_O, B_O, C_O, and D_O, the expected values indicated on the right can be calculated (A_E, B_E, C_E, and D_E). The formulas for calculating simple agreement and Cohen's Kappa agreement (Fleiss 1981) are also presented. Cohen's Kappa values correct for marginal frequencies, for when there is an imbalance between the variables tested.

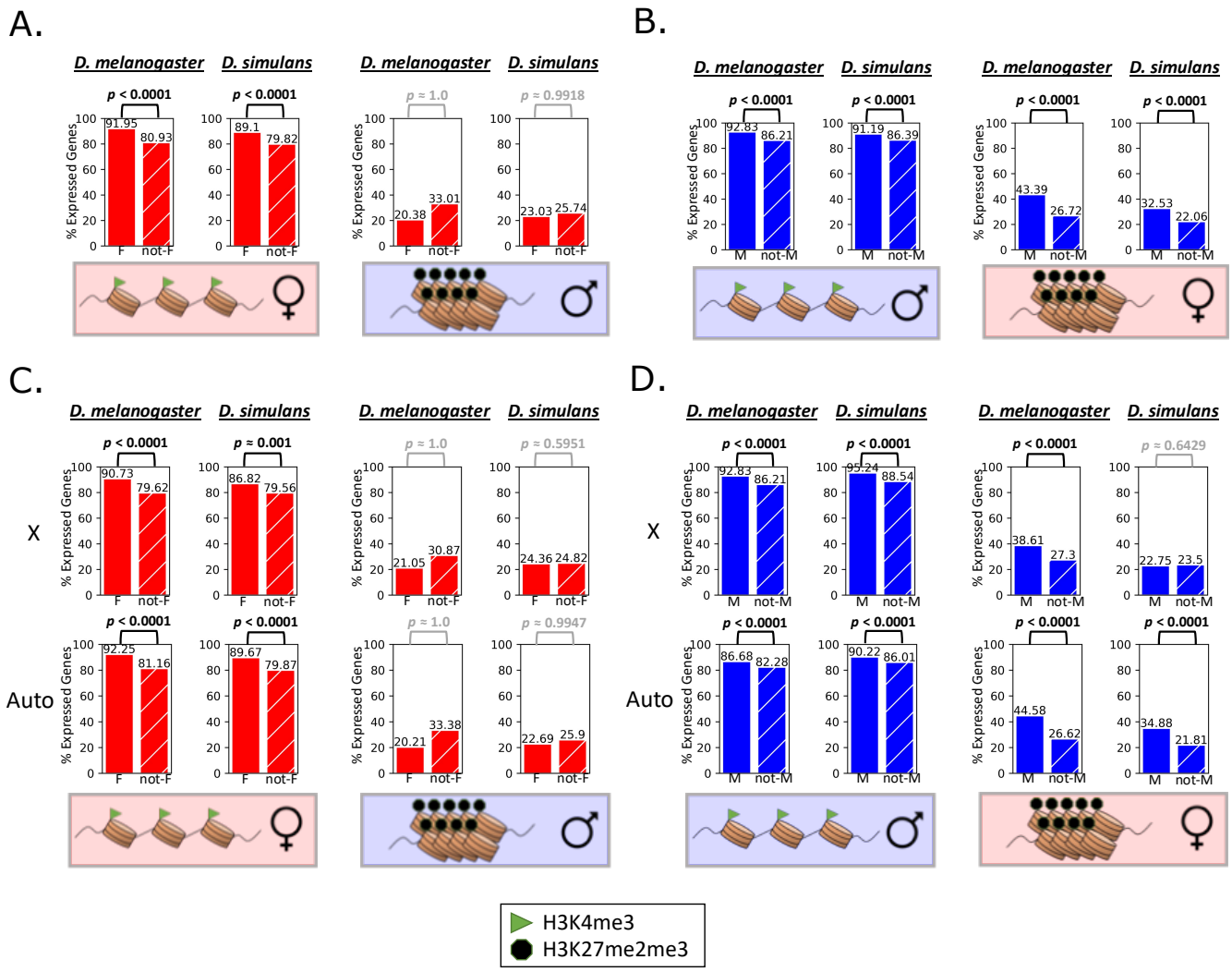


Supplementary Figure 5 – Chromatin marks in males and females. (Panel A) Genes on the X or autosomes (denoted as A) of *D. melanogaster* FlyBase reference r6.17 (n_X=2,556; n_A=14,114; $n_X + n_A$ =16,670) and *D. simulans* FlyBase reference r2.02 (n_X=2,305; n_A=12,504; $n_X + n_A$ =14,809) with the number of genes with H3K4me3 (top) or H3K27me2me3 (bottom) male-limited, female-limited, or detected in both sexes indicated in blue, red, and purple respectively. Note that chromosome 4 is not included in the autosomes. (Panel B) Similar to Panel A, but with selecting for the one-to-one orthologs between *D. melanogaster* and *D. simulans* (n=12,083), excluding genes on chromosome 4 or unmapped scaffolds from further analysis (80 genes in *D. melanogaster* and 137 genes in *D. simulans*), resulting in 1,882 and 10,121 genes are on the *D. melanogaster* X and autosomes respectively, and 1,841 and 10,105 for *D. simulans* X and autosomes.



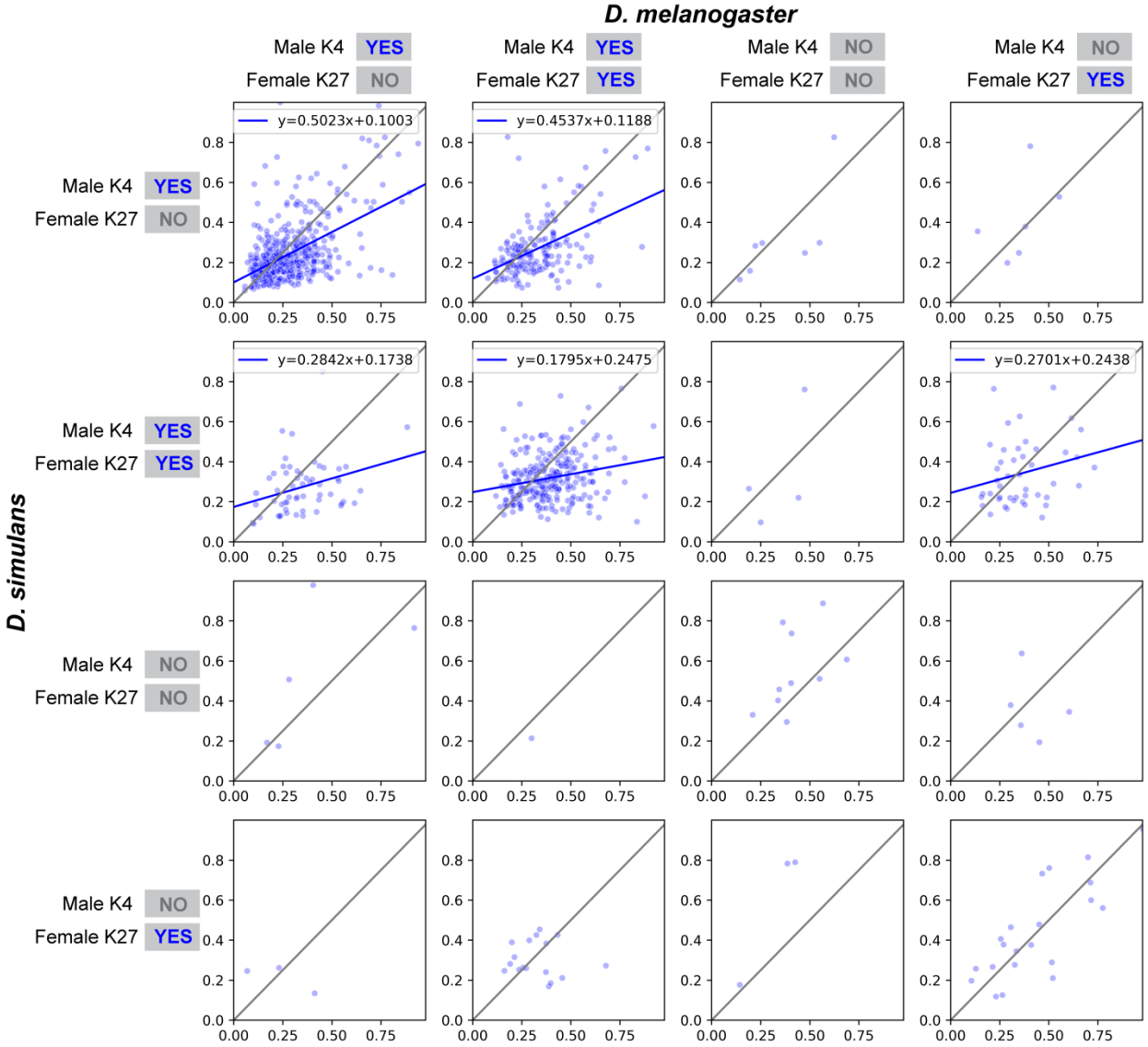
Supplementary Figure 6 –Tests for differential chromatin. H3K4me3 and H3K27me2me3 marks not present (black), present only in females (female-limited, red), present only in males (male-limited, blue), or present in both males and females (purple) on the X chromosome (X) and autosomes (A) for one-to-one orthologous genes of *D. melanogaster* and *D. simulans*. Chromosome 4 is excluded from the autosomes. The number of genes for each group is indicated. The total genes evaluated for are 1,882 and 10,121 for *D. melanogaster* X and autosomes respectively, and 1,841 and 10,105 for *D. simulans* X and autosomes. Tests are performed as follows. Panel A compares the presence of H3K4me3 vs. H3K27me2me3 marks in males/females within each species and chromosomal location. Panel B compares the presence of sex-limited H3K4me3 vs. H3K27me2me3 marks within each species and chromosomal location. Panels C-F compare within H3K4me3 or H3K27me2me3 marks separately. Panel C compares the presence of chromatin marks in either sex on the X vs. the autosomes within each species.

Panel D compares the presence of male-limited or female-limited marks on the X vs. the autosomes within each species. Panel E compares the proportion of male-limited or female-limited marks between D. melanogaster and D. simulans within each chromosomal location. Panel F compares male-limited vs. female-limited within each species and chromosomal location. All tests of X vs. autosomes are evaluated using Pearson's Chi-square (χ^2) test (Pearson 1900). Differences between species, sexes, and H3K4me3 vs. H3K27me2me3 are evaluated using McNemar's test of homogeneity (McNemar 1947).



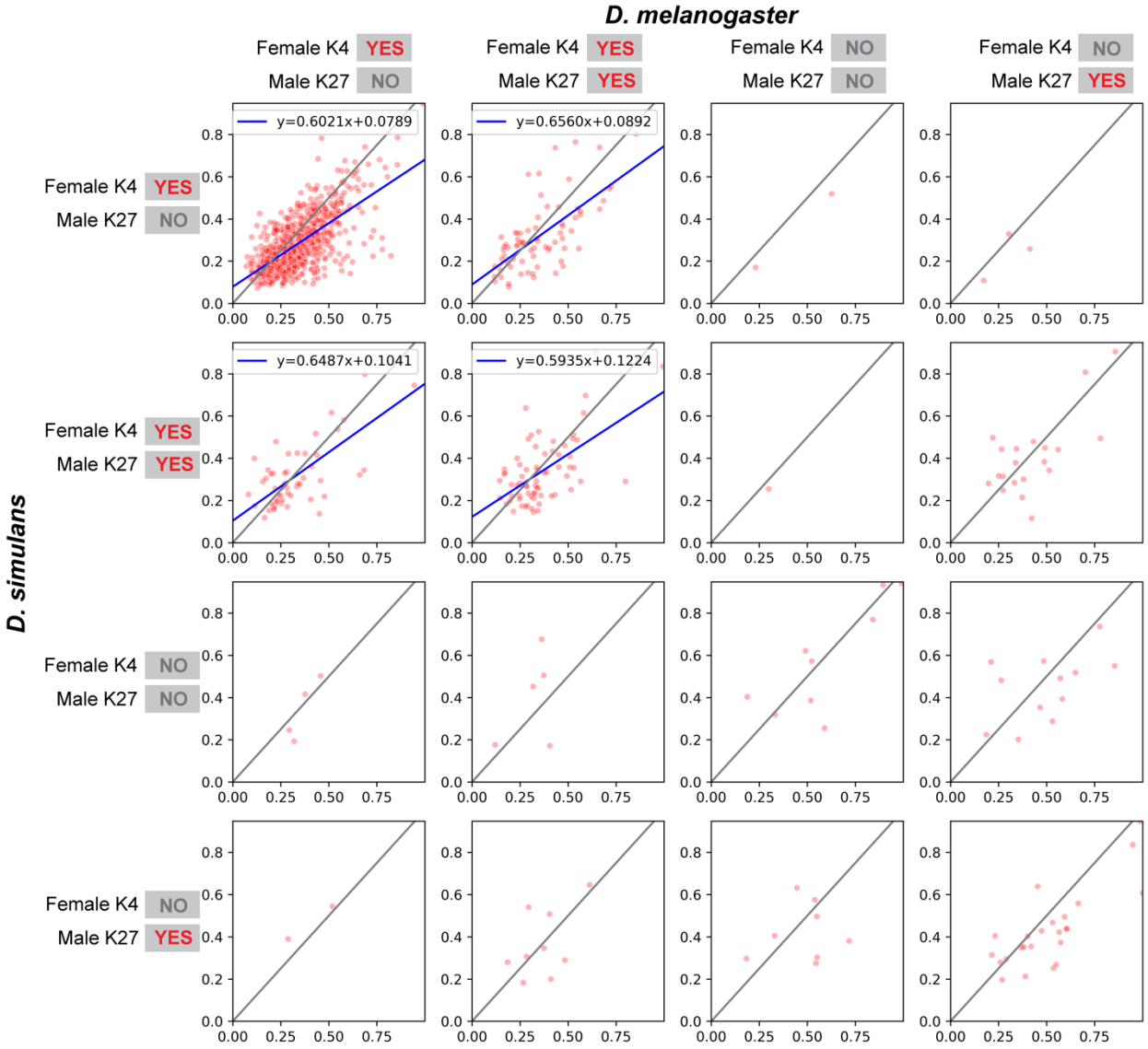
Supplementary Figure 7 – Sex-biased expression is associated with chromatin marks in subset of orthologs. The Y-axis of each graph represents the percent of expressed female-biased (solid red), non-female-biased (hatched red), male-biased (solid blue), or non-male-biased (hatched blue) genes with a one-to-one ortholog within each species with the indicated chromatin (cartoon representations below each set of bars). Consistent with the model presented in Figure 4, (Panel A) Female-biased genes (solid red) are enriched for H3K4me3 (open) chromatin when compared to non-female-biased genes (hatched red) in both species. (Panel B) Male-biased genes (solid blue) are enriched for male open chromatin and female H3K27me2me3 (closed) chromatin when compared to non-male-biased genes (hatched blue) in both species. The model in Figure 4 was also evaluated for X and autosomes separately. (Panel C) Female-biased genes (solid red) are enriched for open chromatin when compared to non-female-biased genes (hatched red) on both the X and autosomes of both species. (Panel D) Male-biased genes (solid blue) are enriched for male open chromatin and female closed chromatin when compared to non-male-biased genes

(hatched blue) on both the X and autosomes of *D melanogaster*. *D simulans* shows the same pattern on the autosomes. On the X chromosome, male-bias genes are enriched for open chromatin in males but not for closed chromatin in females, showing a divergence in the regulatory pattern between the two species. There were 11,937 orthologous genes evaluated, 9,747 (n_X=1,562, n_A=8,182) genes expressed in *D. melanogaster* and Y genes expressed in *D. simulans* (n_X=1,582, n_A=8,320). Each set of female-biased (male-biased) and non-female-biased (non-male-biased) genes were tested for enrichment of the indicated chromatin mark using Fisher exact test (Fisher 1934) with the alternative expectation that the indicated chromatin marks would be more likely in genes with female-biased (male-biased) expression. Significant p-values ($p < 0.001$) are black and p-values above the significance threshold are gray.



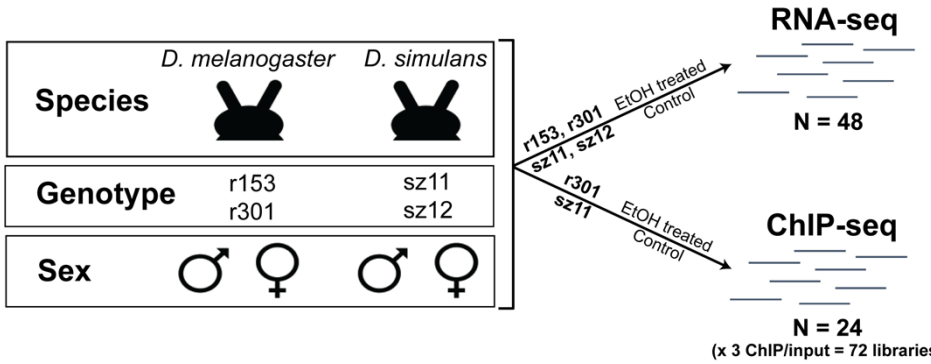
Supplementary Figure 8. Plotted on the interval of (0,1) is the value $(1 - \frac{\hat{f}}{\hat{m}})$ for male-biased orthologs (blue dots), where $\hat{f}$ is average UQ normalized expression across female samples and $\hat{m}$ is average UQ normalized expression across male samples. *D. melanogaster* is on the X-axis and *D. simulans* on the Y axis. Each male-biased ortholog is plotted based on the sex bias ratio observed in each species, and placed in the box corresponding to the chromatin observed in *D. melanogaster* and *D. simulans*. Chromatin of *D.*

melanogaster is indicated at the top of each column of plots and chromatin of *D. simulans* is indicated at the left of each row of plots. Plots along the diagonal from the top left to the bottom right are genes where the observed chromatin is the same between the species. For each row (*D. simulans*) and column (*D. melanogaster*) the presence of H3K4me3 in males is indicated by a blue "YES" next to "Male K4" or a gray "NO" if it is not present. Similarly for the presence of H3K27me2me3 in females indicated by a blue "YES" next to "Female K27" if present, or a gray "NO" otherwise. Linear regression estimates are calculated for plots with at least 25 genes and plotted as a blue line.

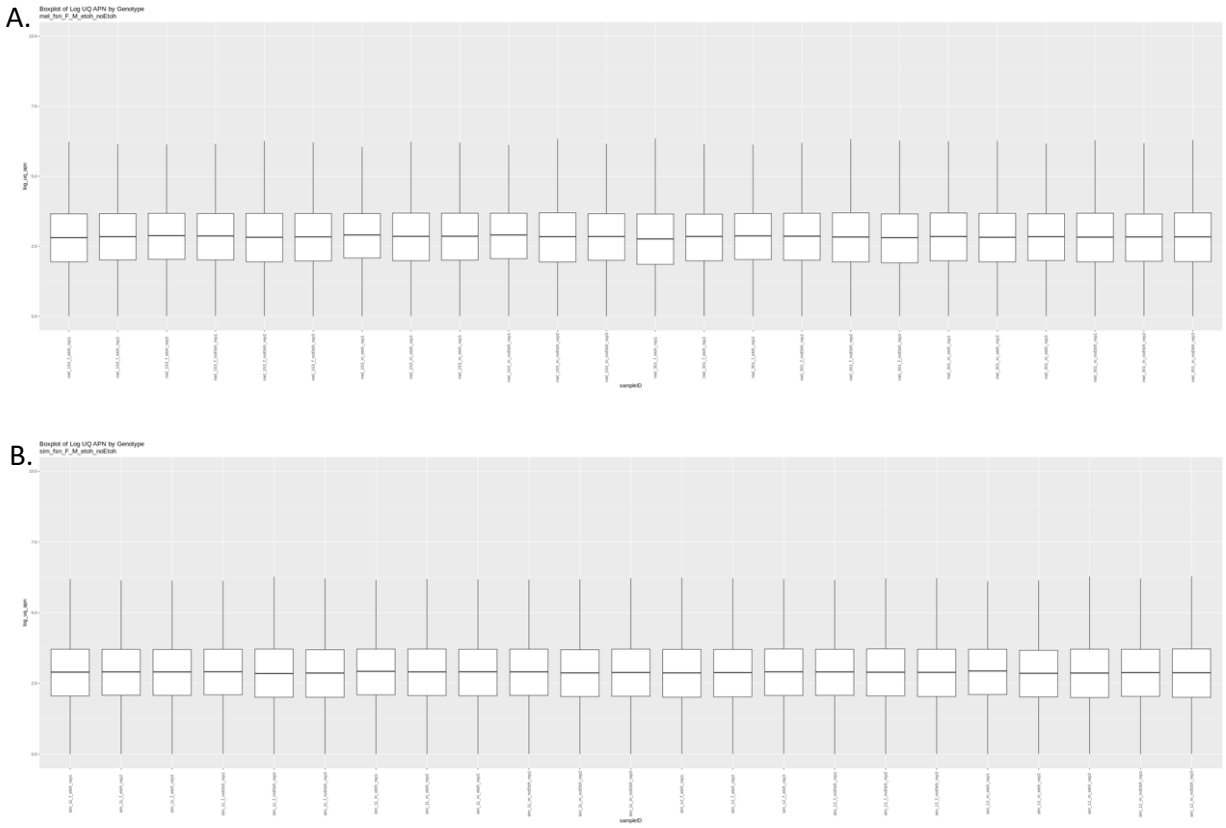


Supplementary Figure 9. Plotted on the interval of (0,1) is the value $(1 - \frac{\hat{m}}{\hat{f}})$ for female-biased orthologs (red dots), where $\hat{f}$ is average UQ normalized expression across female samples and $\hat{m}$ is average UQ normalized expression across male samples. *D. melanogaster* is on the X-axis and *D. simulans* on the Y axis. Each female-biased ortholog is plotted based on the sex bias ratio observed in each species, and placed in the box corresponding to the chromatin observed in *D. melanogaster* and *D. simulans*. Chromatin of *D. melanogaster* is indicated at the top of each column of plots and chromatin of *D. simulans* is indicated at the left of each row of plots. Plots along the diagonal from the top left to the bottom right are genes where the observed chromatin is the same between the species. For each row (*D. simulans*) and column (*D.*

melanogaster) the presence of H3K4me3 in females is indicated by a red "YES" next to "Female K4" or a gray "NO" if it is not present. Similarly for the presence of H3K27me2me3 in males indicated by a red "YES" next to "Male K27" if present, or a gray "NO" otherwise. Linear regression estimates are calculated for plots with at least 25 genes and plotted as a blue line.



Supplementary Figure 10 – Experimental Design. For RNA-seq there were a total of 48 samples (2 species x 2 genotypes x 2 sexes x 6 replicates). For ChIP-seq there were a total of 24 samples (2 species x 1 genotype x 2 sexes x 6 replicates) used for assaying chromatin (3 antibody/inputs per sample). Note that half of the replicates were exposed to ethanol (EtOH) and are included as additional data.



Supplementary Figure 11 – Distributions of RNA-seq expression values after UQ normalization. Upper quartile (UQ) normalization distributions per sample for (Panel A) *D. melanogaster* and (Panel B) *D. simulans* samples excluding the *D. simulans* sz12 male replicate that was removed due to a low median UQ relative to the rest of the samples.

Supplementary Tables

		D. melanogaster (X, A)	*D. simulans* (X, A)	Orthologs (X, A)	
1	Male-biased	2723 (539, 2184)	2160 (433, 1727)	1154 (235, 919)	$p = 0.014$
2	Female-biased	2185 (449, 1736)	1873 (398, 1475)	1038 (215, 823)	
3	Male- and Female-biased	142 (38, 104)	100 (26, 74)	10 (2, 8)	
4	Sex-biased	5050 (1026, 4024)	4133 (857, 3276)	**2202 (452, 1750)**	
5	Unbiased	6666 (893, 5773)	7410 (1036, 6374)	**3816 (430, 3386)**	
6	Switch			**3490 (647, 2843)**	
7	Reversal	Male	Female	113 (37, 76)	
8	Reversal	Female	Male	70 (17, 53)	
9	Gain/Loss	Male	Male and Female	42 (12, 30)	
10	Gain/Loss	Female	Male and Female	16 (4, 12)	
11	Gain/Loss	Male and Female	Male	31 (11, 20)	
12	Gain/Loss	Male and Female	Female	35 (8, 27)	
13	Gain/Loss	Male	Unbiased	1049 (188, 861)	$p < 0.0001$
14	Gain/Loss	Female	Unbiased	872 (161, 711)	
15	Gain/Loss	Male and Female	Unbiased	53 (12, 41)	
16	Gain/Loss	Unbiased	Male	657 (108, 549)	$p \approx 0.0001$
17	Gain/Loss	Unbiased	Female	525 (84, 441)	
18	Gain/Loss	Unbiased	Male and Female	27 (5, 22)	
19	Expressed	**11716 (1919, 9797)**	**11543 (1893, 9650)**	**9508 (1529, 7979)**	

Supplementary Table 1 – Number of genes showing different patterns of expression bias. The number of genes on the X and autosomes (excluding chromosome 4) for each pattern of expression bias for *D. melanogaster* and *D. simulans* head tissue (individual counts of X and autosomes are in parentheses). Sex-biased genes are the sum of male-biased (Male), female-biased (Female), and male- and female-biased (Male and Female) genes. Expression bias of orthologous of the species are indicated in the right-most column. Conserved expression bias, where both species are classified as the same category within the orthologous gene pair, are included in rows 1-5, followed by rows 6-18 with diverged expression bias, where the gene pair is assigned different expression categories between *D. melanogaster* and *D. simulans*. Binomial test probabilities are indicated to the right of the table for the comparison of male-biased vs. female-biased for conserved and species-specific sex-biased genes. Significant p-values are in black if below the significant threshold of $p = 0.001$ and gray if above the threshold.

Phylogeny	*D. melanogaster* Subgroup			*D. melanogaster* Group			12 Species			Total
Model	M1a vs. M2a	M7 vs. M8	M8 vs. M8a	M1a vs. M2a	M7 vs. M8	M8 vs. M8a	M1a vs. M2a	M7 vs. M8	M8 vs. M8a	
Conserved Male-biased Expression	**66**	**83**	4	**44**	**67**	**47**	**13**	**104**	**20**	**1154**
Conserved Male-biased Expression (Male H3K4me3 Both Species)	59	75	4	39	62	41	13	99	19	**1027**
Conserved Male-biased Expression (Male H3K4me3 *D. melanogaster* only)	2	2	0	2	1	2	0	1	1	**26**
Conserved Male-biased Expression (Male H3K4me3 *D. simulans* only)	5	5	0	3	3	3	0	4	0	**62**
Conserved Female-biased Expression	17	22	0	13	25	13	2	51	4	**1038**
Divergent Sex-biased Expression	96	128	6	62	105	68	10	223	23	**3490**
D. melanogaster-specific Sex-biased Expression	49	60	2	29	47	33	5	121	11	**1974**
D. simulans-specific Sex-biased Expression	29	43	2	18	39	21	3	72	8	**1209**
Reversal of Sex-biased Expression	6	9	2	5	7	5	0	14	2	**183**
Female-biased Expression in One Species	27	39	0	17	33	21	3	79	6	**1397**
Male-biased Expression in One Species	47	59	3	26	50	30	5	107	13	**1706**
Conserved Presence of Male H3K4me3	187	249	13	132	226	141	**33**	**467**	**58**	**8462**
Conserved Presence of Female H3K4me3	185	241	12	128	216	139	30	**449**	**56**	**8022**
Conserved Presence of Male H3K27me2me3	**111**	**137**	3	**71**	**109**	**73**	14	**162**	16	**2687**
Conserved Presence of Female H3K27me2me3	**111**	**139**	6	**64**	**104**	**70**	15	160	14	**2762**

Supplementary Table 2 – Enrichment of genes with positive selection. Summary of enrichment tests performed between genes with evidence of positive selection from flyDIVas (Stanley and Kulathinal 2016; Clark 2007) and genes with conserved/diverged expression or conserved presence of chromatin marks described in this study. The number of genes with evidence of positive selection for the 3 phylogenetic levels (*D. melanogaster* subgroup, *D. melanogaster* group, and 12 species) and 3 models tested (M1a vs. M2a, M7 vs. M8, and M8 vs. M8a) in flyDIVas is provided for each group. Gene numbers in red are those that were significantly enriched (χ^2: $p < 0.001$) for genes with positive selection. More detailed descriptions of the models tested can be found in Table 2 of the PAML manual (http://abacus.gene.ucl.ac.uk/software/pamlDOC.pdf). Briefly, M1a vs. M2a compares nearly neutral evolution and positive selection, M7 vs. M8 compares where dN/dS (ω) varies according to a beta distribution vs. a beta distribution plus a discrete ω class where ω > 1 (positive selection), and M8 vs. M8a which compares where ω varies according to a beta distribution with a discrete ω class where ω > 1 vs. a beta distribution with ω=1.

Comparison	Feature	Description	All	X	Autosomes
D. melanogaster *vs.* *D. simulans*	Gene	Male H3K4me3	0.67	0.65	0.67
		Female H3K4me3	0.73	0.75	0.72
		Male H3K27me2me3	0.52	0.45	0.54
		Female H3K27me2me3	0.54	0.55	0.54
		Male-limited H3K4me3	0.19	0.30	0.16
		Female-limited H3K4me3	0.07	0.05	0.07
		Male-limited H3K27me2me3	0.08	0.05	0.09
		Female-limited H3K27me2me3	0.09	0.15	0.08
Male *vs.* *Female*	3' UTR	*D. melanogaster* H3K4me3	0.63	-	-
		D. simulans H3K4me3	0.54	-	-
		D. melanogaster H3K27me2me3	0.31	-	-
		D. simulans H3K27me2me3	0.27	-	-
	5' UTR	*D. melanogaster* H3K4me3	0.72	-	-
		D. simulans H3K4me3	0.68	-	-
		D. melanogaster H3K27me2me3	0.29	-	-
		D. simulans H3K27me2me3	0.29	-	-
	Exon	*D. melanogaster* H3K4me3	0.63	-	-
		D. simulans H3K4me3	0.58	-	-
		D. melanogaster H3K27me2me3	0.34	-	-
		D. simulans H3K27me2me3	0.30	-	-
	Intron	*D. melanogaster* H3K4me3	0.58	-	-
		D. simulans H3K4me3	0.55	-	-
		D. melanogaster H3K27me2me3	0.36	-	-
		D. simulans H3K27me2me3	0.31	-	-
	TSS (300bp Windows)	*D. melanogaster* H3K4me3	0.74	-	-
		D. simulans H3K4me3	0.68	-	-
		D. melanogaster H3K27me2me3	0.39	-	-
		D. simulans H3K27me2me3	0.30	-	-
	Intergenic	*D. melanogaster* H3K4me3	0.64	-	-
		D. simulans H3K4me3	0.69	-	-
		D. melanogaster H3K27me2me3	0.55	-	-
		D. simulans H3K27me2me3	0.59	-	-
	Gene	*D. melanogaster* H3K4me3	0.73	0.67	0.74
		D. simulans H3K4me3	0.68	0.63	0.69
		D. melanogaster H3K27me2me3	0.58	0.53	0.59
		D. simulans H3K27me2me3	0.54	0.49	0.54
H3K4me3 *vs.* *H3K27me2me3*	3' UTR	*D. melanogaster* Males	-0.10	-	-
		D. simulans Males	-0.08	-	-
		D. melanogaster Females	-0.11	-	-
		D. simulans Females	-0.12	-	-
	5' UTR	*D. melanogaster* Males	-0.09	-	-
		D. simulans Males	-0.07	-	-

		D. melanogaster Females	-0.11	-	-
		D. simulans Females	-0.10	-	-
	Exon	*D. melanogaster* Males	-0.16	-	-
		D. simulans Males	-0.10	-	-
		D. melanogaster Females	-0.19	-	-
		D. simulans Females	-0.14	-	-
	Intron	*D. melanogaster* Males	-0.15	-	-
		D. simulans Males	-0.08	-	-
		D. melanogaster Females	-0.16	-	-
		D. simulans Females	-0.12	-	-
	TSS (300bp Windows)	*D. melanogaster* Males	-0.20	-	-
		D. simulans Males	-0.10	-	-
		D. melanogaster Females	-0.22	-	-
		D. simulans Females	-0.14	-	-
	Intergenic	*D. melanogaster* Males	-0.31	-	-
		D. simulans Males	-0.21	-	-
		D. melanogaster Females	-0.30	-	-
		D. simulans Females	-0.21	-	-
	Genes	*D. melanogaster* Males	-0.22	-0.11	-0.25
		D. simulans Males	-0.12	-0.05	-0.13
		D. melanogaster Females	-0.26	-0.28	-0.26
		D. simulans Females	-0.22	-0.22	-0.22
Head tissue vs. elav-expressing neurons	Genes	H3K4me3 in *D. melanogaster* Males	0.28	-	-
		H3K4me3 in *D. melanogaster* Females	0.37	-	-
		H3K27me2me3 in *D. melanogaster* Males	0.26	-	-
		H3K27me2me3 in *D. melanogaster* Females	0.42	-	-
Head tissue vs. fru-P1-expressing neurons	Genes	H3K4me3 in *D. melanogaster* Males	0.27	-	-
		H3K4me3 in *D. melanogaster* Females	-0.04	-	-
		H3K27me2me3 in *D. melanogaster* Males	0.33	-	-
		H3K27me2me3 in *D. melanogaster* Females	0.32	-	-

Supplementary Table 3 – Summary of Kappa values for the indicated comparisons.
Cohen's Kappa values (Fleiss 1981) indicating chance corrected agreement of the comparison described the "Comparison" column for the feature described in the "Feature" column and group in the "Description" column. Kappa values are presented for all chromosomes (X and autosomes combined) for all comparisons, as well as X chromosomes and autosomes separately for several indicated comparisons. Chromosome 4 is excluded from the autosomes.

Feature Type	***D. melanogaster***	***D. simulans***
Genes	17737	15385
Transcripts	35254	26261
TSS (300bp Windows)	22893	21069
5'UTR	28479	25081
3'UTR	21600	16231
Exonic Features	87473	79405
Intronic Features	44769	47236
Intergenic Features	11356	16174

Supplementary Table 4 – Number of annotated genomic features in *D. melanogaster* and *D. simulans*. Counts of features within *D. melanogaster* and *D. simulans* genome annotation files. 5' UTR and 3'UTR were determined for each transcript using the references described in the Genome Annotation section of the Methods. A transcription start site (TSS) was defined as a 300 bp region, 150 bp upstream and downstream from each annotated transcript start. In *D. melanogaster* there were three pairs of genes where the members in each pair had the same start position but opposite strands: i) *bug* (FBgn0034050) and *Diap2* (FBgn0015247), ii) *lncRNA:CR44456* (FBgn0265649) and *lncRNA:CR44455* (FBgn0265648), and iii) *CR43482* (FBgn0263493) and *CR43483* (FBgn0263494). Event analysis (Newman, et al. 2018) was used to determine exonic and intronic features. Intergenic features were defined by subtracting the genic features from the entire genome with a length greater than 50 bp.

Species	Feature Type	# Detected in Males	# Detected in Females	# Detected in Either Sex	# Detected in Both Sexes
D. melanogaster	3UTR	14505 (67.15%)	14280 (66.11%)	14700 (68.06%)	14085 (65.21%)
	5UTR	18640 (65.45%)	18198 (63.9%)	19066 (66.95%)	17772 (62.4%)
	TSS	15761 (68.85%)	15323 (66.93%)	16161 (70.59%)	14923 (65.19%)
	Exonic	69373 (79.31%)	68038 (77.78%)	70568 (80.67%)	66843 (76.42%)
	Intergenic	6000 (52.84%)	5633 (49.6%)	6260 (55.13%)	5373 (47.31%)
	Intronic	29555 (66.02%)	28483 (63.62%)	30576 (68.3%)	27462 (61.34%)
D. simulans	3UTR	11820 (72.82%)	11717 (72.19%)	12032 (74.13%)	11505 (70.88%)
	5UTR	16108 (64.22%)	16054 (64.01%)	16653 (66.4%)	15509 (61.84%)
	TSS	13806 (65.53%)	13712 (65.08%)	14291 (67.83%)	13227 (62.78%)
	Exonic	61777 (77.8%)	61482 (77.43%)	63283 (79.7%)	59976 (75.53%)
	Intergenic	6769 (41.85%)	6616 (40.91%)	7144 (44.17%)	6241 (38.59%)
	Intronic	30010 (63.53%)	30013 (63.54%)	31531 (66.75%)	28492 (60.32%)

Supplementary Table 5 – Summary of features detected by RNA-seq. The number (percent) of features detected in males (irrespective of females), in females (irrespective of males), in either males or females (union), and in both males and females (intersection) for each species mapped to the associated reference

genome. Percent (in parentheses) is calculated by dividing the number detected by the total number for each feature type (see Supplementary Table 4 for feature totals).

A.

Species	*D. melanogaster*			
Genome	***D. melanogaster* FB r6.17**		***D. simulans* FB r2.02**	
Sex	**Male**	**Female**	**Male**	**Female**
Mean # mapped reads per replicate	16,368,252	16,479,280	16,877,562	16,915,308
Mean % mapped reads per replicate	91.37%	92.76%	94.35%	95.22%

Species	*D. simulans*			
Genome	***D. melanogaster* FB r6.17**		***D. simulans* FB r2.02**	
Sex	**Male**	**Female**	**Male**	**Female**
Mean # mapped reads per replicate	14,774,793	15,598,854	15,498,853	16,423,578
Mean % mapped reads per replicate	90.44%	94.61%	94.83%	94.61%

B.

Species	*D. melanogaster*					
Sex	**Male**			**Female**		
ChIP/Input	**Input**	**H3K4me3**	**H3K27me2me3**	**Input**	**H3K4me3**	**H3K27me2me3**
Mean # mapped reads per replicate	10,915,497	13,688,173	12,666,807	12,239,391	13,817,377	14,380,575
Mean % mapped reads per replicate	78.82%	86.08%	76.38%	81.83%	87.73%	78.70%

Species	*D. simulans*					
Sex	**Male**			**Female**		
ChIP/Input	**Input**	**H3K4me3**	**Input**	**H3K4me3**	**Input**	**H3K4me3**
Mean # mapped reads per replicate	10,435,104	14,728,518	10,435,104	14,728,518	10,435,104	14,728,518
Mean % mapped read per replicate	80.25%	92.01%	80.25%	92.01%	80.25%	92.01%

Supplementary Table 6 – Summary of read mapping counts and percentages. (A) RNA-seq mapped reads. All RNA-seq samples were mapped to both the *D. melanogaster* FlyBase 6.17 genome and the *D. simulans* FlyBase r.202 genome. The mean number and percent of processed reads across replicates after mapping to the indicated genome are given. (B) ChIP-seq mapped reads. All ChIP-seq samples were mapped to the associated reference genome based on the species of the sample (*D. melanogaster* FlyBase 6.17 genome or *D. simulans* FlyBase r.202). The mean number and percent of mapped processed reads across replicates for the indicated ChIP mark or input control are given.

Supplementary Files:

Supplementary File 1 - Gene-level expression and chromatin accessibility results for *D. melanogaster*. All column variables are defined in Supplementary File 9.

Supplementary File 2 – Gene-level expression and chromatin accessibility results for *D. simulans*. All column variables are defined in Supplementary File 9.

Supplementary File 3 – ChIP-seq protocol

Supplementary File 4 – Orthologous gene pairs of *D. melanogaster* to *D. simulans* selected from FlyBase OrthoDB report (Waterhouse, et al. 2013) in release 2017_04 (dmel_orthologs_in_drosophila_species_fb_2017_04.tsv.gz, downloaded 4/17/19). The original FlyBase file was modified to have individual columns for coordinates, +/- values for strand (compared to 1/-1), and "Dsim\" removed from Ortholog_GeneSymbol elements.

Supplementary File 5 – Upper quartile values used in for RNA-seq quantification.

Supplementary File 6 – Feature-level expression and chromatin accessibility results for *D. melanogaster*.

Supplementary File 7 – Feature-level expression and chromatin accessibility results for *D. simulans*.

Supplementary File 8 – Gene-level expression and chromatin accessibility results for *D. melanogaster* and *D. simulans* orthologs as identified by the FlyBase OrthoDB report (Waterhouse, et al. 2013). All column variables are defined in Supplementary File 10.

Supplementary File 9 – Gene-level variable definitions for species result files (Supplementary Files 1, 2).

Supplementary File 10 – Gene-level variable definitions for the *D. melanogaster* and *D. simulans* ortholog result file (Supplementary File 8).

Supplementary File 11 – For all gene numbers called out in the main text, the descriptions and the flags needed to identify those genes in Supplementary Files 1 or 8.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403-410.

Amrein H, Axel R. 1997. Genes expressed in neurons of adult male Drosophila. Cell 88:459-469.

An WQ, Wensink PC. 1995. Three protein binding sites form an enhancer that regulates sex-and fat body-specific transcription of Drosophila yolk protein genes. Embo Journal 14:1221-1230.

Bashaw GJ, Baker BS. 1995. The msl-2 dosage compensation gene of Drosophila encodes a putative DNA-binding protein whose expression is sex specifically regulated by Sex-lethal. Development 121:3245-3258.

Bashaw GJ, Baker BS. 1997. The regulation of the Drosophila msl-2 gene reveals a function for Sex-lethal in translational control. Cell 89:789-798.

Bell LR, Maine EM, Schedl P, Cline TW. 1988. Sex-lethal, a Drosophila sex determination switch gene, exhibits sex-specific RNA splicing and sequence similarity to RNA binding proteins. Cell 55:1037-1046.

Boggs RT, Gregor P, Idriss S, Belote JM, McKeown M. 1987. Regulation of sexual differentiation in D. melanogaster via alternative splicing of RNA from the transformer gene. Cell 50:739-747.

Burtis KC, Baker BS. 1989. Drosophila doublesex Gene Controls Somatic Sexual Differentiation by Producing Alternatively Spticed mRNAs Encoding Related Sex-Specific Polypeptides. Cell 56:997-1010.

Burtis KC, Coschigano KT, Baker BS, Wensink PC. 1991. The doublesex proteins of Drosophila melanogaster bind directly to a sex-specific yolk protein gene enhancer. Embo Journal 10:2577-2582.

Byron K. 2010. Mining roX1 RNA in Drosophila genomes using covariance models.

Cline TW. 1978. Two closely linked mutations in Drosophila melanogaster that are lethal to opposite sexes and interact with daughterless. Genetics 90:683-698.

Coschigano KT, Wensink PC. 1993. Sex-specific transcriptional regulation by the male and female doublesex proteins of Drosophila. Genes & Development 7:42-54.

Erdman SE, Chen HJ, Burtis KC. 1996. Functional and genetic characterization of the oligomerization and DNA binding properties of the Drosophila doublesex proteins. Genetics 144:1639-1652.

Fleiss JL. 1981. Statistical methods for rates and proportions. Second edition: Wiley, John and Sons, Inc., New York, N.Y.

Flickinger TW, Salz HK. 1994. The Drosophila sex determination gene snf encodes a nuclear protein with sequence and functional similarity to the mammalian U1A snRNP protein. Genes & Development 8:914-925.

Garrett-Engele CM, Siegal ML, Manoli DS, Williams BC, Li H, Baker BS. 2002. intersex, a gene required for female sexual development in Drosophila, is expressed in both sexes and functions together with doublesex to regulate terminal differentiation. Development 129:4661-4675.

Gebauer F, Merendino L, Hentze MW, Valcarcel J. 1998. The Drosophila splicing regulator sex-lethal directly inhibits translation of male-specific-lethal 2 mRNA. Rna-a Publication of the Rna Society 4:142-150.

Gorman M, Franke A, Baker BS. 1995. Molecular characterization of the male-specific lethal-3 gene and investigations of the regulation of dosage compensation in Drosophila. Development 121:463-475.

Granadino B, Campuzano S, Sanchez L. 1990. The Drosophila melanogaster fl(2)d gene is needed for the female-specific splicing of Sex-lethal RNA. Embo Journal 9:2597-2602.

Granadino B, Penalva LOF, Sanchez L. 1996. The gene fl(2)d is needed for the sex-specific splicing of transformer pre-mRNA but not for double-sex pre-mRNA in Drosophila melanogaster. Molecular and General Genetics 253:26-31.

Heinrichs V, Ryner LC, Baker BS. 1998. Regulation of sex-specific selection of fruitless 5 ' splice sites by transformer and transformer-2. Molecular and Cellular Biology 18:450-458.

Hilfiker A, Amrein H, Dubendorfer A, Schneiter R, Nothiger R. 1995. The gene virilizer is required for female-specific splicing controlled by Sxl, the master gene for sexual development in Drosophila. Development 121:4017-4026.

Inoue K, Hoshijima K, Higuchi I, Sakamoto H, Shimura Y. 1992. Binding of the Drosophila transformer and transformer-2 proteins to the regulatory elements of doublesex primary transcript for sex-specific RNA processing. Proceedings of the National Academy of Sciences of the United States of America 89:8092-8096.

Inoue K, Hoshijima K, Sakamoto H, Shimura Y. 1990. Binding of the Drosophila Sex-lethal gene product to the alternative splice site of transformer primary transcript. Nature 344:461-463.

Kelley RL, Solovyeva I, Lyman LM, Richman R, Solovyev V, Kuroda MI. 1995. Expression of Msl-2 causes assembly of dosage compensation regulators on the X-chromosomes and female-lethality in Drosophila. Cell 81:867-877.

Kelley RL, Wang JW, Bell L, Kuroda MI. 1997. Sex lethal controls dosage compensation in Drosophila by a non-splicing mechanism. Nature 387:195-199.

Kuroda MI, Kernan MJ, Kreber R, Ganetzky B, Baker BS. 1991. The maleless Protein Associates with the X Chromosome to Regulate Dosage Compensation in Drosophila. Cell 66:935-947.

Lallena MJ, Chalmers KJ, Llamazares S, Lamond AI, Valcarcel J. 2002. Splicing regulation at the second catalytic step by sex-lethal involves 3 ' splice site recognition by SPF45. Cell 109:285-296.

Li H, Baker BS. 1998. hermaphrodite and doublesex function both dependently and independently to control various aspects of sexual differentiation in Drosophila. Development 125:2641-2651.

McNemar Q. 1947. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika 12:153-157.

Meller VH, Wu KH, Roman G, Kuroda MI, Davis RL. 1997. roX1 RNA paints the X chromosome of male Drosophila and is regulated by the dosage compensation system. Cell 88:445-457.

Newman J, Concannon P, Tardaguila M, Conesa A, McIntyre L. 2018. Event Analysis: Using Transcript Events To Improve Estimates of Abundance in RNA-seq Data. G3-Genes Genomes Genetics 8:2923-2940.

Palmateer CM, Moseley SC, Ray S, Brovero SG, Arbeitman MN. 2021. Analysis of cell-type-specific chromatin modifications and gene expression in Drosophila neurons that direct reproductive behavior. Plos Genetics 17.

Park SW, Kang YIe, Sypula JG, Choi J, Oh H, Park Y. 2007. An evolutionarily conserved domain of roX2 RNA is sufficient for induction of H4-Lys16 acetylation on the Drosophila X chromosome. Genetics 177:1429-1437.

Pearson K. 1900. On the Criterion that a given System of Deviations from the Probable in the Case of a Correlated System of Variables is such that it can be reasonably supposed to have arisen from Random Sampling. Philosophical Magazine 50:157-175.

Quinn JJ, Zhang QFC, Georgiev P, Ilik IA, Akhtar A, Change HY. 2016. Rapid evolutionary turnover underlies conserved lncRNA-genome interactions. Genes & Development 30:191-207.

Ryner LC, Goodwin SF, Castrillon DH, Anand A, Villella A, Baker BS, Hall JC, Taylor BJ, Wasserman SA. 1996. Control of male sexual behavior and sexual orientation in Drosophila by the fruitless gene. Cell 87:1079-1089.

Sosnowski BA, Belote JM, McKeown M. 1989. Sex-Specific Alternative Splicing of RNA from the transformer Gene Results from Sequence-Dependent Splice Site Blockage. Cell 58:449-459.

Waterhouse R, Tegenfeldt F, Li J, Zdobnov E, Kriventseva E. 2013. OrthoDB: a hierarchical catalog of animal, fungal and bacterial orthologs. Nucleic Acids Research 41:D358-D365.

Zhou SB, Yang YF, Scott MJ, Pannuti A, Fehr KC, Eisen A, Koonin EV, Fouts DL, Wrightsman R, Manning JE, et al. 1995. Male-specific lethal 2, a dosage compensation gene of Drosophila, undergoes sex-specific regulation and encodes a protein with a RING finger and a metallothionein-like cysteine cluster. Embo Journal 14:2884-2895.