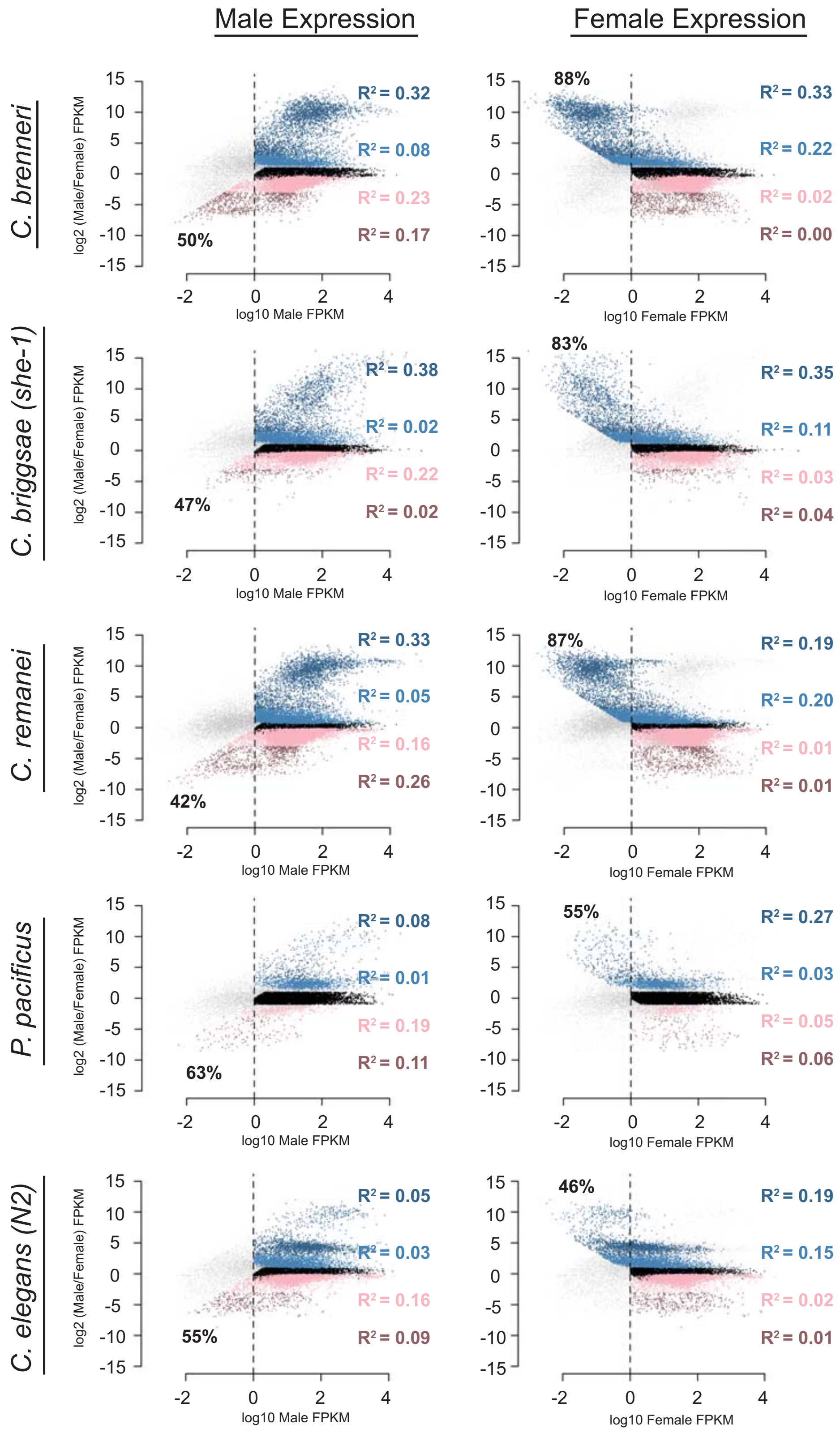

Figure S1 For each species, the magnitude of sex-biased expression (log2 sex expression ratio, (Male/Female) FPKM) is plotted against the level of expression (log10 FPKM) in males (left panels) and females (right panels). Dashed line indicates FPKM =1. Percentages of high sex-biased genes with low expression (FPKM < 1) in the opposite sex are indicated. Non-biased genes are plotted in black. Male-biased genes are plotted in blue; female-biased genes are plotted in pink. Darker colors indicate high magnitude of bias. R-squared values for each category are indicated.

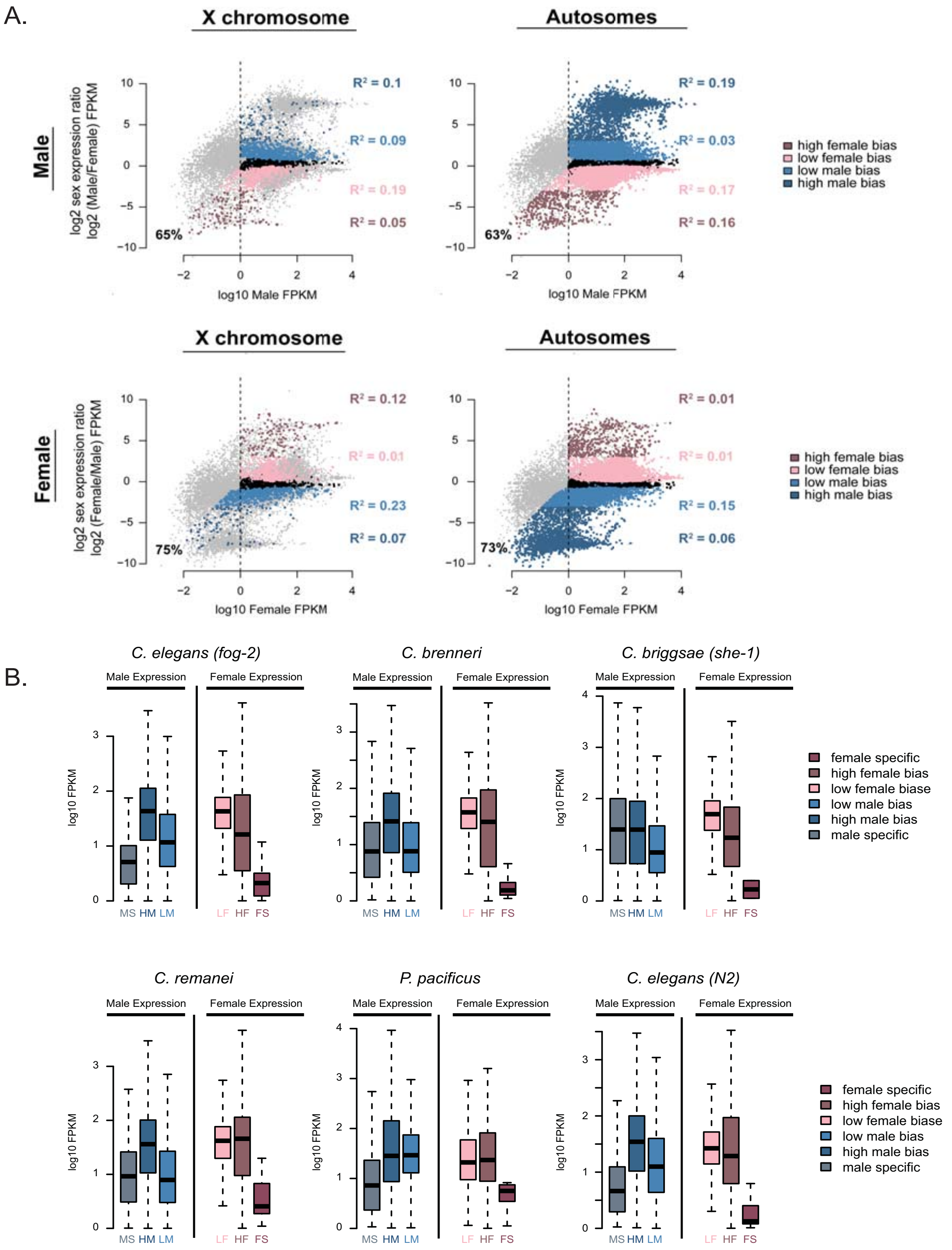

Figure S2 **A)** As in Supplemental Figure 1, for *C. elegans* (*fog-2*) data. Plots are separated by X (left panels) and autosomes (right panels). **B)** For all species, boxplots indicate the range of expression (calculated as the log10 FPKM) of each sex-bias category. Male expression is plotted for male-biased genes (left 3 boxes); female expression is plotted for female-biased genes (right 3 boxes). Overall, sex-specific genes are expressed at low levels in the corresponding sex. (MS – Male-Specific, HM – High Male, LM – Low Male, LF – Low Female, HF – High Female, FS – Female Specific).

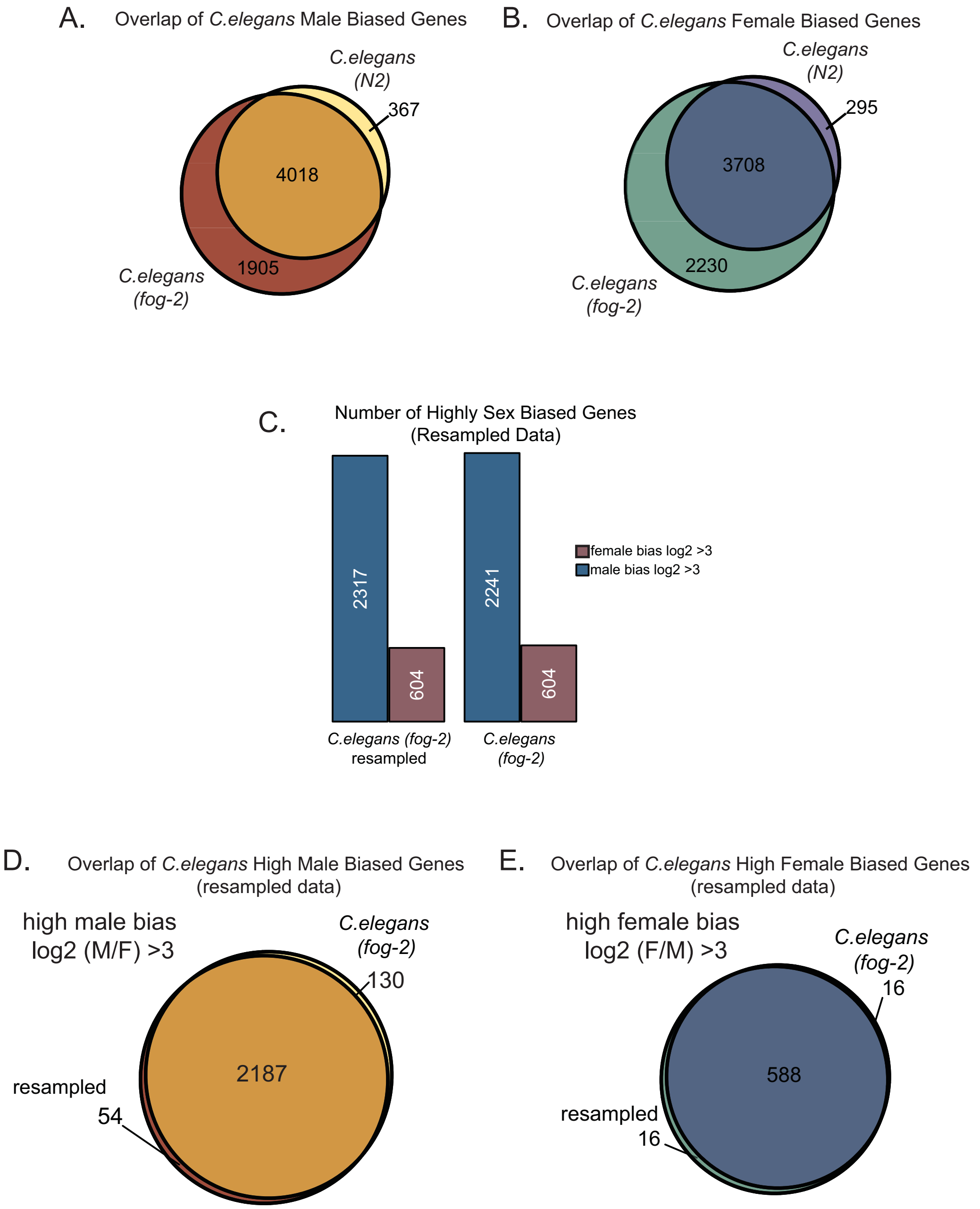

Figure S3 **A)** Sets of male and female-biased genes were determined individually using *C. elegans* (*N2)* (male-hermaphrodite) and (*fog-2)* (male-female) data. Sex-biased genes (including sex-specific genes) are those called differentially expressed by DESeq (q value < 0.05) and with FPKM > 1 in at least one sex. Overlap of male-biased genes is plotted. **B)** Same as in **A** but overlap of female-biased genes is plotted. **C)** *C. elegans (fog-2)* mapped reads were resampled to match the number of mapped reads in *P. pacificus*. Using the resampled data, we identified genes with sex-biased expression. Plot indicates the number of genes with high male and high female-bias (log2 sex expression ratio > 3) for the resampled data (left) and for the original *C. elegans (fog-2)* data. **D)** Overlap of high male-biased genes in the original and the resampled *C. elegans (fog-2)* data. **E)** Same as in **D** but for high female-biased genes**.**

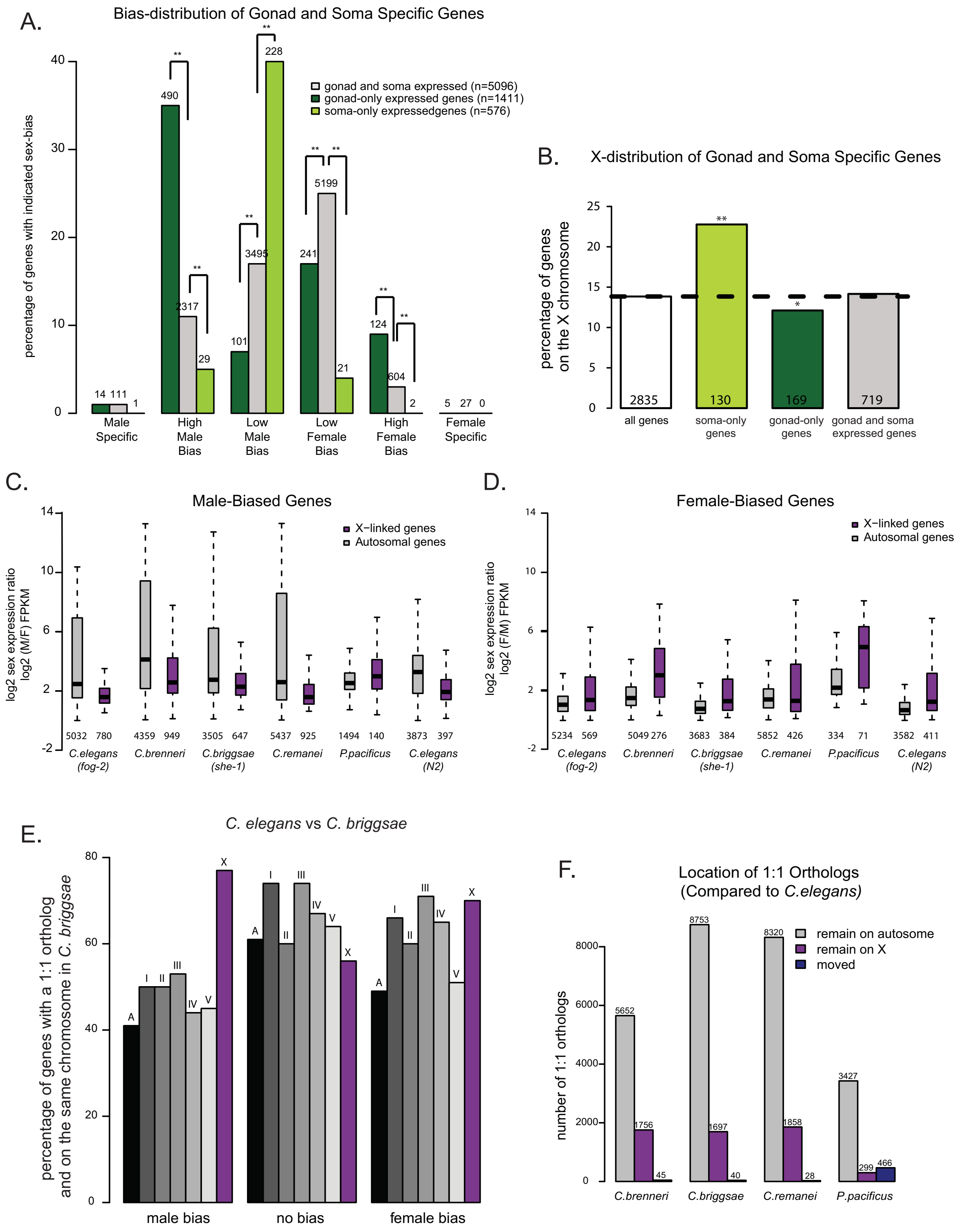

Figure S4 **A)** *C. elegans* genes were grouped into genes expressed in gonad and soma (gray), soma-only (light green), and gonad-only (dark green). For each category, the percentage of genes that fall into the indicated sex-bias category is plotted. Significance by Fisher test: (**) indicates p value less than 0.001. **B)** For each expression category the percentage of genes located on the X chromosome was plotted. Significance of enrichment or depletion was calculated using Fisher test: (*) indicates p value less than 0.05; (**) indicates p value less than 0.001. Number of X-linked genes is indicated at the bottom of each bar. **C)** As in Figure 4C, magnitude of male-biased expression (log2 male over female expression) was calculated for each male-biased gene. Here, bias magnitude of male-biased genes is plotted. Number of genes analyzed is indicated below each box. **D)** Same as **C,** but for all female-biased genes. **E)** In Figure 6A we used a defined set of 1:1:1:1 orthologs to determine locational conservation of biased and unbiased genes. Here we define a set of 1:1 orthologs between *C. elegans* and *C. briggsae*. This less stringent definition of orthology gave a larger list of genes, and all chromosomes could be evaluated individually. The number of 1:1 orthologs located on the same chromosome was calculated. For each chromosome, the conservation is plotted as the percentage of *C. elegans* genes that have a 1:1 ortholog in *C. briggsae* located on the same chromosome. **F)** Macrosynteny is high amongst the *Caenorhabditis* species. Between *C. elegans* and each of the other four species, the number of 1:1 orthologs that remain autosomal (grey), remain X-linked (purple) or move between X and autosomes (dark blue) in the two species are plotted.

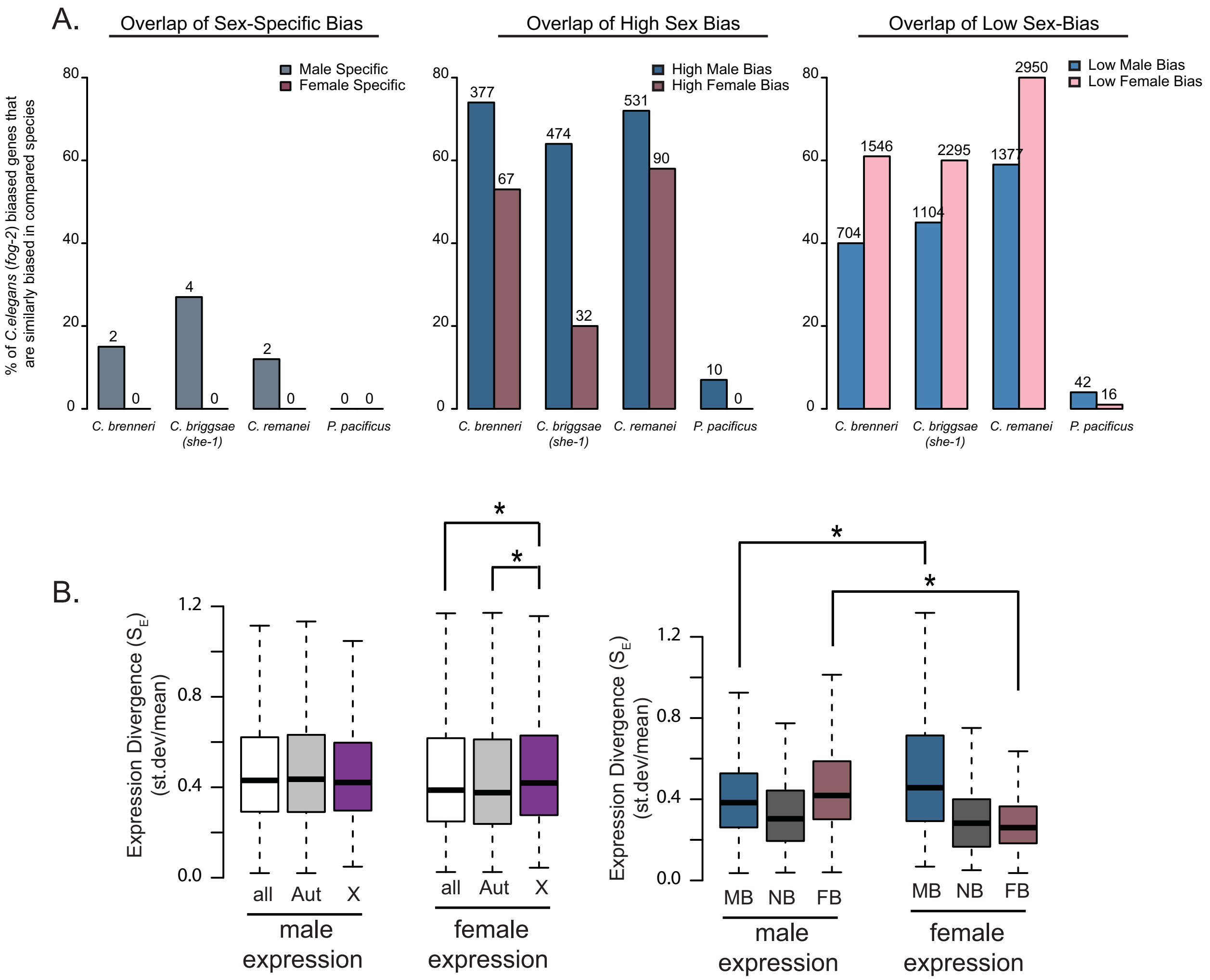

Figure S5 **A)** The percentage of genes in *C. elegans* with a similarly sex-biased 1:1 ortholog in the indicated species is plotted. Numbers above each bar indicated the number of 1:1 orthologs with similar bias. Percentage of overlap is plotted for *C. elegans (fog-2)* sex-specific genes **(Left)**, high sex-biased genes **(Middle)**, and low sex-biased genes **(Right)**. **B)** Using *Caenorhabditis* 1:1:1:1 orthologs, we calculated the interspecies expression divergence for each gene as the coefficient of variation (σ/μ). Left panel shows male and female expression divergence for all (white), autosomal (grey) and X-linked (purple) genes. Right panel shows male and female expression divergence for male-biased (MB, blue), female-biased (FB, pink), and non-biased (NB, dark grey) genes. (*) indicates significant difference in distribution (p-value < 0.01) as calculated by Mann-Whitney test.

Tables S1-S7
Available for download as Excel files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.163311/-/DC1

Table S1	Overview of DNAseq Data, DNAseq Replicates, RNAseq data - read numbers, and GEO Numbers
Table S2	Contig Assignments
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