Supplementary Material: Disentangling the relationship between sex-biased gene expression and X-linkage

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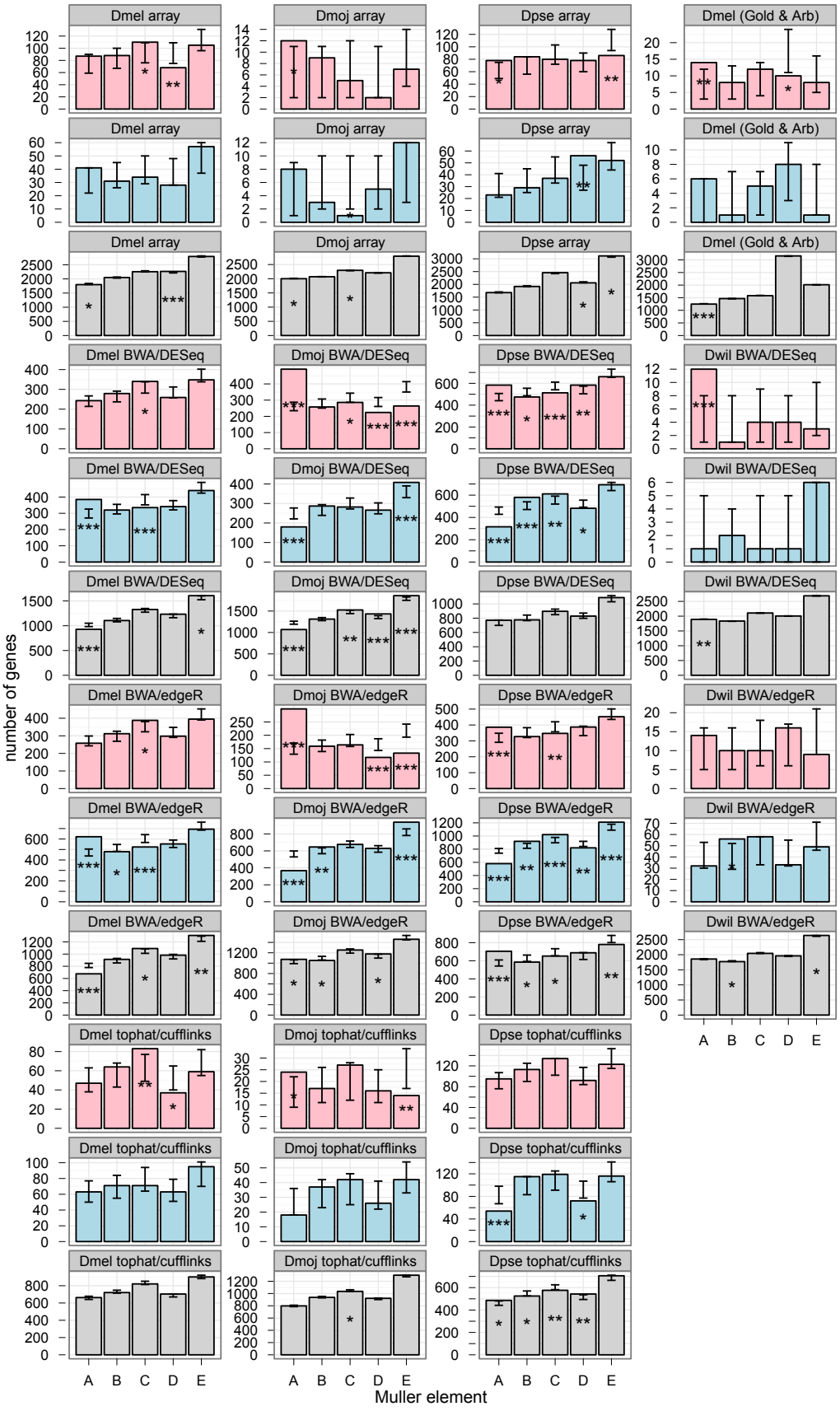
Supplementary Figures

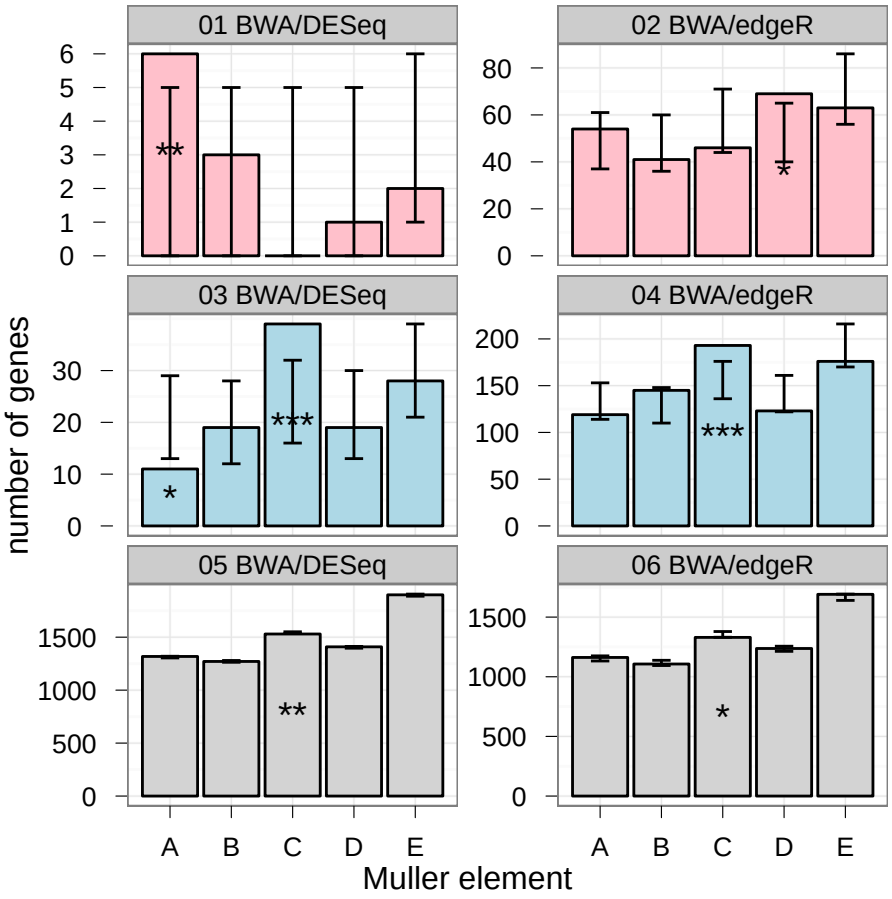
Supplementary Figure 1: Counts of genes with sex-biased expression in head on *Drosophila* chromosome arms

The number of genes on each chromosome arm that have female-biased (pink), male-biased (blue), or unbiased (gray) expression (with an FDR cutoff of 0.05) in head are indicated by the height of the bars. Genes with sex-biased expression in head were determined using RNA-seq and microarrays. Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using either BWA or tophat, and tests for differential expression between males and females were carried out using DESeq and edgeR (for the BWA alignments) or cufflinks (for the tophat alignments). P-values of tests for differential expression between males and females for previously published microarray data (Goldman and Arbeitman, 2007) were taken from SEBIDA and FDR corrected (Benjamini and Hochberg, 1995). The 95% confidence interval of the expected number of genes in each sex-bias class on each chromosome arm (determined by a permutation test based on the number of sex-biased genes in the genome and the relative sizes of the chromosome arms) are indicated by error bars. Significant deviations between observed and expected counts (as determined by the permutation test) are indicated by asterisks ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Chromosome arms are indicated by their Muller element nomenclature with the dot chromosome (Muller element F) omitted. Muller elements correspond to *D. melanogaster* chromosome arms as follows: A=X, B=2L, C=2R, D=3L, E=3R. (Dmel: *D. melanogaster*; Dpse: *D. pseudoobscura*; Dmoj: *D. mojavensis*; Dwil: *D. willistoni.*)


Dmel array
Dmoj array
Dpse array
Dmel (Gold & Arb)
Dmel BWA/DESeq
Dmoj BWA/DESeq
Dpse BWA/DESeq
Dwil BWA/DESeq
Dmel BWA/edgeR
Dmoj BWA/edgeR
Dpse BWA/edgeR
Dwil BWA/edgeR
Dmel tophat/cufflinks
Dmoj tophat/cufflinks
Dpse tophat/cufflinks
Dmel tophat/cufflinks
Dmoj tophat/cufflinks
Dpse tophat/cufflinks
number of genes
A B C D E
Muller element

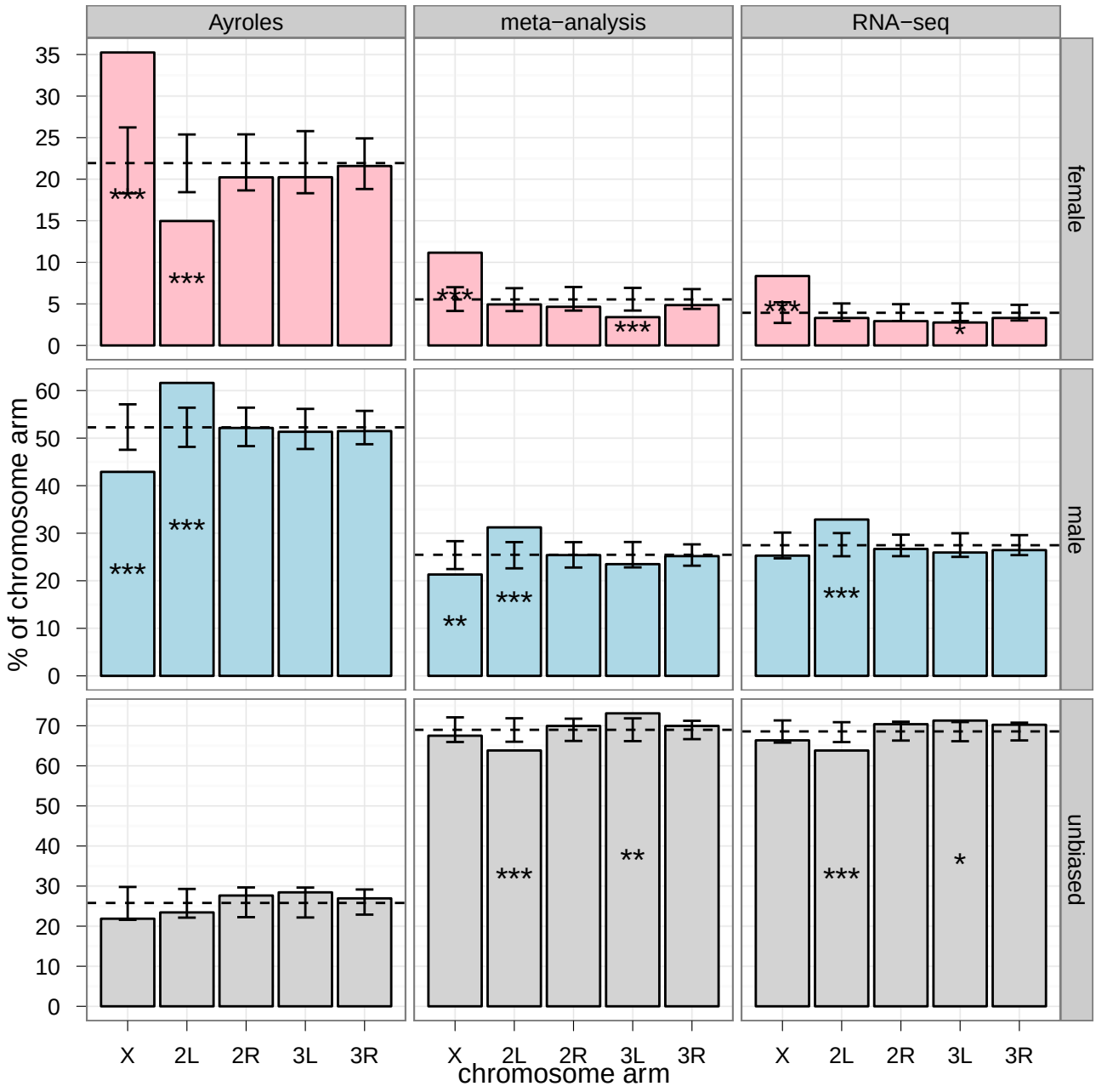
Supplementary Figure 2: Counts of genes on each chromosome arm with sex-biased expression in *D. willistoni* thorax

The number of genes on each chromosome arm that have female-biased (pink), male-biased (blue), or unbiased (gray) expression (with an FDR cutoff of 0.05) in *D. willistoni* thorax are indicated by the height of the bars. Genes with sex-biased expression in thorax were determined using RNA-seq. Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using BWA, and tests for differential expression between males and females were carried out using DESeq and edgeR. The 95% confidence intervals of the expected number of genes in each sex-bias class on each chromosome arm (determined by a permutation test based on the number of sex-biased genes in the genome and the relative sizes of the chromosome arms) are indicated by error bars. Significant deviations between observed and expected counts (as determined by the permutation test) are indicated by asterisks ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Chromosome arms are indicated by their Muller element nomenclature with the dot chromosome (Muller element F) omitted. Muller elements correspond to *D. willistoni* chromosome arms as follows: A=XL, B=2R, C=2L, D=XR, E=3.


01 BWA/DESeq
02 BWA/edgeR
03 BWA/DESeq
04 BWA/edgeR
05 BWA/DESeq
06 BWA/edgeR
number of genes
Muller element
A
B
C
D
E

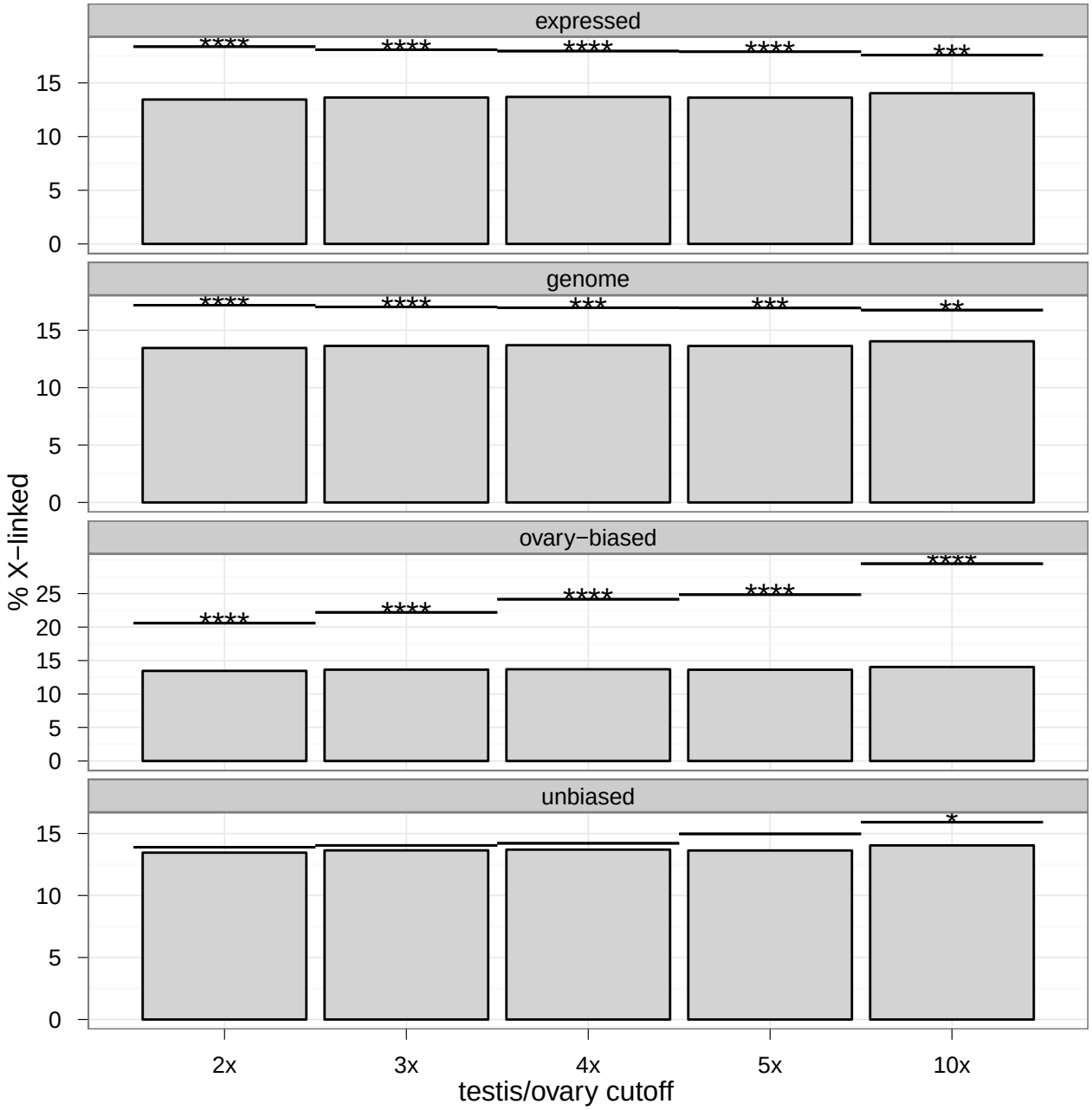
Supplementary Figure 3: Percent of genes with sex-biased expression on each chromosome arm in both larval and adult *D. melanogaster*

The percent of genes on each chromosome arm that have female-biased (pink), male-biased (blue), or unbiased (gray) expression (with an FDR cutoff of 0.05) in both larval and adult *D. melanogaster* are indicated by the height of the bars. Genes with sex-biased expression in adult were determined based on Ayroles et al. (2009), a meta-analysis of multiple microarray data sets (Gnad and Parsch, 2006), or our RNA-seq data (and corrected for an FDR of 0.05). Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using BWA, and tests for differential expression between males and females were carried out using edgeR. The 95% confidence interval of the expected percent of genes in each sex-bias class on each chromosome arm (determined by a permutation test based on the number of sex-biased genes in the genome and the relative sizes of the chromosome arms) are indicated by error bars. The expected percent of genes in each sex-bias class on each chromosome arm, indicated by a dashed line, was determined based on the percent of the entire genome in each sex-biased class. Significant deviations between observed and expected counts (as determined by the permutation test) are indicated by asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


Ayroles
meta−analysis
RNA−seq
female
male
unbiased
% of chromosome arm
chromosome arm
X
2L
2R
3L
3R

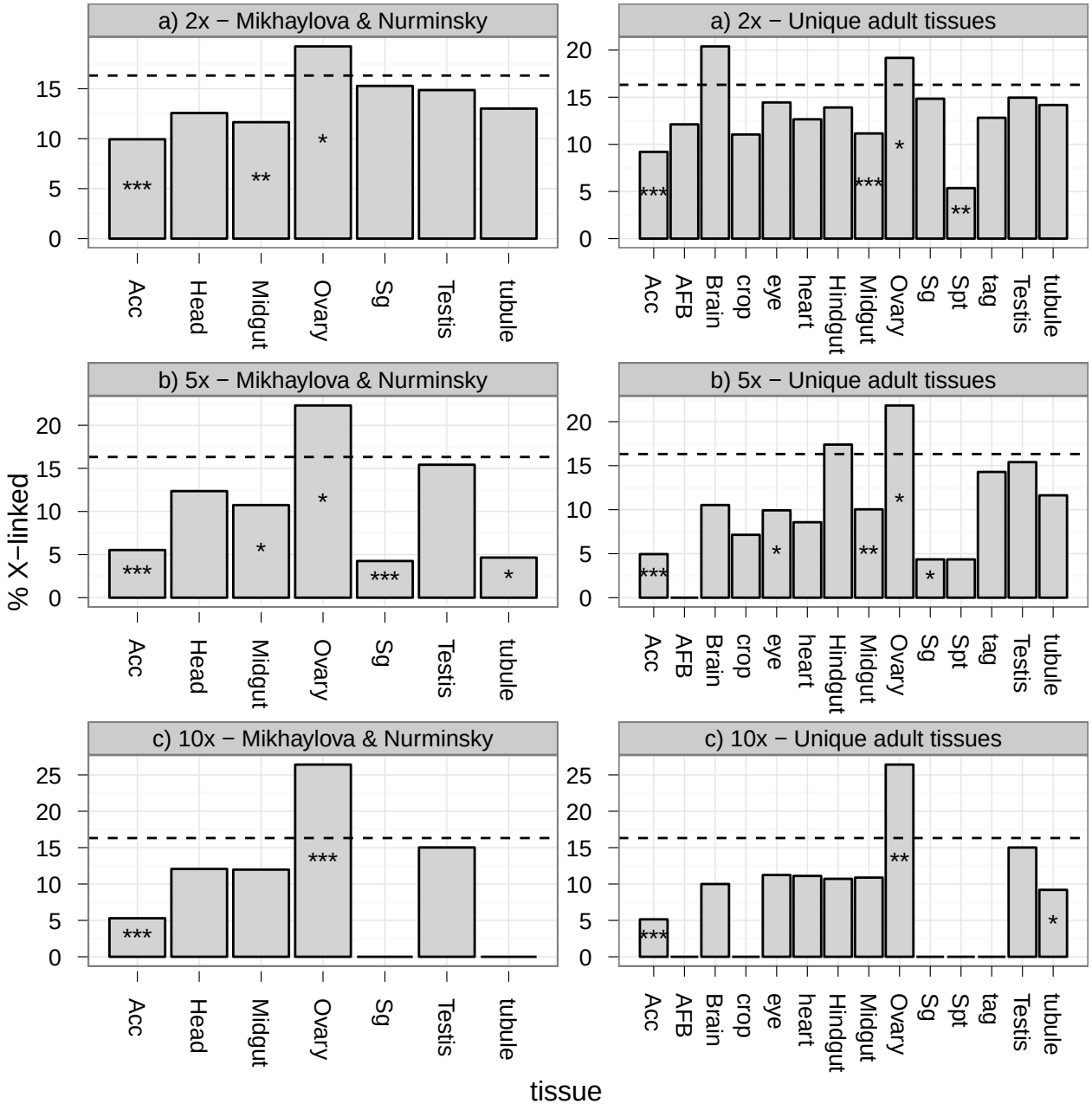
Supplementary Figure 4: Testis-enriched expression and X-linkage in *Drosophila*

D. melanogaster genes with testis-enriched expression were determined based on the relative expression between testis and ovary at 5 different fold-change cutoffs (two-fold, three-fold, four-fold, five-fold, and ten-fold). Barplots show the percent of genes with testis-enriched expression that are X-linked, with the x-axis indicating the testis/ovary fold-change cutoff. Horizontal lines represent the percent of genes in the control group that are X-linked, and asterisks indicate significant differences between the frequency of X-linked genes with testis-enriched expression and X-linked genes in the control group determined by Fisher's exact test ($^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$, $^{****}P < 0.00005$). Four control groups were used: all genes whose expression was measured in either testis or ovary in all four replicates (expressed), all genes in the genome (genome), genes with ovary-enriched expression (ovary-biased), and all genes expressed in testis or ovary but without testis- or ovary-enriched expression (unbiased).


expressed
genome
ovary−biased
unbiased
% X−linked
testis/ovary cutoff
2x
3x
4x
5x
10x

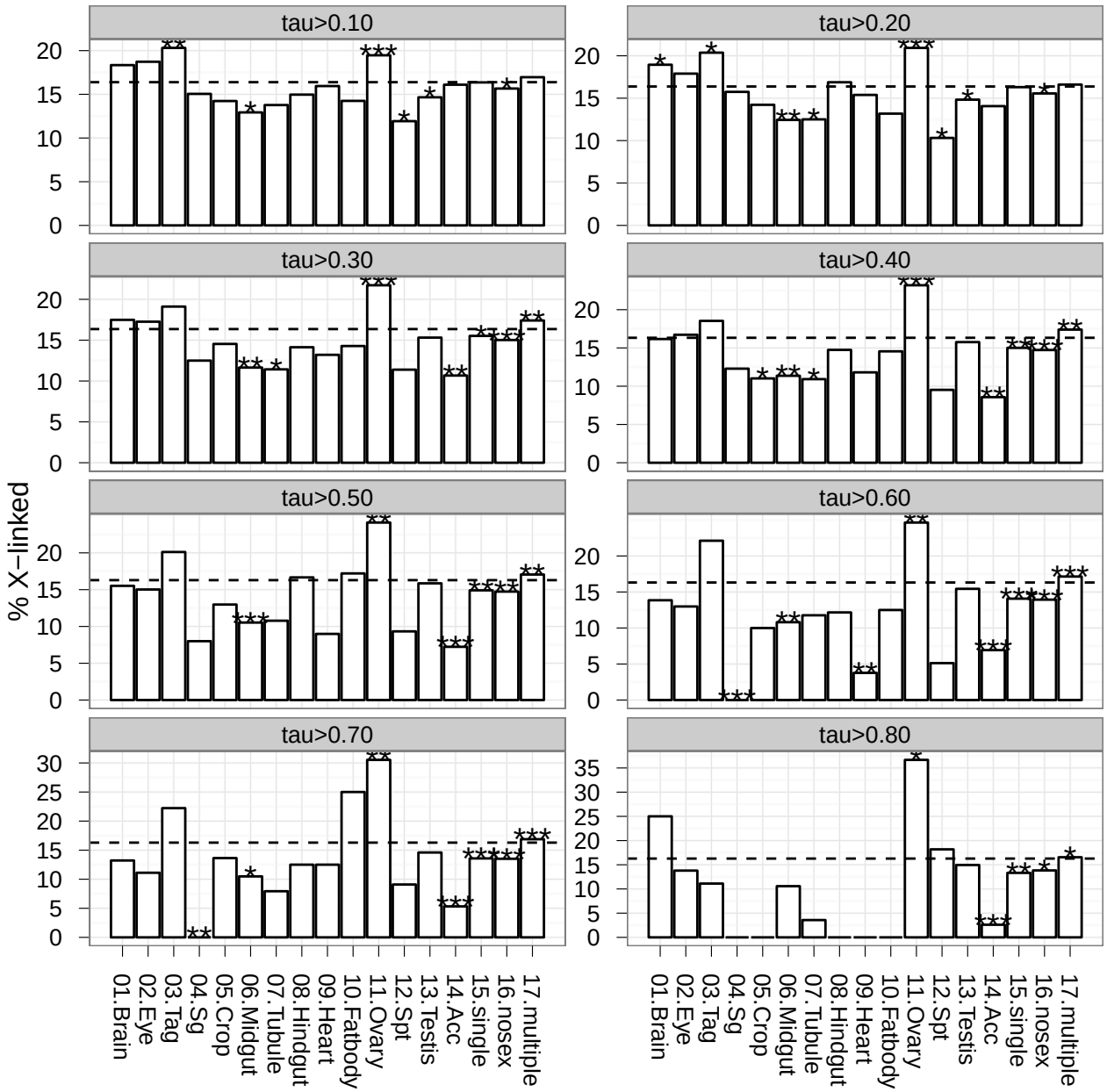
Supplementary Figure 5: Expression breadth and X-linkage in *Drosophila* using Mikhaylova and Nurminsky's method

Narrowly expressed *D. melanogaster* genes were identified using the method of Mikhaylova and Nurminsky (2011) and data from FlyAtlas (Chintapalli et al., 2007). Three cutoffs of the expression in the focal tissue to expression in other tissues were used: (a) two-fold, (b) five-fold, (c) ten-fold. Barplots show the percent of genes that are narrowly expressed in either the tissues used by Mikhaylova and Nurminksy (left column) or 14 unique adult tissues (right column) (Acc: accessory gland; Sg: salivary gland; AFB: adult fat body; Spt: spermatheca; tag: thoracicoabdominal ganglion). The dashed lines indicate the percent of the entire genome that is X-linked. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


a) 2x − Mikhaylova & Nurminsky
a) 2x − Unique adult tissues
b) 5x − Mikhaylova & Nurminsky
b) 5x − Unique adult tissues
c) 10x − Mikhaylova & Nurminsky
c) 10x − Unique adult tissues
% X−linked
tissue
Acc
AFB
Brain
crop
eye
Head
heart
Hindgut
Midgut
Ovary
Sg
Spt
tag
Testis
tubule

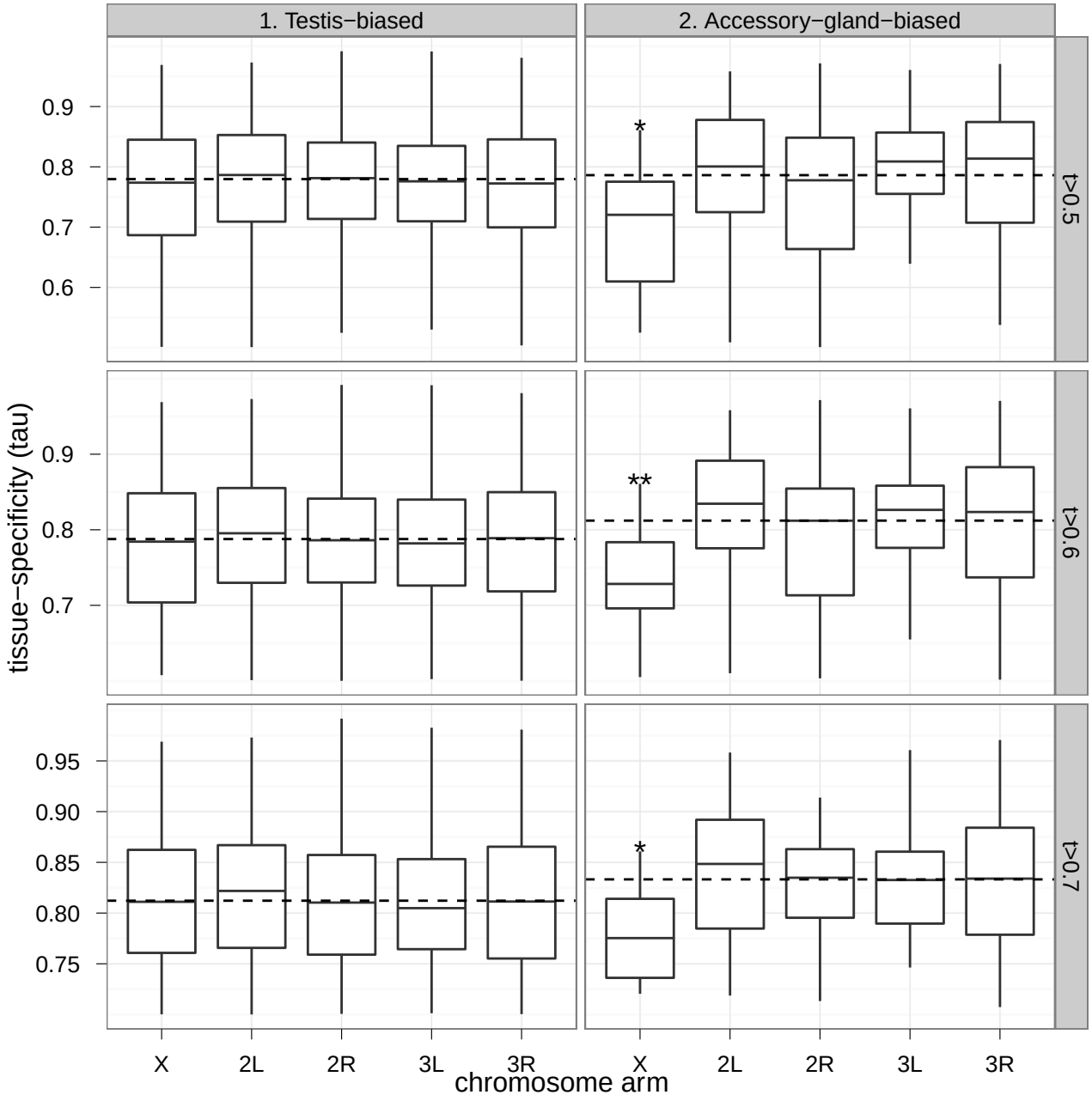
Supplementary Figure 6: Expression breadth and X-linkage in *Drosophila* using τ

Narrowly and broadly expressed *D. melanogaster* genes were identified using various τ cutoffs (Acc: accessory gland; Sg: salivary gland; AFB: adult fat body; Spt: spermatheca; tag: thoracicoabdominal ganglion). Barplots show the percent of narrowly- (1-16) and broadly- (17) expressed genes genes that are X-linked. The dashed lines indicate the percent of the entire genome that is X-linked. Low τ cutoffs are better for inferring broadly expressed genes, while higher τ cutoffs are better for inferring narrowly expressed genes. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


tau>0.10
tau>0.20
tau>0.30
tau>0.40
tau>0.50
tau>0.60
tau>0.70
tau>0.80
% X−linked
01.Brain
02.Eye
03.Tag
04.Sg
05.Crop
06.Midgut
07.Tubule
08.Hindgut
09.Heart
10.Fatbody
11.Ovary
12.Spt
13.Testis
14.Acc
15.single
16.nosex
17.multiple

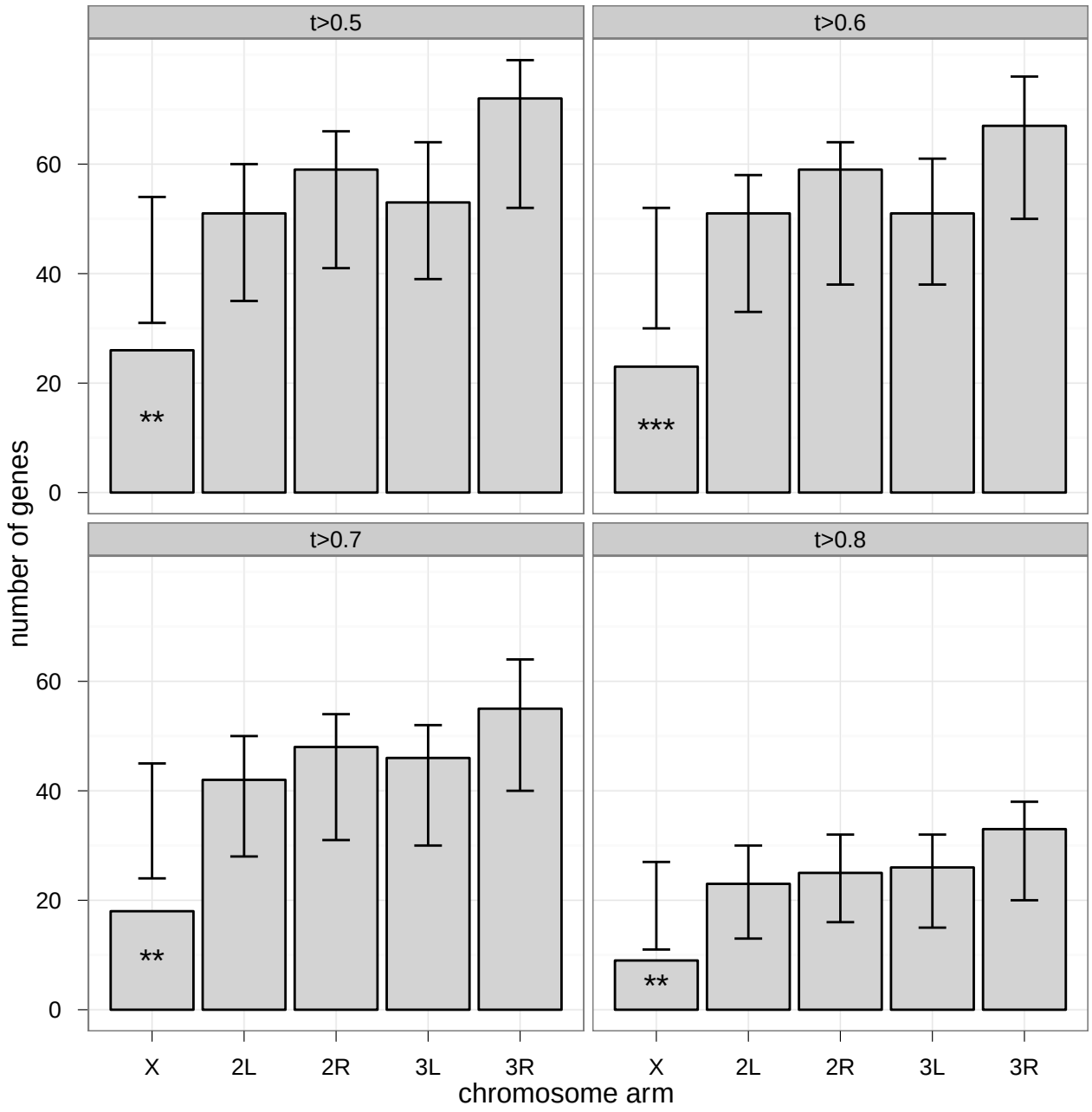
Supplementary Figure 7: Distribution of τ for genes with testis- and accessory-gland-biased expression

Boxplots show the distribution of τ for genes on each chromosome arm in *D. melanogaster* (chromosome 4 was omitted). Boxes indicate the interquartile range, the horizontal line in the middle of each box represents the median value, and the whiskers extend to 1.5x the interquartile range (outliers were omitted). The dashed line indicates the genome-wide average. Significant differences between τ for a given chromosome arm and the rest of the genome were assessed using a Mann-Whitney test ($^*P < 0.05$; $^{**}P < 0.005$). Distributions of τ were calculated for genes with testis-biased expression (left) and genes with accessory-gland-biased expression (right) using various τ cutoffs (listed along right side).


1. Testis−biased
2. Accessory−gland−biased
tissue−specificity (tau)
0.9
0.8
0.7
0.6
0.95
0.90
0.85
0.80
0.75
*
**
*
t>0.5
t>0.6
t>0.7
X
2L
2R
3L
3R
chromosome arm

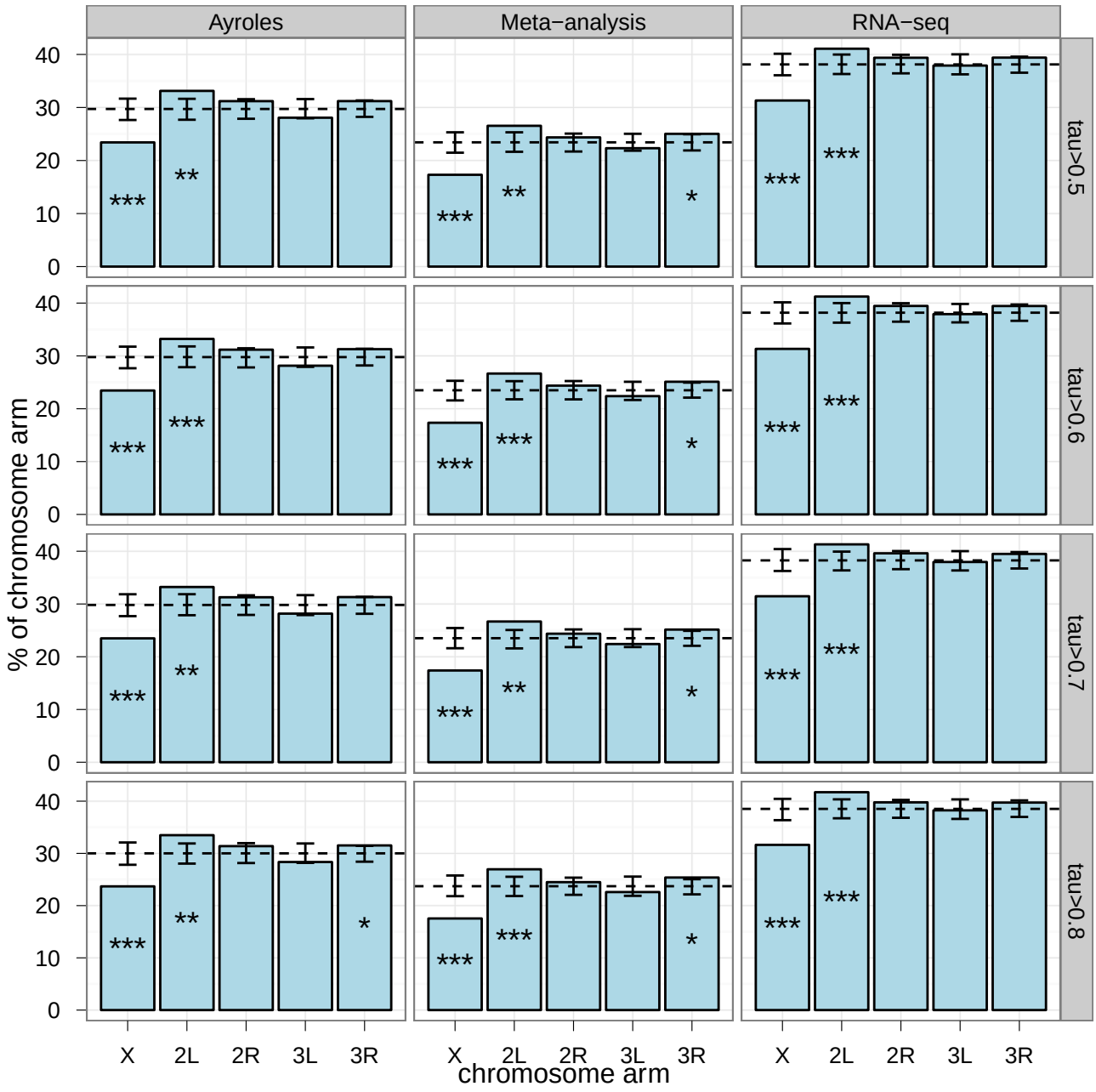
Supplementary Figure 8: Counts of genes on each *D. melanogaster* chromosome arm with with testis-biased expression that encode components of the sperm proteome

The number of genes on each chromosome arm that have testis-biased expression and encode a component of the sperm proteome are indicated by the height of the bars. Testis-biased expression was determined using four different τ cutoffs, and genes were said to encode a component of the sperm proteome if they were detected in either SPI (Dorus et al., 2006) or SPII (Wasbrough et al., 2010). The 95% confidence intervals of the expected number of genes on each chromosome arm (determined by a permutation test based on the relative sizes of the chromosome arms) are indicated by error bars. Significant deviations between observed and expected counts (as determined by the permutation test) are indicated by asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


t>0.5
t>0.6
t>0.7
t>0.8
number of genes
0
20
40
60
**
***
**
**
X
2L
2R
3L
3R
chromosome arm

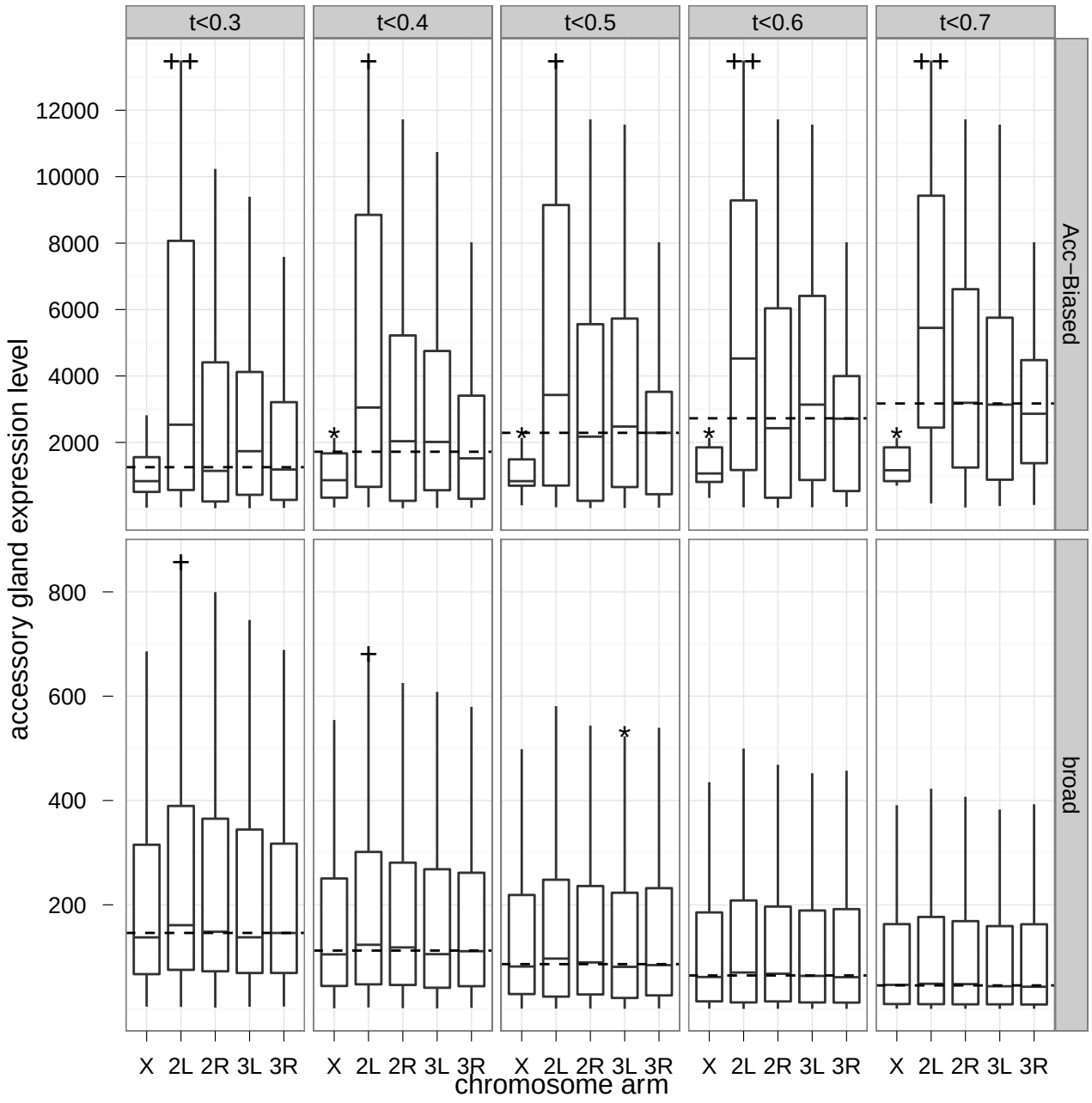
Supplementary Figure 9: Percent of genes with male-biased expression that do not have accessory-gland-biased expression on each *D. melanogaster* chromosome arm

The percent of genes on each chromosome arm that have male-biased expression but do not have accessory-gland-biased expression in *D. melanogaster* are indicated by the height of the bars. Genes with sex-biased expression were determined based on Ayroles et al. (2009), a meta-analysis of multiple microarray data sets (Gnad and Parsch, 2006), or our RNA-seq data (and corrected for an FDR of 0.05). Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using BWA, and tests for differential expression between males and females were carried out using edgeR. Accessory-gland-biased expression was determined using four different τ cutoffs (indicated along the right side of each rown). The 95% confidence interval of the expected percent of genes in each sex-bias class on each chromosome arm (determined by a permutation test based on the relative sizes of the chromosome arms) are indicated by error bars. The expected percent of genes in each sex-bias class on each chromosome arm, indicated by a dashed line, was determined based on the percent of the entire genome in each sex-biased class. Significant deviations between observed and expected counts (as determined by the permutation test) are indicated by asterisks ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


Ayroles
Meta−analysis
RNA−seq
tau>0.5
tau>0.6
tau>0.7
tau>0.8
% of chromosome arm
chromosome arm
X
2L
2R
3L
3R
0
10
20
30
40

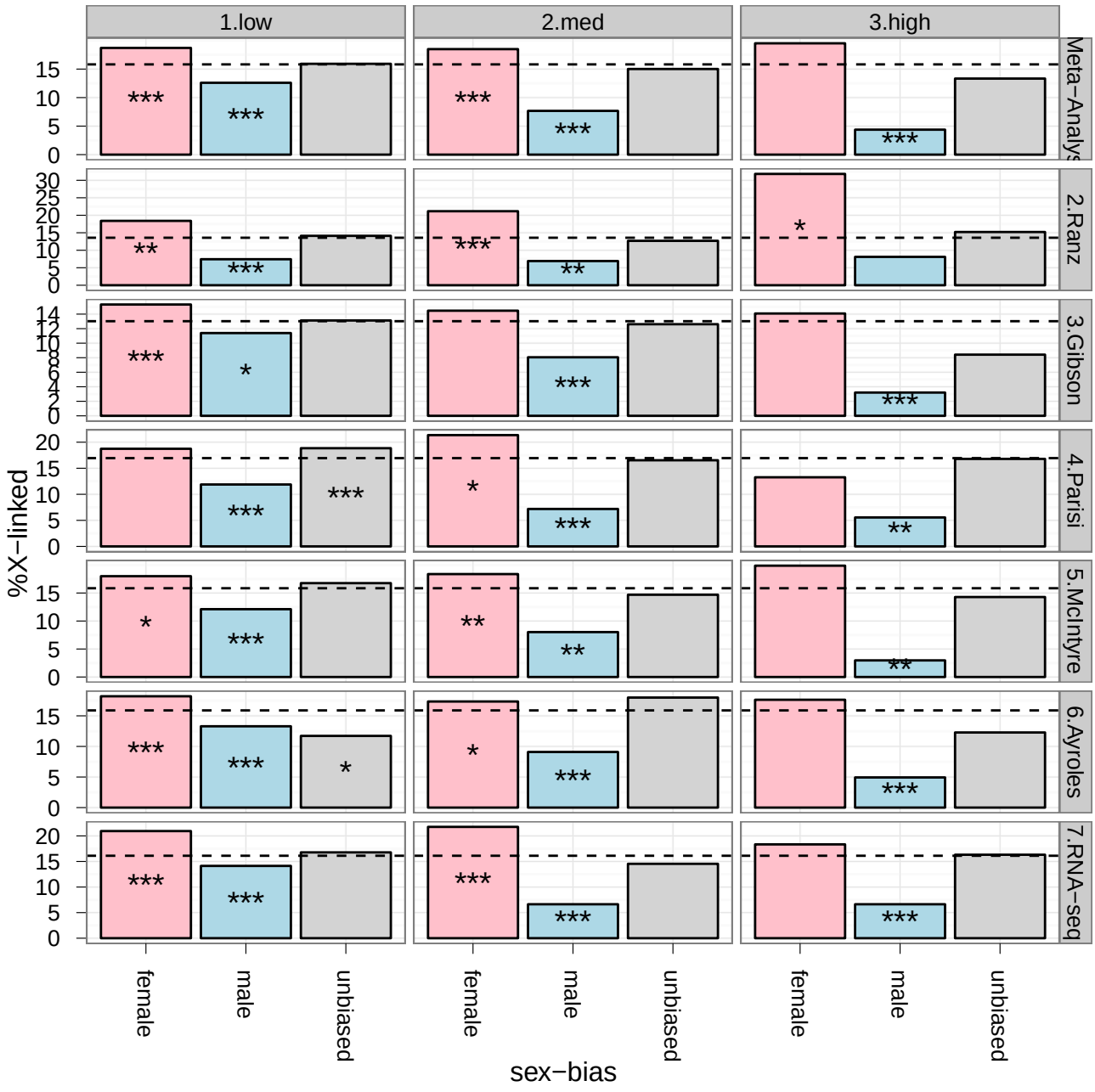
Supplementary Figure 10: Accessory-gland expression level on each chromosome arm

Boxplots show the distribution of the accessory gland expression level for genes on each chromosome arm in *D. melanogaster* (chromosome 4 was omitted). Boxes indicate the interquartile range, the horizontal line in the middle of each box represents the median value, and the whiskers extend to 1.5x the interquartile range (outliers were omitted). Genes were divided into those with accessory-gland-biased expression or those that are broadly expressed using the τ cutoffs indicated along the top of each column. Significant differences between the expression level for a given chromosome and the rest of the genome were assessed using a Mann-Whitney test; asterisks indicate that the expression level on a chromosome is significantly lower than the genome-wide average ($^*P < 0.05$), and crosses indicate the expression level on a chromosome is signifantly greater ($+P < 0.05$; $++P < 0.005$).


t<0.3
t<0.4
t<0.5
t<0.6
t<0.7
Acc−Biased
broad
accessory gland expression level
12000
10000
8000
6000
4000
2000
800
600
400
200
X 2L 2R 3L 3R
chromosome arm

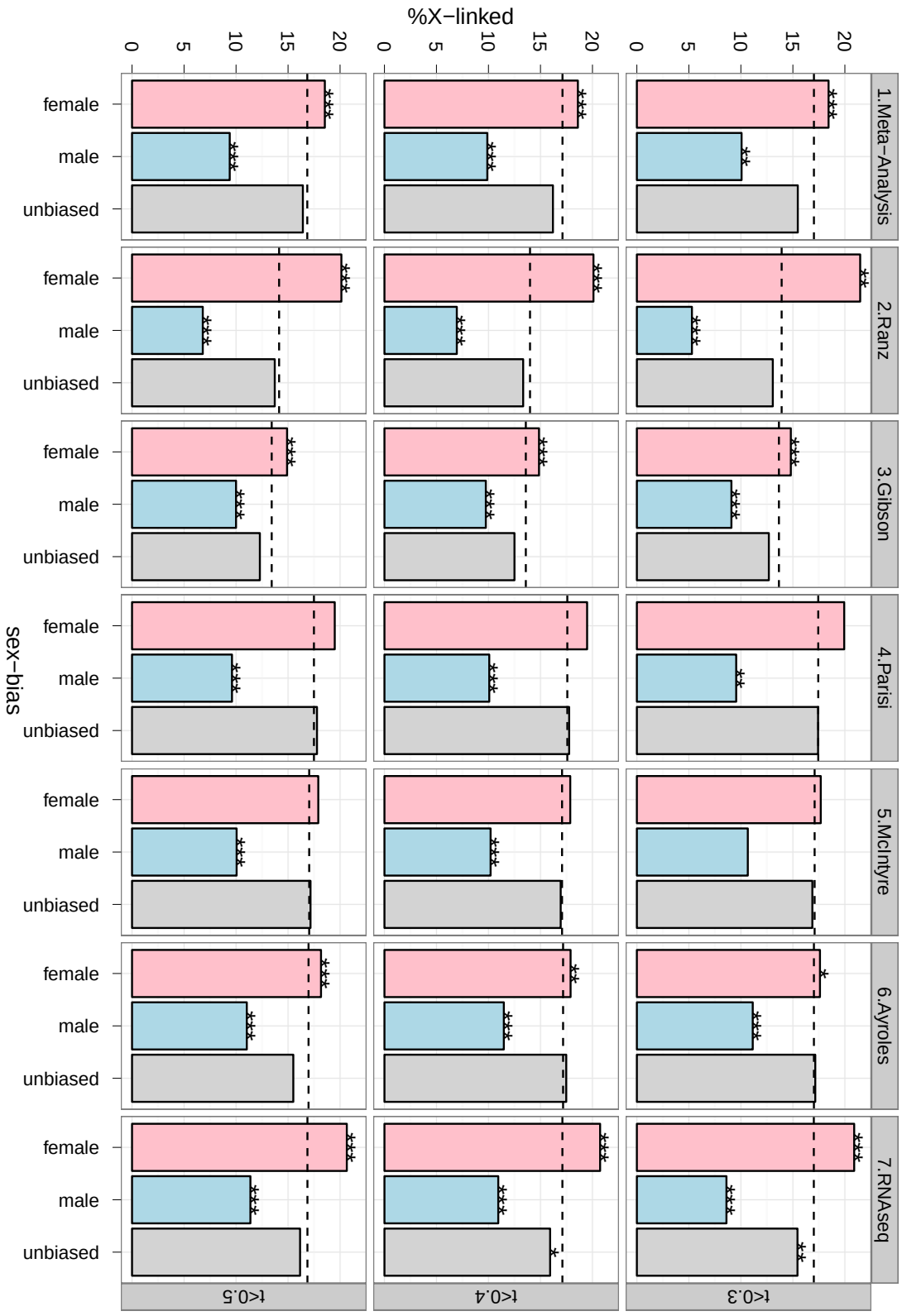
Supplementary Figure 11: Accessory gland expression, sex-bias, and X-linkage

D. melanogaster genes were divided into those with low expression in accessory gland ($S < 100$), moderate expression in accessory gland ($100 \leq S < 1000$), and highly expressed in accessory gland ($S \geq 1000$). Genes with sex-biased expression were identified using seven different data sets (labeled along the right side of the rows) and an FDR cutoff of 0.05. Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using BWA, and tests for differential expression between males and females were carried out using edgeR. Within each panel is shown the percent of genes with female-biased (pink), male-biased (blue), and unbiased (gray) expression that are X-linked. The dashed lines indicate the percent of the entire genome that is X-linked. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


1.low
2.med
3.high
Meta−Analys
2.Ranz
3.Gibson
4.Parisi
5.McIntyre
6.Ayroles
7.RNA−seq
%X−linked
sex−bias
female
male
unbiased

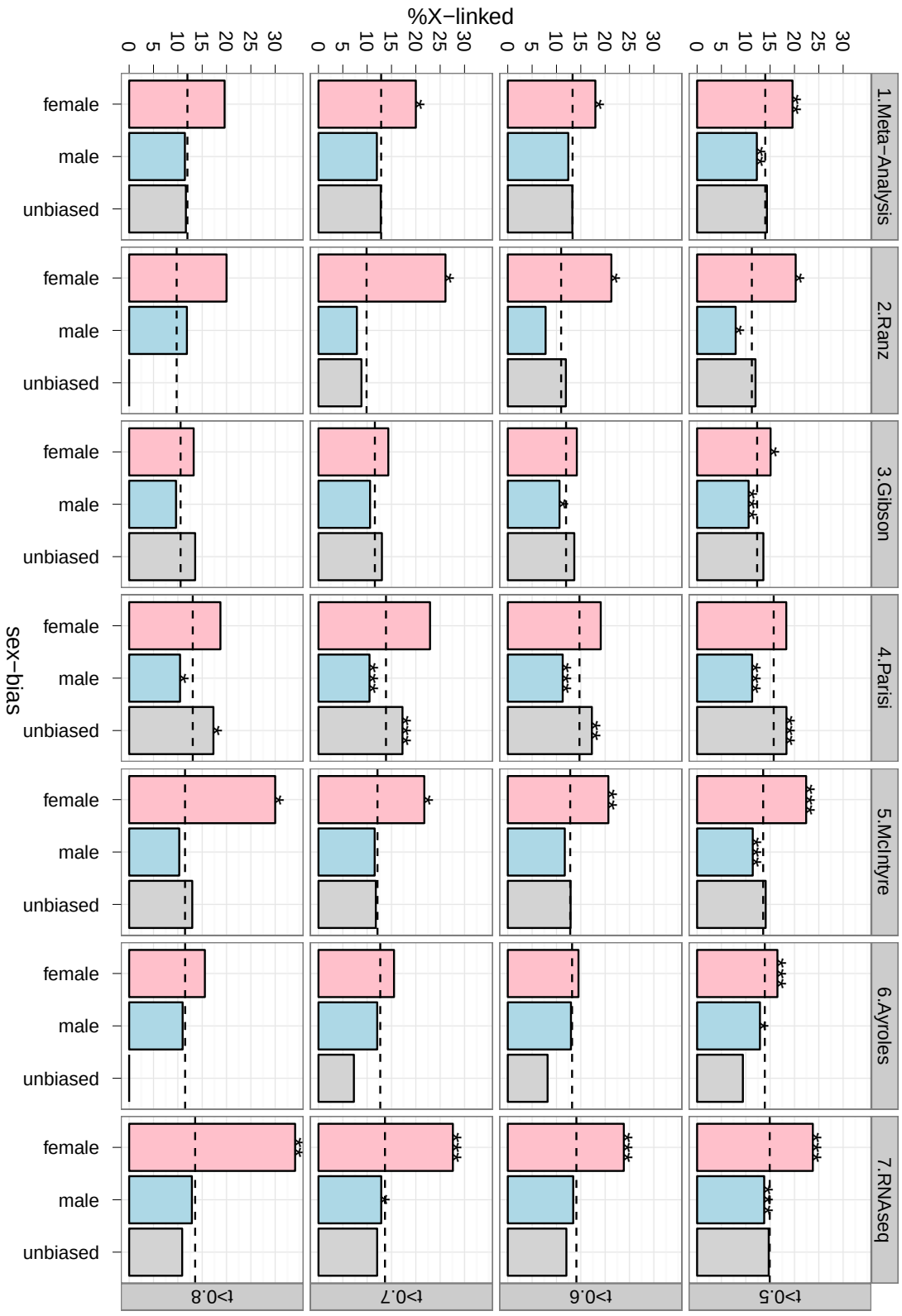
Supplementary Figure 12: X-linkage of *Drosophila* genes with sex-biased expression that are broadly expressed

Barplots show the percent of broadly expressed genes with female- (pink), male- (blue), and un- (gray) biased expression that are X-linked. Broadly expressed *D. melanogaster* genes were identified using various τ cutoffs (labeled along the right side of the rows), and genes with sex-biased expression were identified using seven different data sets (labeled along the columns) and an FDR cutoff of 0.05. Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using BWA, and tests for differential expression between males and females were carried out using edgeR. The dashed lines indicate the percent of the entire genome that is X-linked. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


%X−linked
0
5
10
15
20
sex−bias
female
male
unbiased
1.Meta−Analysis
2.Ranz
3.Gibson
4.Parisi
5.McIntyre
6.Ayroles
7.RNAseq
t<0.3
t<0.4
t<0.5

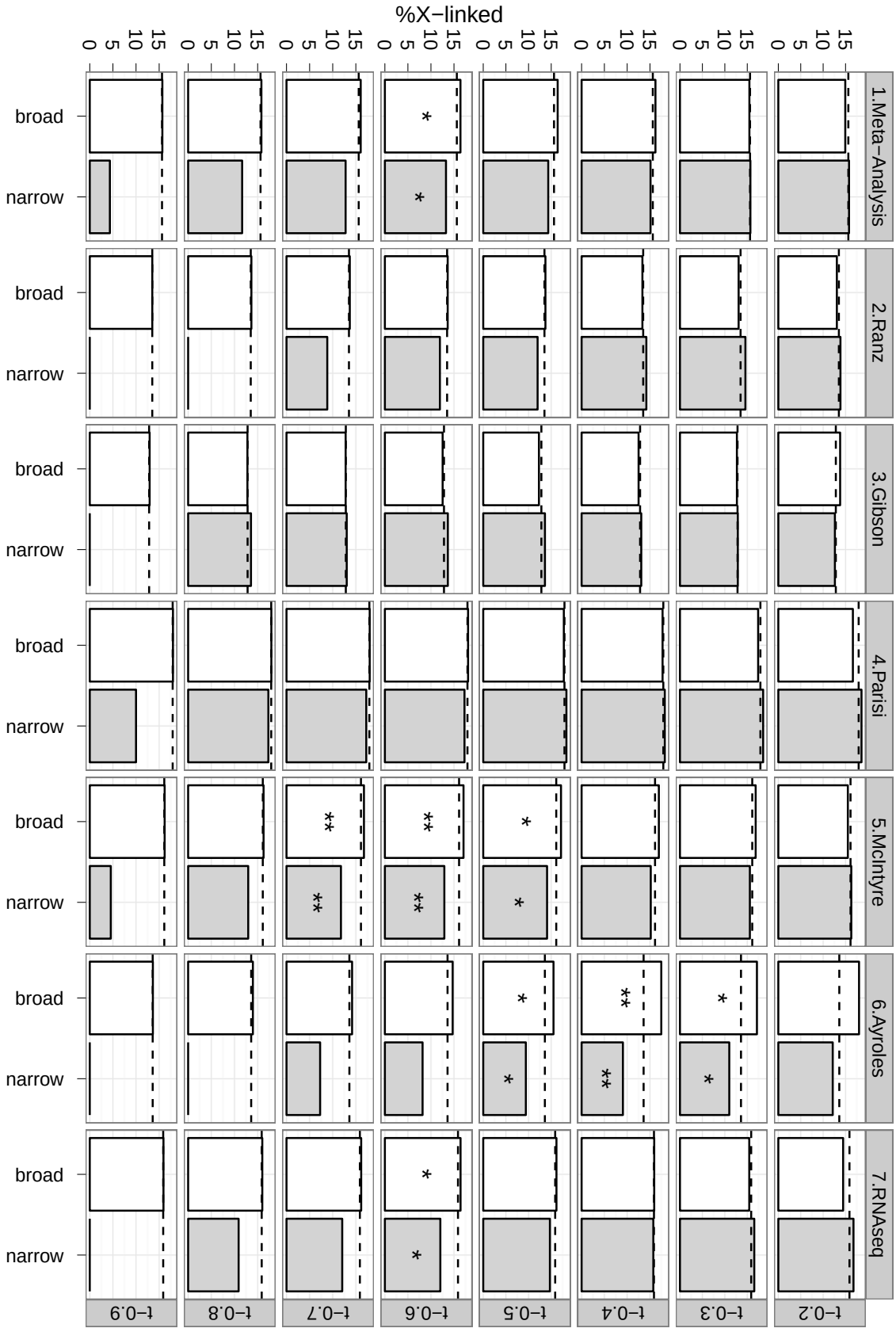
Supplementary Figure 13: X-linkage of *Drosophila* genes with sex-biased expression that are narrowly expressed

Barplots show the percent of narrowly expressed genes with female- (pink), male- (blue), and un- (gray) biased expression that are X-linked. Narrowly expressed *D. melanogaster* genes were identified using various τ cutoffs (labeled along the right side of the rows), and genes with sex-biased expression were identified using seven different data sets (labeled along the columns) and an FDR cutoff of 0.05. Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using BWA, and tests for differential expression between males and females were carried out using edgeR. The dashed lines indicate the percent of the entire genome that is X-linked. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


%X−linked
sex−bias
female
male
unbiased
1.Meta−Analysis
2.Ranz
3.Gibson
4.Parisi
5.McIntyre
6.Ayroles
7.RNAseq
t>0.5
t>0.6
t>0.7
t>0.8

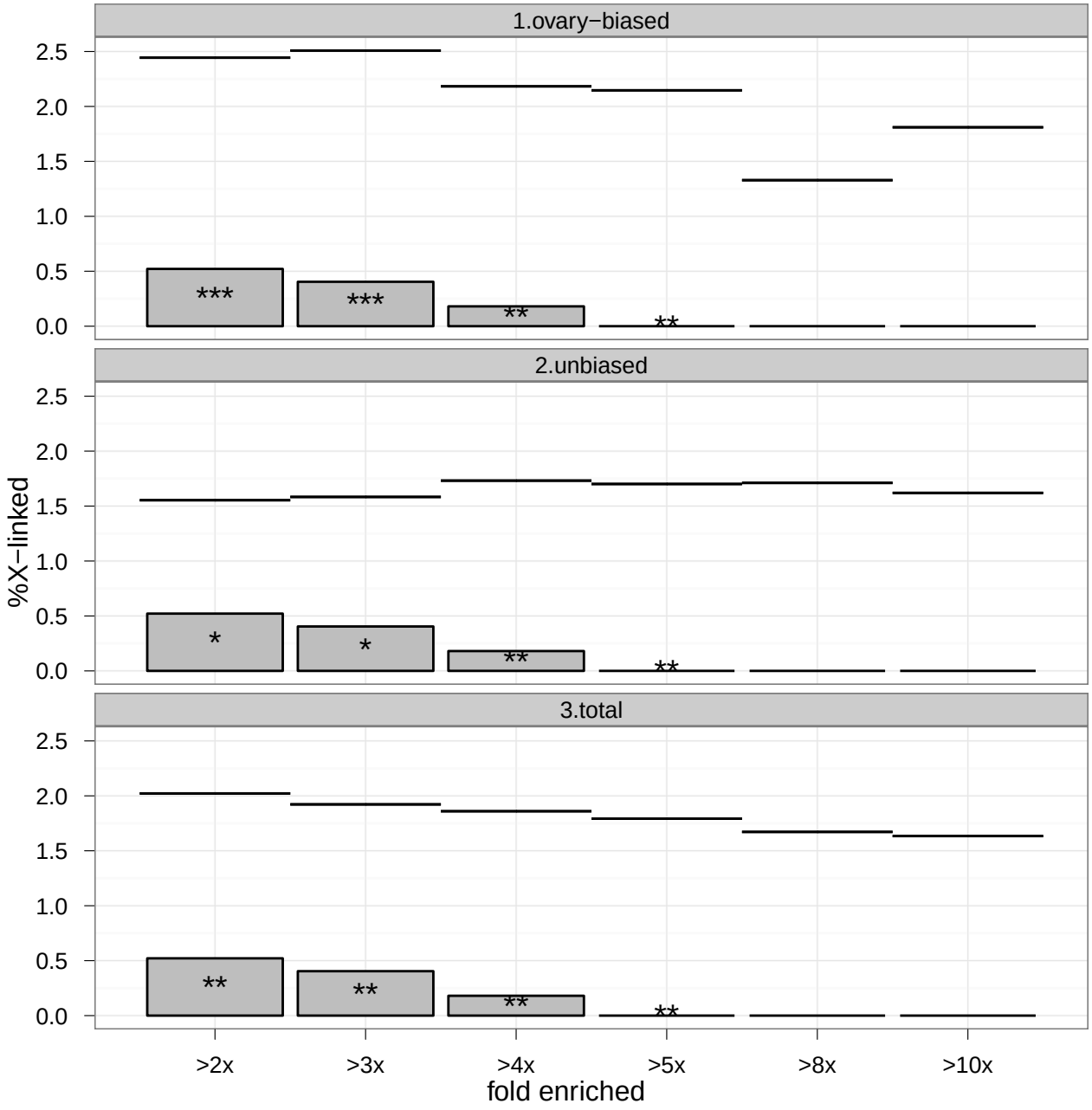
Supplementary Figure 14: X-linkage of broadly- and narrowly-expressed *Drosophila* genes without sex-biased expression

Barplots show the percent of broadly (white) and narrowly (gray) expressed genes with non-sex-biased expression that are X-linked. Broadly and narrowly expressed *D. melanogaster* genes were identified using various τ cutoffs (labeled along the right side of the rows), and genes with non-sex-biased expression were identified using seven different data sets (labeled along the top of the columns) and an FDR cutoff of 0.05. Alignments of the RNA-seq reads to the annotated protein-coding transcriptome were performed using BWA, and tests for differential expression between males and females were carried out using edgeR. The dashed lines indicate the percent of the entire genome that is X-linked. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


%X−linked
1.Meta−Analysis
2.Ranz
3.Gibson
4.Parisi
5.McIntyre
6.Ayroles
7.RNAseq
t−0.2
t−0.3
t−0.4
t−0.5
t−0.6
t−0.7
t−0.8
t−0.9
broad
narrow
0
5
10
15

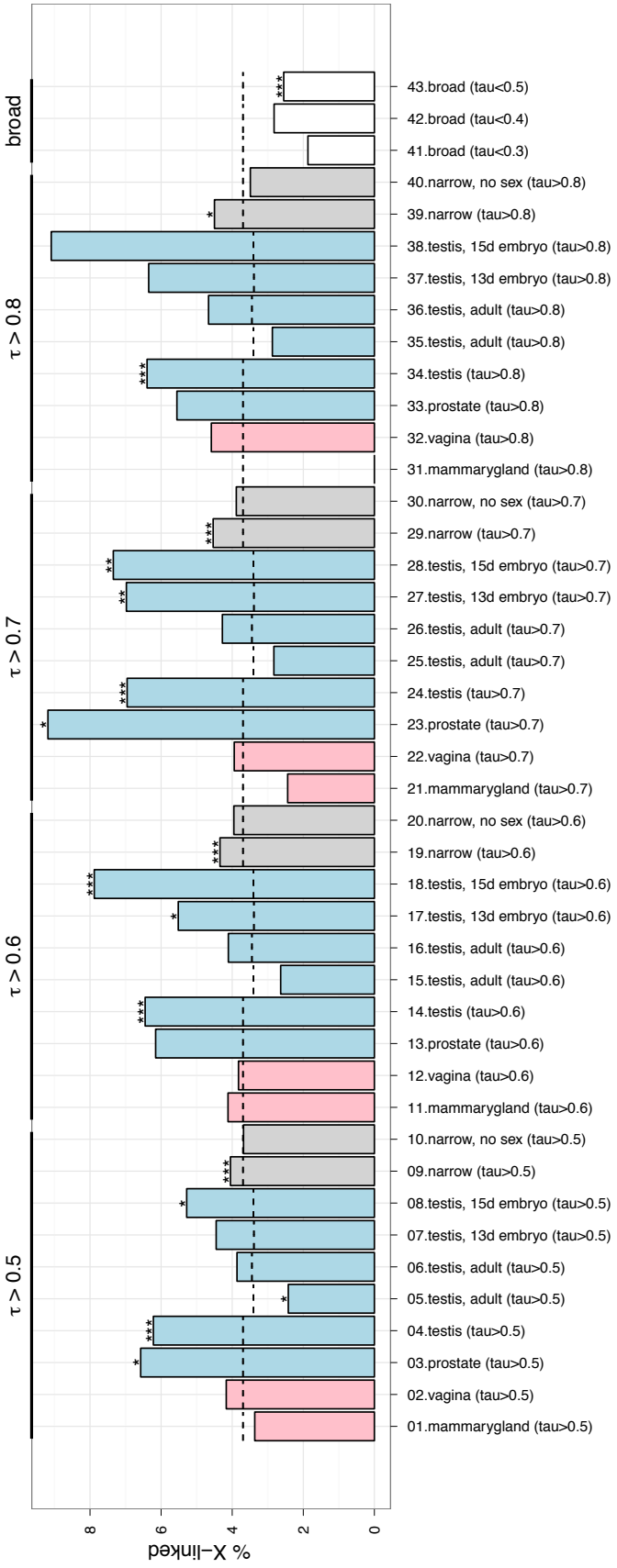
Supplementary Figure 15: Testis-enriched expression and X-linkage in mouse

Mouse genes with testis-enriched expression were identified based on the relative expression between adult testis and unfertilized ovary at 6 different fold-change cutoffs (two-fold, three-fold, four-fold, five-fold, eight-fold, and ten-fold). Barplots show the percent of genes with testis-enriched expression that are X-linked, with the X-axis indicating the testis/ovary fold-change cutoff. Horizontal lines represent the percent of genes in the control group that are X-linked, and asterisks indicate significant differences between the frequency of X-linked genes with testis-enriched expression and the control group (determined by Fisher's exact test; $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$). Three control groups were used: genes with ovary-enriched expression at the same fold cutoff (ovary-biased), all genes expressed in testis or ovary but without testis- or ovary-enriched expression (unbiased), and all genes expressed in testis or ovary but without testis-enriched expression (total). Genes were included in the analysis if they had at least five mapped ESTs in the testis and ovary libraries.


1.ovary−biased
2.unbiased
3.total
%X−linked
2.5
2.0
1.5
1.0
0.5
0.0
***
***
**
**
*
*
**
**
**
**
**
**
>2x
>3x
>4x
>5x
>8x
>10x
fold enriched

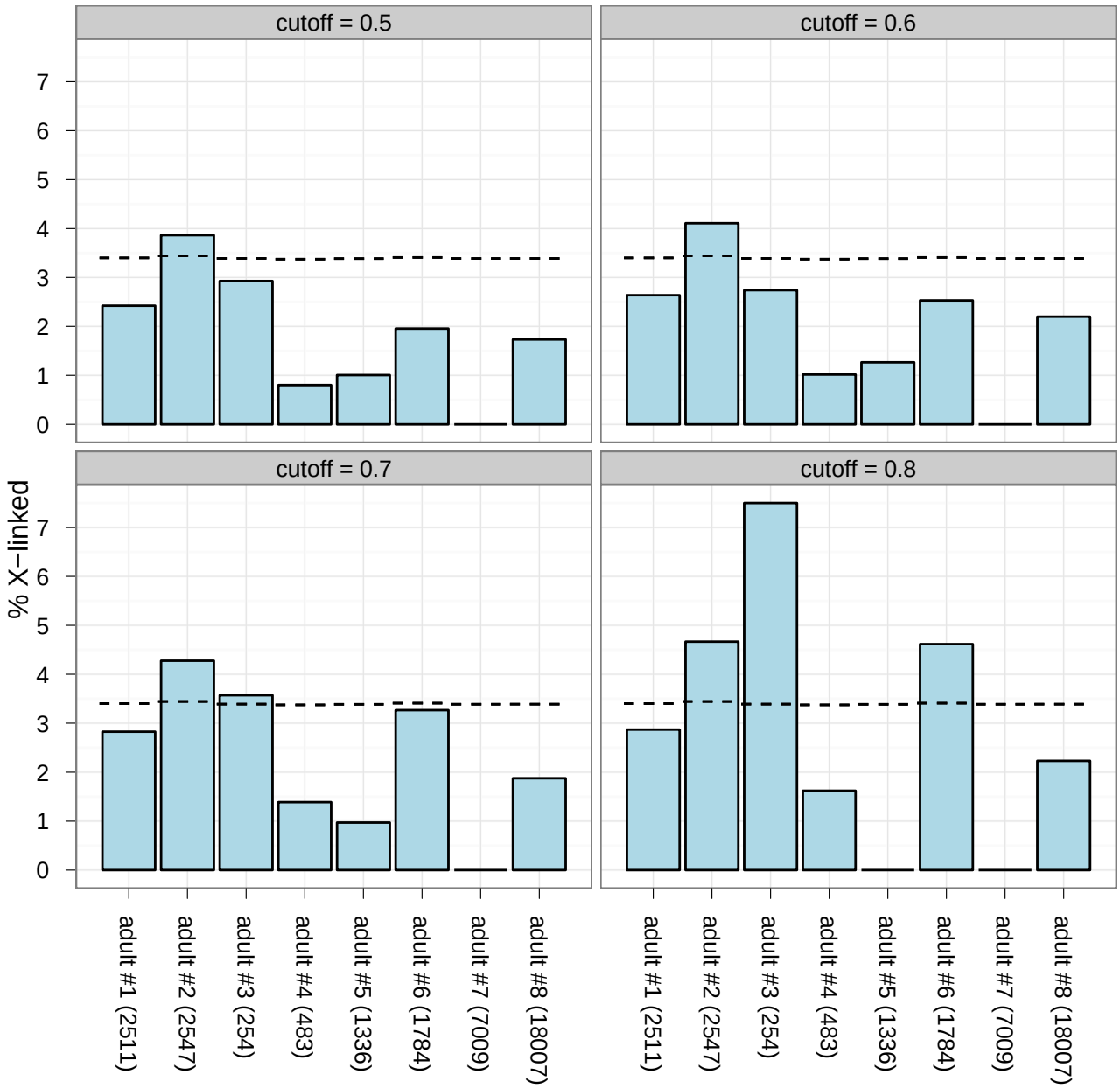
Supplementary Figure 16: Expression breadth and X-linkage in mouse

Barplots show the percent of mouse genes with narrow expression in each of four sex-limited tissues (pink and blue bars), across all 25 tissues (gray bars), across 21 non-sex-limited tissues (gray bars), and broadly expressed genes (white bars) that are X-linked. Six different τ cutoffs were used to assign genes to expression breadth classes; cutoffs used for narrowly expressed genes are indicated along the top of the graph and in the parentheses, while those for the broadly expressed genes are in parentheses. Genes with testis-biased expression were identified using all testis EST libraries, EST libraries from adult testis, or EST libraries from embryonic testis. The dashed lines indicate the percent of the entire genome that is X-linked. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).


broad
τ > 0.8
τ > 0.7
τ > 0.6
τ > 0.5
43.broad (tau<0.5)
42.broad (tau<0.4)
41.broad (tau<0.3)
40.narrow, no sex (tau>0.8)
39.narrow (tau>0.8)
38.testis, 15d embryo (tau>0.8)
37.testis, 13d embryo (tau>0.8)
36.testis, adult (tau>0.8)
35.testis, adult (tau>0.8)
34.testis (tau>0.8)
33.prostate (tau>0.8)
32.vagina (tau>0.8)
31.mammarygland (tau>0.8)
30.narrow, no sex (tau>0.7)
29.narrow (tau>0.7)
28.testis, 15d embryo (tau>0.7)
27.testis, 13d embryo (tau>0.7)
26.testis, adult (tau>0.7)
25.testis, adult (tau>0.7)
24.testis (tau>0.7)
23.prostate (tau>0.7)
22.vagina (tau>0.7)
21.mammarygland (tau>0.7)
20.narrow, no sex (tau>0.6)
19.narrow (tau>0.6)
18.testis, 15d embryo (tau>0.6)
17.testis, 13d embryo (tau>0.6)
16.testis, adult (tau>0.6)
15.testis, adult (tau>0.6)
14.testis (tau>0.6)
13.prostate (tau>0.6)
12.vagina (tau>0.6)
11.mammarygland (tau>0.6)
10.narrow, no sex (tau>0.5)
09.narrow (tau>0.5)
08.testis, 15d embryo (tau>0.5)
07.testis, 13d embryo (tau>0.5)
06.testis, adult (tau>0.5)
05.testis, adult (tau>0.5)
04.testis (tau>0.5)
03.prostate (tau>0.5)
02.vagina (tau>0.5)
01.mammarygland (tau>0.5)
% X−linked
0
2
4
6
8

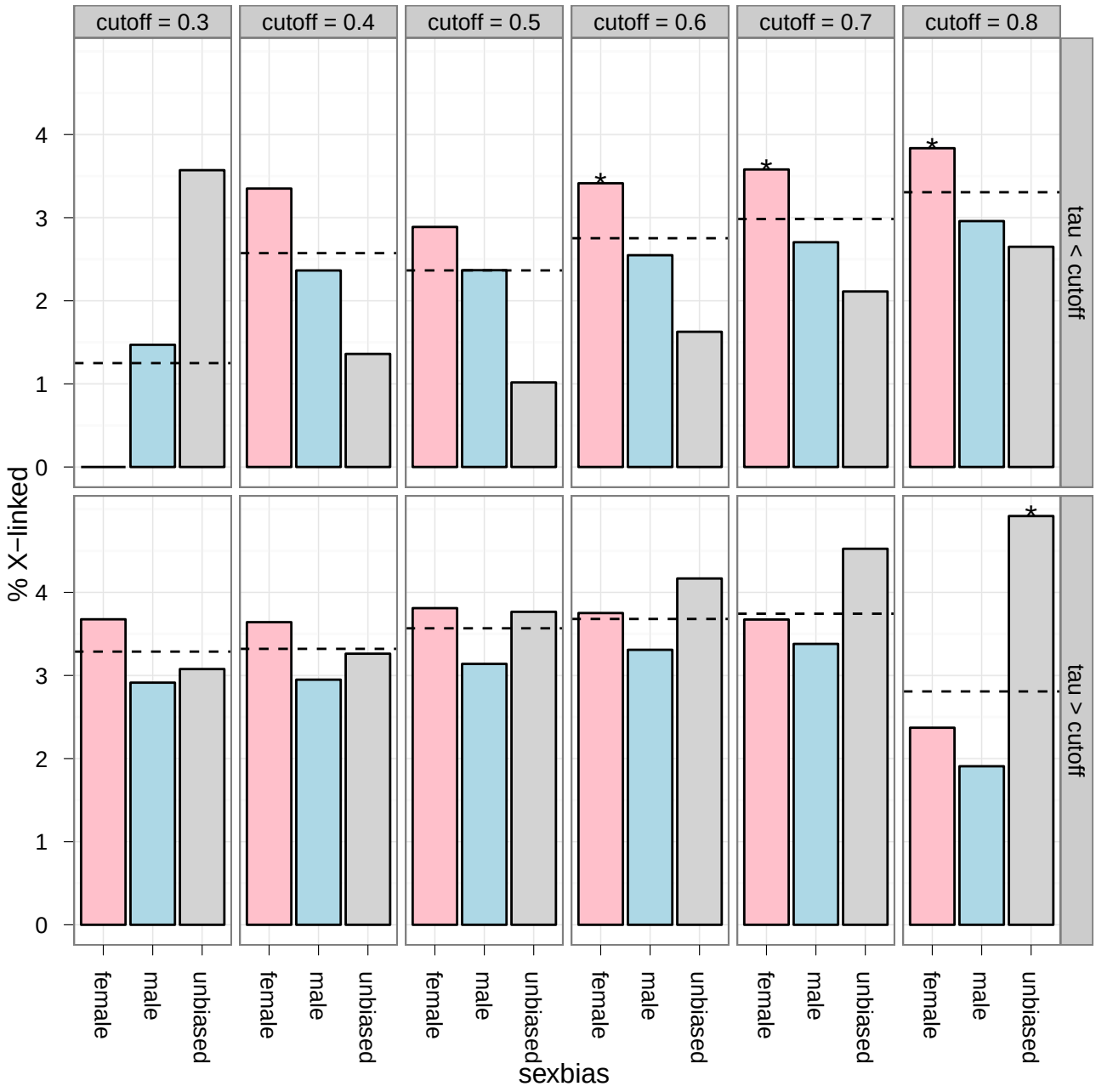
Supplementary Figure 17: Testis-biased expression and X-linkage in adult mouse testis

Barplots show the percent of mouse genes with biased expression in adult testis, determined using eight different EST libraries (UniGene IDs are in parentheses), that are X-linked. Four different τ cutoffs were used to identify genes with testis-biased expression (indicated at the top of each panel). The dashed lines indicate the percent of the entire genome that is X-linked.


cutoff = 0.5
cutoff = 0.6
cutoff = 0.7
cutoff = 0.8
% X−linked
0
1
2
3
4
5
6
7
adult #1 (2511)
adult #2 (2547)
adult #3 (254)
adult #4 (483)
adult #5 (1336)
adult #6 (1784)
adult #7 (7009)
adult #8 (18007)

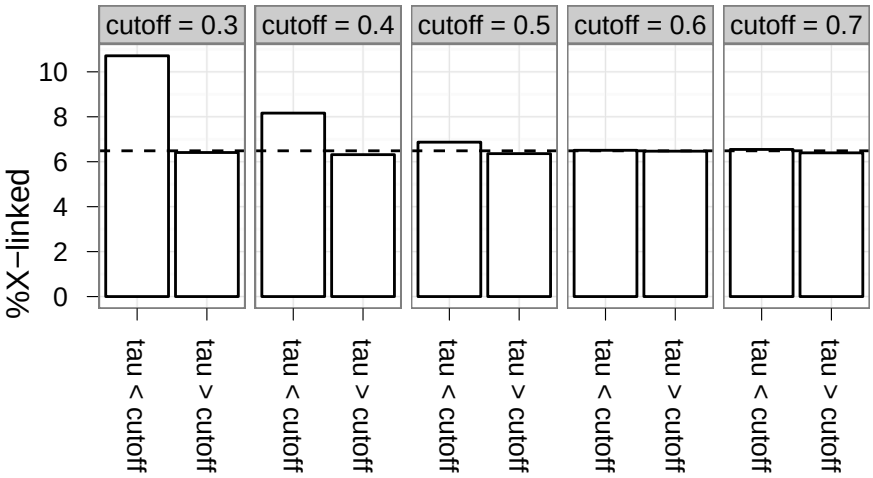
Supplementary Figure 18: X-linkage, sex-bias, and expression breadth of mouse genes

Barplots show the percent of broadly (top row) or narrowly (bottom row) expressed mouse genes with female- (pink), male- (blue), or un- (gray) biased expression that are X-linked. The dashed lines indicate the percent of all broadly/narrowly expressed genes that are X-linked. The asterisks show observed values that significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test ($^*P < 0.05$). Various τ cutoffs, given at the top of each column, were used to assign genes to expression breadth classes. Lower τ cutoffs are good for identifying genes that are broadly expressed, while higher cutoffs are better for identifying genes that are narrowly expressed. Genes whose expression breadth falls below a high τ cutoff are not narrowly expressed, while those whose expression breadth is above a low τ cutoff are not broadly expressed.


cutoff = 0.3
cutoff = 0.4
cutoff = 0.5
cutoff = 0.6
cutoff = 0.7
cutoff = 0.8
tau < cutoff
tau > cutoff
% X−linked
0
1
2
3
4
female
male
unbiased
sexbias

Supplementary Figure 19: X-linkage and expression breadth for mouse genes without sex-biased expression

Barplots show the percent of broadly or narrowly expressed mouse genes with non-sex-biased expression that are X-linked. The dashed lines indicate the percent of genes with non-sex-biased expression that are X-linked. Lower τ cutoffs are better for identifying broadly expressed genes ($\tau <$ cutoff), while higher τ cutoffs are better for identifying narrowly expressed genes ($\tau >$ cutoff). None of the observed values significantly differ from the expectation based on the size of the X chromosome as determined by a permutation test.


cutoff = 0.3
cutoff = 0.4
cutoff = 0.5
cutoff = 0.6
cutoff = 0.7
%X−linked
0
2
4
6
8
10
tau < cutoff
tau > cutoff

Supplementary Tables

Supplementary Table 1: Expression breadth and X-linkage of *D. melanogaster* genes without sex-biased expression

sex-bias	broad	narrow	X (broad)	A (broad)	X (narrow)	A (narrow)	P value
Meta-analysis	$\tau < 0.3$	$\tau \geq 0.6$	188	1027	119	778	0.16946
Meta-analysis	$\tau < 0.3$	$\tau \geq 0.7$	188	1027	61	416	0.17029
Meta-analysis	$\tau < 0.3$	$\tau \geq 0.8$	188	1027	19	144	0.24255
Meta-analysis	$\tau < 0.4$	$\tau \geq 0.6$	271	1402	119	778	0.04996
Meta-analysis	$\tau < 0.4$	$\tau \geq 0.7$	271	1402	61	416	0.07261
Meta-analysis	$\tau < 0.4$	$\tau \geq 0.8$	271	1402	19	144	0.14377
RNA-seq	$\tau < 0.3$	$\tau \geq 0.6$	389	2130	62	453	0.04874
RNA-seq	$\tau < 0.3$	$\tau \geq 0.7$	389	2130	34	248	0.13689
RNA-seq	$\tau < 0.3$	$\tau \geq 0.8$	389	2130	11	90	0.25874
RNA-seq	$\tau < 0.4$	$\tau \geq 0.6$	471	2486	62	453	0.02422
RNA-seq	$\tau < 0.4$	$\tau \geq 0.7$	471	2486	34	248	0.10209
RNA-seq	$\tau < 0.4$	$\tau \geq 0.8$	471	2486	11	90	0.21067

Genes without sex-biased expression were identified using a meta-analysis of multiple microarray datasets (Gnad and Parsch, 2006) or RNA-seq (BWA alignments and edgeR to call differential expression). Broadly expressed and narrowly expressed genes were identified using a variety of τ cutoffs. The P values are from Fisher's exact tests that were performed on the counts of broadly- and narrowly-expressed genes on the X chromosome (X) and the autosomes (A).

Supplementary Table 2: SRA accessions for RNA-seq data

Species	Sample	Sex	SRA run ID
D. melanogaster	head	female	SRR039433
D. melanogaster	head	female	SRR039434
D. melanogaster	head	female	SRR039435
D. melanogaster	head	female	SRR039445
D. melanogaster	head	male	SRR039436
D. melanogaster	head	male	SRR039437
D. melanogaster	head	male	SRR039438
D. melanogaster	head	male	SRR039452
D. melanogaster	whole fly	female	SRR166807
D. melanogaster	whole fly	female	SRR166808
D. melanogaster	whole fly	male	SRR166809
D. melanogaster	whole fly	male	SRR166810
D. mojavensis	head	female	SRR037497
D. mojavensis	head	female	SRR037504
D. mojavensis	head	female	SRR037505
D. mojavensis	head	female	SRR037506
D. mojavensis	head	female	SRR037507
D. mojavensis	head	male	SRR037508
D. mojavensis	head	male	SRR037515
D. mojavensis	head	male	SRR037516
D. mojavensis	head	male	SRR037517
D. mojavensis	head	male	SRR037518
D. mojavensis	whole fly	female	SRR166832
D. mojavensis	whole fly	female	SRR166833
D. mojavensis	whole fly	male	SRR166834
D. mojavensis	whole fly	male	SRR166835

Supplementary Table 2 (continued)

Species	Sample	Sex	SRA run ID
D. pseudoobscura	head	female	SRR034782
D. pseudoobscura	head	female	SRR034789
D. pseudoobscura	head	female	SRR034790
D. pseudoobscura	head	female	SRR034791
D. pseudoobscura	head	male	SRR034792
D. pseudoobscura	head	male	SRR034799
D. pseudoobscura	head	male	SRR034800
D. pseudoobscura	head	male	SRR034801
D. pseudoobscura	whole fly	female	SRR166828
D. pseudoobscura	whole fly	female	SRR166829
D. pseudoobscura	whole fly	male	SRR166830
D. pseudoobscura	whole fly	male	SRR166831
D. willistoni	head	female	SRX095355
D. willistoni	head	female	SRX095357
D. willistoni	head	male	SRX095356
D. willistoni	head	male	SRX095358
D. willistoni	thorax	female	SRX095359
D. willistoni	thorax	male	SRX095360
D. willistoni	abdomen	female	SRX095361
D. willistoni	abdomen	male	SRX095362

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