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

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