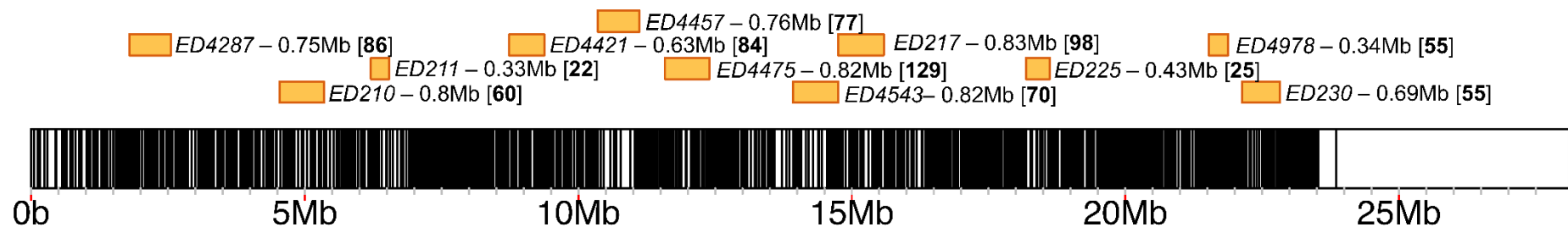# Heightened condition dependence of the sexual transcriptome as a function of genetic quality in *Drosophila melanogaster* head tissue

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Electronic Supplementary Material 1. Genomic position and size of deletions.


ESM1. Genomic position and size of the 11 deletions used in the low genetic quality treatment along chromosome 3L. The yellow boxes depict the position of each deletion, with information on the identity, the extension (in Megabases) and the number of genes deleted or partially deleted (bold).

Electronic Supplementary Material 2. Results using OreR as control line.

ESM2-A. Number of sex-biased genes in the control (OreR) and each deletion line. Within each bias class, all deletions showed a significant reduction in the number of sex-biased genes compared to controls ($P_{adj} < 0.001$, FDR corrected, except where $†P_{adj} = 0.04$). Between brackets we report the number of genes that are common with the control line (where numbers are not reported, no common genes were found).

Deletion	Female-biased genes (*n*)	Male-biased genes (*n*)	Total (*n*)
Control	330	144	474
ED210	28(3)	41	69(3)
ED211	22(2)	20(2)	42(4)
ED217	7	57	64
ED225	5	14	19
ED230	75(15)	86(2)	161(17)
ED4287	145(99)	112†(31)	257(130)
ED4421	15	13(1)	28(1)
ED4457	44	36	80
ED4475	26(2)	35	61(2)
ED4543	3	8	11
ED4978	22(1)	21(1)	43(2)

ESM2-B. Differential gene expression analysis conducted by contrasting each deletion towards the OreR controls, with genes grouped on the x-axis according to their sex-bias in the controls (FB = female biased, MB = male biased, UB = unbiased). Differences between specific changes in gene expression were tested using a Bayesian linear mixed-model. Values expressed in (A) absolute $\log_2$ fold changes and (B) $\log_2$ fold changes (*** $P < 0.001$). Both MB and FB genes in males showed a higher magnitude of change compared to the respective class in females (both $P < 0.001$). Within each sex we compared the two bias classes and found greater condition dependence of FB genes than MB genes in both males and females (both $P < 0.001$).
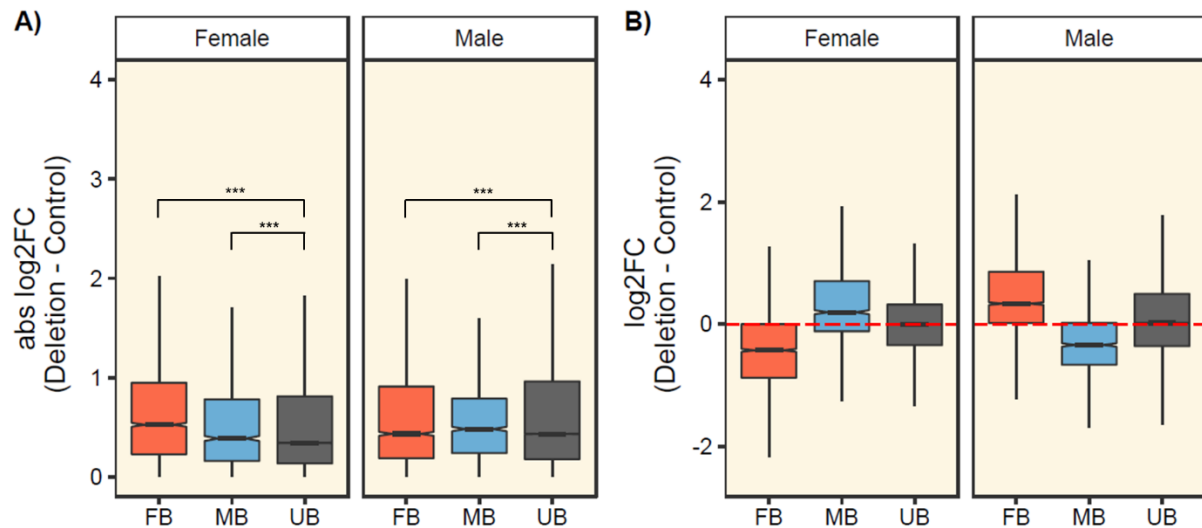

ESM2-C. Model coefficients from the linear mixed-effect model fit to data on log2FC between OreR control and deletion lines using Bayesian Hamiltonian Markov chain Monte Carlo.

Effect	Estimate	95% CI
Intercept	-0.30	(-0.36, -0.25)
Sex *Male*	1.2	(1.15, 1.25)
Bias *Male biased*	0.79	(0.74, 0.85)
Bias *Unbiased*	0.34	(0.31, 0.38)
Sex *Male* × **Bias** *Male biased*	-1.92	(-2.01, -1.85)
Sex *Male* × **Bias** *Unbiased*	-1.11	(-1.17, -1.06)

Electronic Supplementary Material 3. Volcano plots showing sex-biased genes using P<0.01 as threshold.

ESM3. Volcano plot highlighting sex-biased genes in control and each deletion line, using $P_{adj} < 0.01$ as threshold.
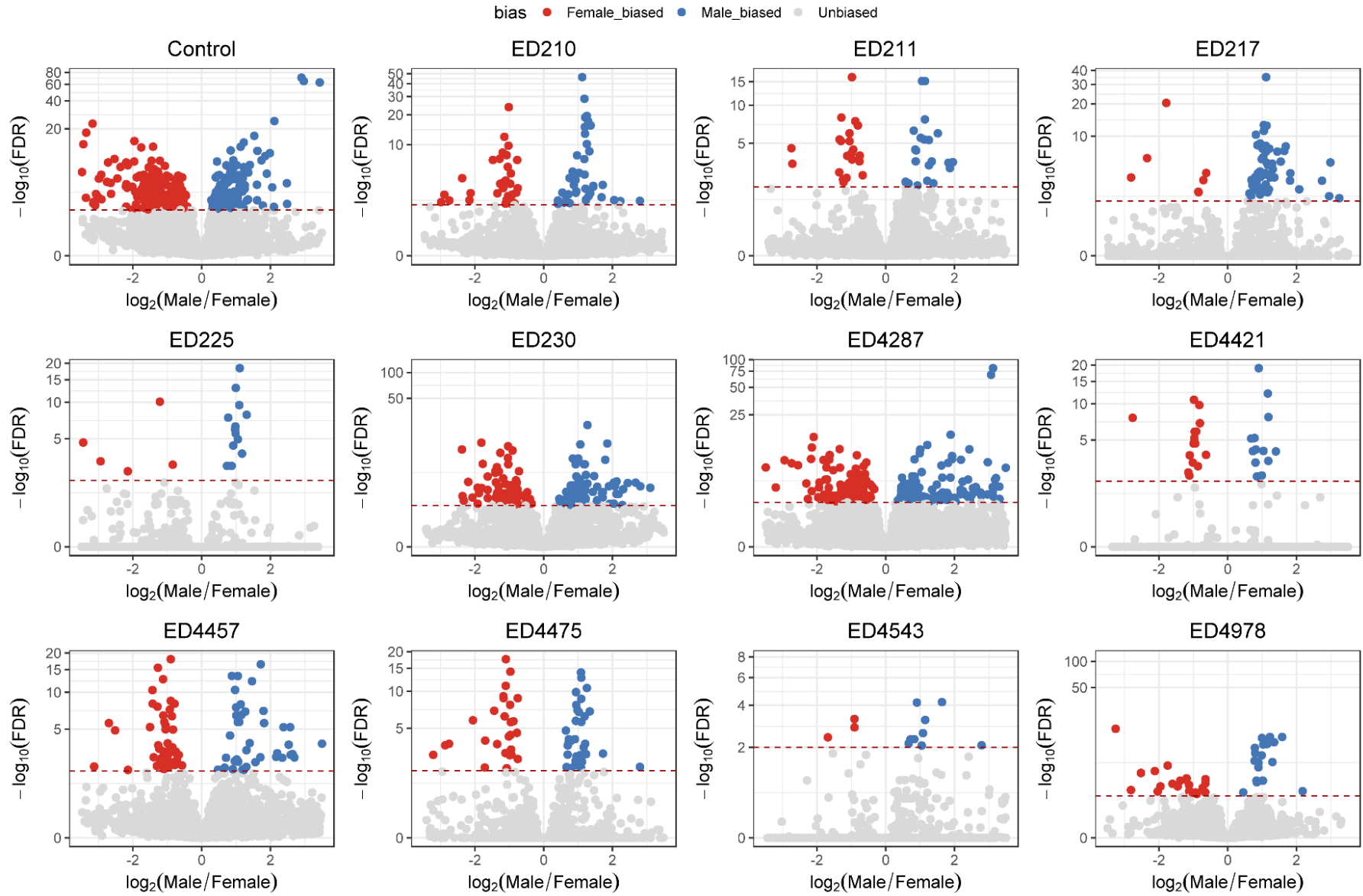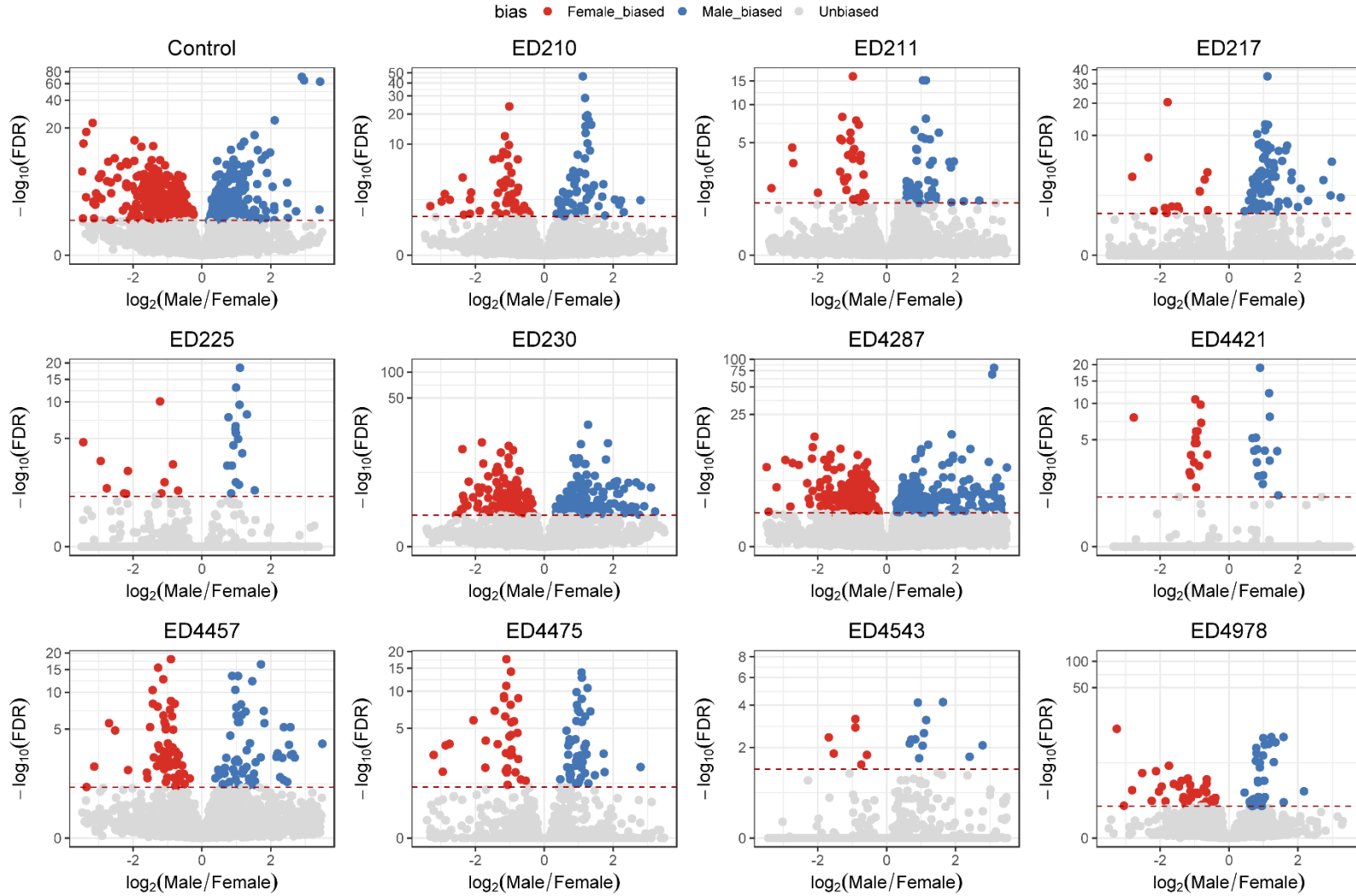
Electronic Supplementary Material 4. Sex-bias classification using different thresholds.

ESM4-A. Number of sex-biased genes in control and each deletion line, using $P_{adj} < 0.05$ as threshold during classification. Within each bias class, all deletions showed a significant reduction in the number of sex-biased genes compared to control ($P_{adj} < 0.001$, FDR corrected), excluding groups marked with († $P_{adj} > 0.05$).

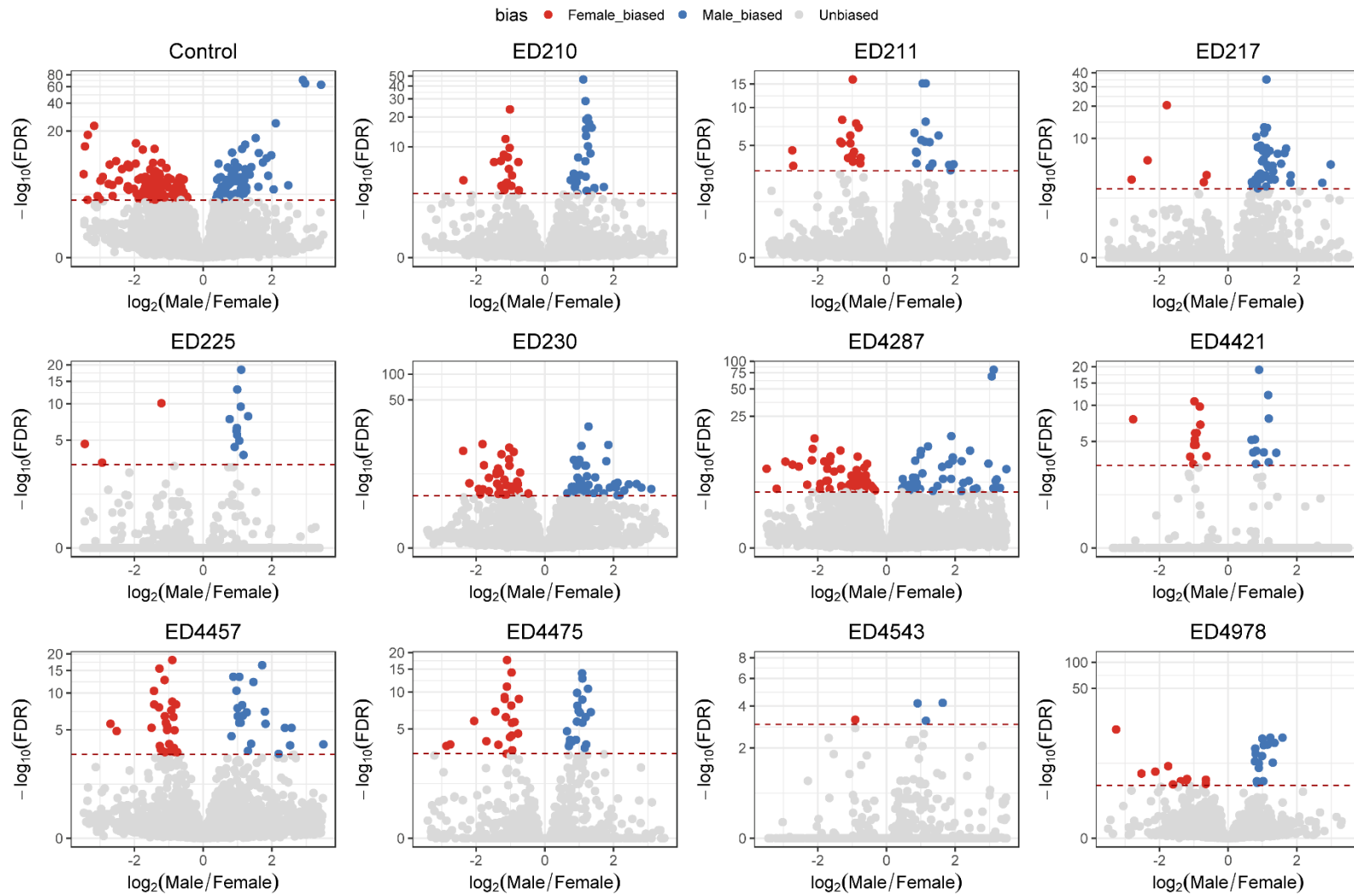| Deletion	Female-biased genes (*n*)	Male-biased genes (*n*)	Total (*n*)
Control	349	320	669
ED210	53	59	112
ED211	30	44	74
ED217	14	76	90
ED225	11	17	28
ED230	145	176	321
ED4287	326†	227	553
ED4421	16	15	31
ED4457	61	58	119
ED4475	34	50	84
ED4543	6	11	17
ED4978	41	36	77

ESM4-B. Number of sex-biased genes in control and each deletion line, using $P_{adj} < 0.001$ as threshold during classification. Within each bias class, all deletions showed a significant reduction in the number of sex-biased genes compared to control ($P_{adj} < 0.001$, FDR corrected).

Deletion	Female-biased genes (*n*)	Male-biased genes (*n*)	Total (*n*)
Control	121	77	198
ED210	17	25	42
ED211	17	16	33
ED217	5	44	49
ED225	3	11	14
ED230	36	41	77
ED4287	57	43	100
ED4421	12	11	23
ED4457	27	22	49
ED4475	21	21	42
ED4543	1	3	4
ED4978	10	17	27

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2 **ESM4-C**. Volcano plot highlighting sex-biased genes in control and each deletion line, using $P_{adj} < 0.05$ as threshold.
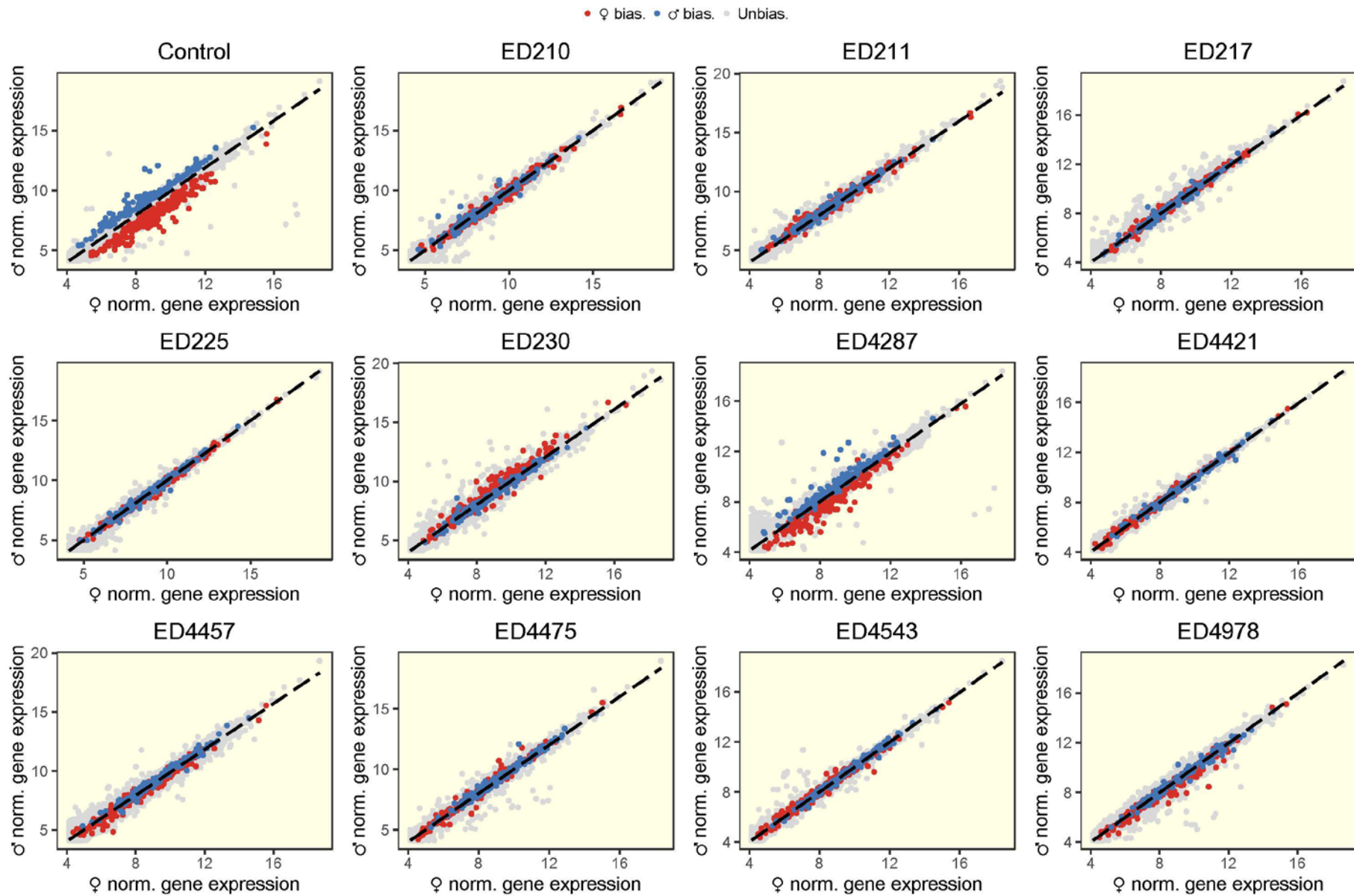
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ESM4-D. Volcano plot highlighting sex-biased genes in control and each deletion line, using $P_{adj} < 0.001$ as threshold.

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Electronic Supplementary Material 5. Scatterplots of gene expression in male and female head tissue.

ESM5. Scatterplots of gene expression in male and female head tissue for the control line and each of the 11 deletion lines. Genes with significant sex-biased expression in the control line are displayed in color. Gene expression level is estimated using normalized counts, which were generated from raw count data using only normalization by size factors in *DESeq2*.

Legend: ♀ bias. ♂ bias. Unbias.

Control | ED210 | ED211 | ED217
ED225 | ED230 | ED4287 | ED4421
ED4457 | ED4475 | ED4543 | ED4978

All plots: x-axis ♀ norm. gene expression; y-axis ♂ norm. gene expression

Electronic Supplementary Material 6. Model coefficients.

ESM6. Model coefficients from the linear mixed-effect model fit to data on log2FC between control and deletion lines using Bayesian Hamiltonian Markov chain Monte Carlo.

Effect	Estimate	95% CI
Intercept	-0.29	(-0.35, -0.24)
Sex *Male*	1.21	(1.16, 1.26)
Bias *Male biased*	0.79	(0.74, 0.84)
Bias *Unbiased*	0.34	(0.3, 0.37)
Sex *Male* × **Bias** *Male biased*	-1.94	(-2.01, -1.86)
Sex *Male* × **Bias** *Unbiased*	-1.12	(-1.17, -1.07)

Electronic Supplementary Material 7. Tissue specificity analysis.

ESM7. Condition dependence of male-, female-, and un-biased genes when gene tissue specificity is taken into account. Differences between specific changes in gene expression were tested using a Bayesian generalized linear model (*** $P < 0.001$, * $P < 0.05$, FB = female biased, MB = male biased, UB = unbiased).
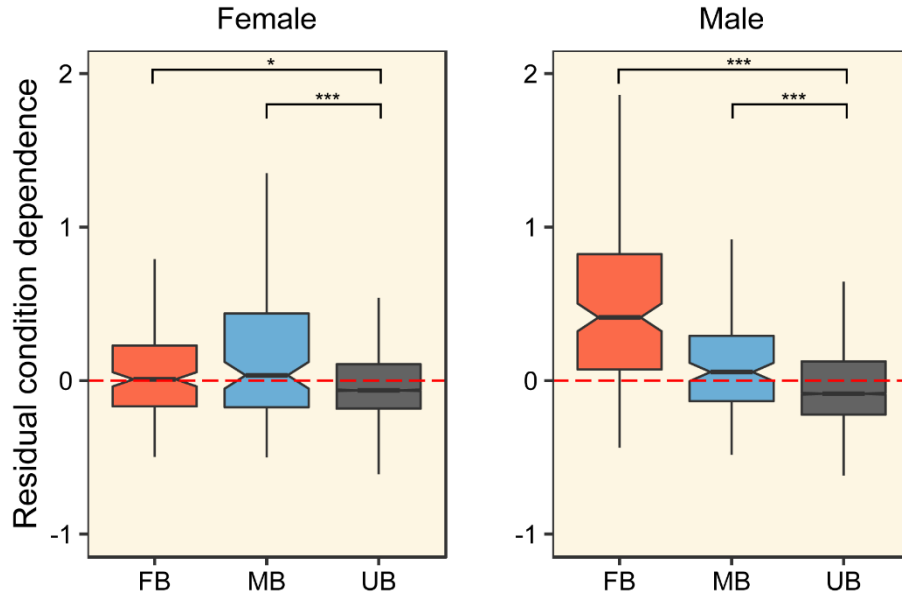
Sex-biased genes often show elevated tissue-specific expression. Our result could thus potentially be driven by properties associated with tissue-specific genes rather than sex-biased genes. To test for this, we calculated the tissue specificity (τ) for each gene using the following equation:

$$\tau = \frac{\sum_{i=1}^{N}(1 - \log(S_i)/\log(S_{max}))}{N - 1}$$

where S_i is the gene expression in tissue *i*, S_{max} is the highest expression value gene *i* takes in any tissue, and *N* is the number of tissues analysed (Larracuente et al. 2008). Gene expression values from different tissues were obtained from FlyAtlas (http://www.flyatlas.org/; Chintapalli et al. 2007) and we sampled non-overlapping tissues following Meisel (2011). We then regressed absolute condition dependence (abs log2FC(Deletion-Control)) on τ, for all genes in each sex separately. Next we built a generalized linear model using Bayesian Hamiltonian Markov chain Monte Carlo in the package *rstanarm*, with Sex and Bias (FB, MB, or UB) as fixed effect and the residuals from above linear models as the response. We used weakly informative normally distributed priors for both the intercept (mean = 0, scale = 10) and coefficients (mean = 0, scale = 2.5), running four chains with 2000 iterations each and discarding the first 1000 as warm-up. Comparisons between classes of genes were performed using the function *hypothesis* of the *brms* package, with the equivalent of a two-tailed *P*-value reported. Below we report the model coefficients.

Effect	Estimate	95% CI
Intercept	0.04	(-0.001, 0.09)
Sex *Male*	0.44	(0.38, 0.51)
Bias *Male biased*	0.13	(0.05, 0.2)
Bias *Unbiased*	-0.04	(-0.09, -0.002)
Sex *Male* × **Bias** *Male biased*	-0.49	(-0.59, -0.38)
Sex *Male* × **Bias** *Unbiased*	-0.45	(-0.52, -0.38)

References

Chintapalli VR, Wang J, Dow JAT. 2007 Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. Nat Genet. **39**, 715–720.

Larracuente AM, Sackton TB, Greenberg AJ, Wong A, Singh ND, Sturgill D, Zhang Y, Oliver B, Clark AG. 2008 Evolution of protein coding genes in *Drosophila*. Trends Genet. **24**, 114–123.

Meisel RP. 2011 Towards a more nuanced understanding of the relationship between sex-biased gene expression and rates of protein-coding sequence evolution. Molecular Biology and Evolution **28**, 1893-1900.

Electronic Supplementary Material 8. Correlation between degree of condition dependence and degree of sex-bias for each line.

ESM8. Correlation (r = Pearson's correlation coefficient) between degree of condition dependence ($\log_2$ fold change value from the contrast *Control/Deletion*) and degree of sex-bias in the control, calculated for each sex and deletion line for male-biased and female-biased genes separately.

| Deletion | Male | | | | Female | | | |
| | Male-biased genes | | Female-biased genes | | Male-biased genes | | Female-biased genes | |
	r	*P* value	r	*P* value	r	*P* value	r	*P* value
ED210	0.03	0.71	-0.66	< 0.001	-0.37	< 0.001	-0.07	0.33
ED211	0.12	0.13	-0.68	< 0.001	-0.24	< 0.001	0.00	0.98
ED217	0.22	< 0.01	-0.70	< 0.001	-0.31	< 0.001	0.04	0.61
ED225	0.25	< 0.01	-0.63	< 0.001	-0.39	< 0.001	-0.04	0.59
ED230	0.21	< 0.01	-0.62	< 0.001	-0.29	< 0.001	0.14	0.04
ED4287	-0.35	< 0.001	-0.05	0.45	-0.28	< 0.001	0.16	0.02
ED4421	-0.28	< 0.001	-0.13	0.07	-0.74	< 0.001	0.65	< 0.001
ED4457	-0.38	< 0.001	-0.27	< 0.001	-0.74	< 0.001	0.55	< 0.001
ED4475	-0.26	< 0.001	-0.11	0.11	-0.75	< 0.001	0.69	< 0.001
ED4543	-0.21	< 0.01	-0.19	0.01	-0.73	< 0.001	0.60	< 0.001
ED4978	-0.22	< 0.01	-0.05	0.46	-0.74	< 0.001	0.62	< 0.001