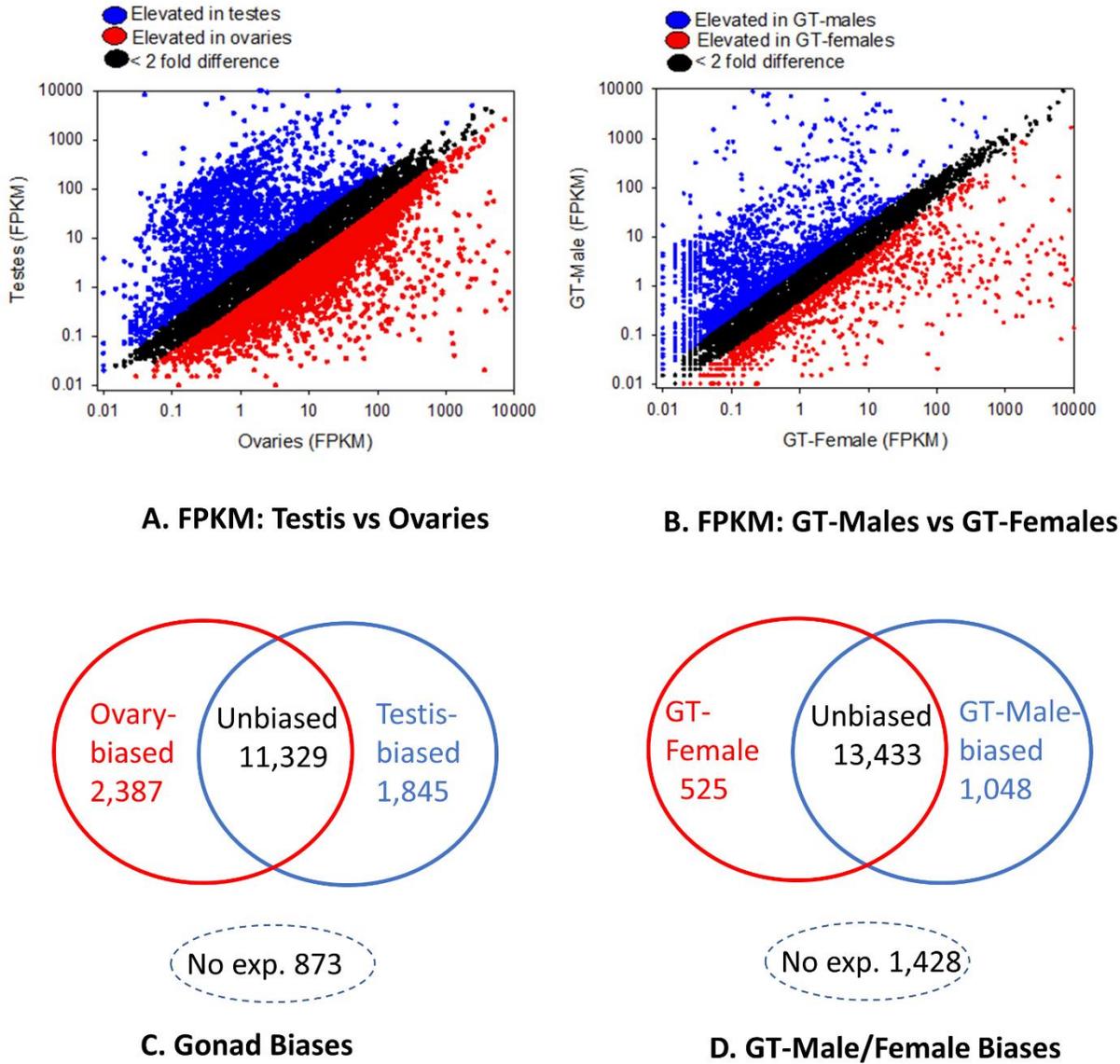


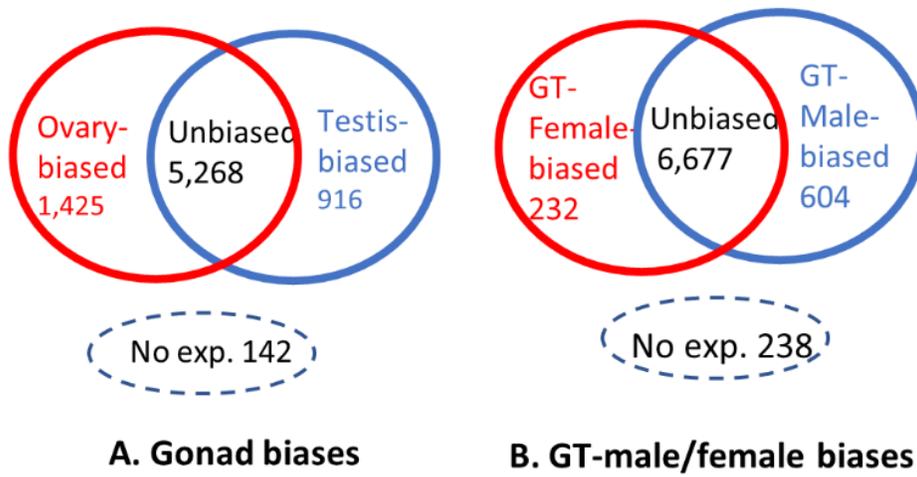
SUPPLEMENTAL MATERIAL

Absence of a faster-X effect in beetles (*Tribolium*, Coleoptera)

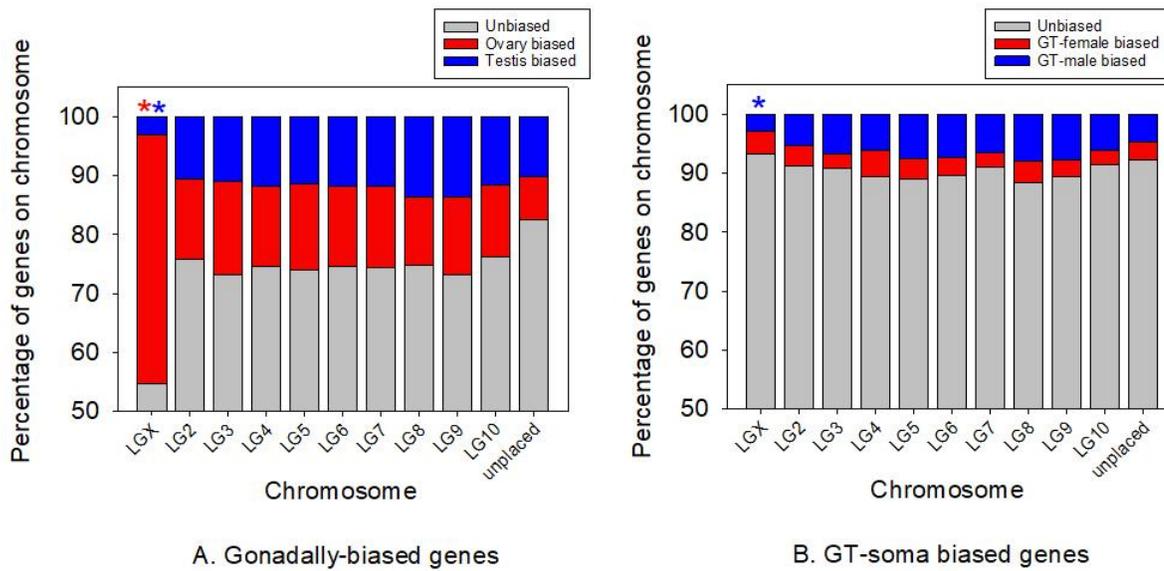
Whittle, Kulkarni, and Extavour 2020



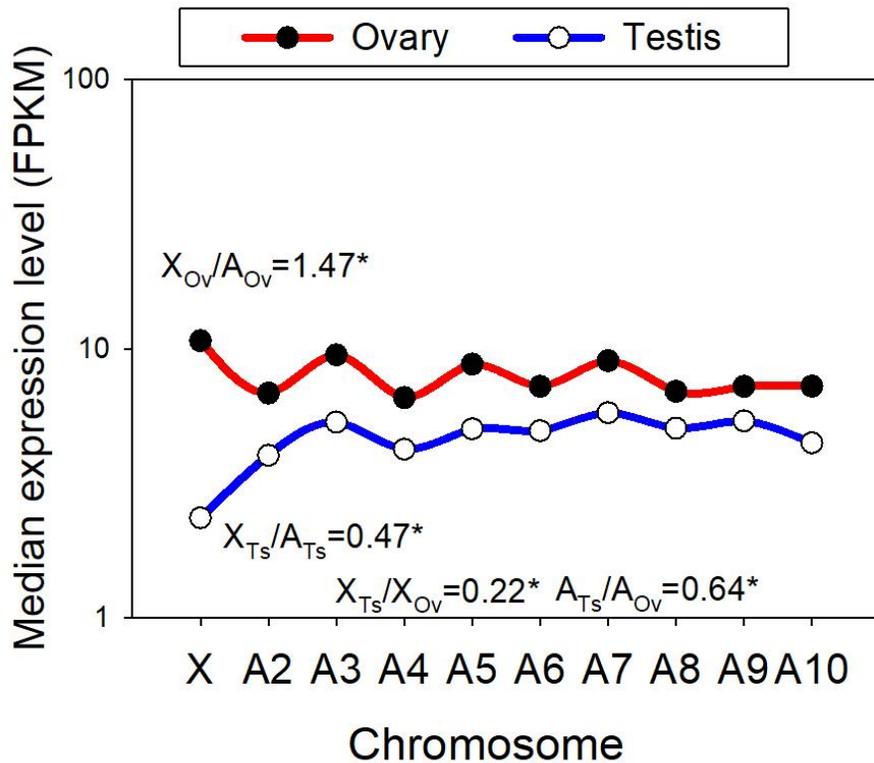
**Figure S1. Sex-biased expression in 16,434 genes of *T. castaneum*.** A) Expression level (FPKM) in the testes versus ovaries; B) expression level (FPKM) in GT-males versus GT-females; C) Venn diagram of sex-biased expression the gonads and; D) Venn diagram of sex-biased gene expression in the GT-soma. In A and B, all expressed genes are shown and those with two-fold or greater difference in expression in the male and female tissues are in blue and red respectively (those statistically significant in C and D). Genes with no expression in both tissues were excluded in A and B and are shown in C and D (no. exp).



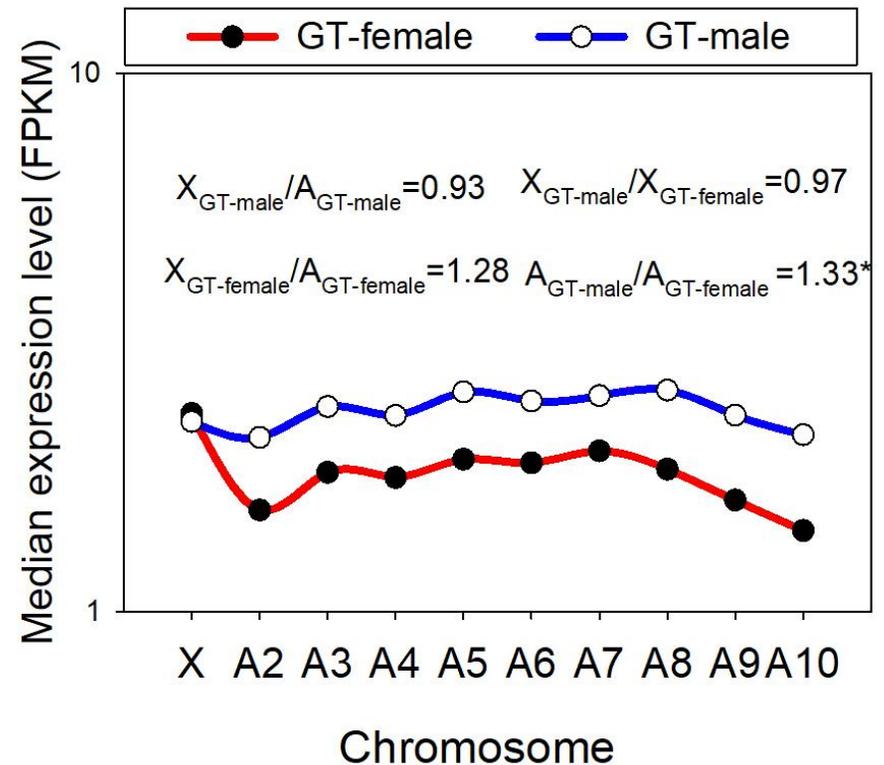
**Figure S2.** A Venn diagram showing the number of sex-biased and unbiased genes in the A) gonads; B) GT-soma. Genes with no expression were defined as unbiased for dN/dS analyses.



**Figure S3.** The frequency of sex-biased genes and unbiased genes on the X chromosome and autosomes when all 16,434 genes of *T. castaneum* are included in assessment. A) Gonadally biased genes; B) GT-soma biased genes. In A, the red and blue asterisks indicate more ovary-biased and fewer testis- (and fewer GT-male biased in B) biased genes respectively on the X-chromosomes than on pooled autosomes (Chi<sup>2</sup>-P with Yates' correction P<0.05 for each contrast). Unbiased genes include those with no observed difference (<two-fold, or P>0.05) in male-female expression or no expression. NOTE: The percentage of ovary-biased genes on the X-chromosome in our 7,751 *T. castaneum* genes with orthologs in *T. freemani* was 53.9%, while for all 16,434 *T. castaneum* genes it was 42.2%. This reflects the fact that in the former case the studied genes had high confidence orthologs between species, which are apt to be more frequently identified for ovary-biased genes due to their slowed evolution, an effect that was pronounced on the X-chromosome.



A. Median expression in gonads  
(all 16,434 genes)



B. Median expression in GT-soma  
(all 16,434 genes)

**Figure S4. Median expression in the male and female tissues on each of the ten chromosomes in *T. castaneum* using all 16,434 genes in the genome.** A) Gonads; B) GT-soma. For panel A, the ratio of median expression on the X chromosome (X) and autosomes (A) for testis-biased genes and for ovary-biased genes are shown ( $X_{TS}/A_{TS}$  and  $X_{OV}/A_{OV}$ ). Also shown are  $X_{TS}/X_{OV}$  and  $A_{TS}/A_{OV}$ . Panel B contains the equivalent results for the GT-soma. Unmapped genes on chromosomes were excluded. \*Indicates a statistically significant difference between the two groups contained in each ratio using MWU-tests.

**Table S1.** RNA-seq data used in present study before and after adapter and quality trimming with BBDuk (<https://jgi.doe.gov/data-and-tools/bbtools/>). The Short Read Archive (SRA) Biosample identifiers are also shown (<https://www.ncbi.nlm.nih.gov/sra>).

Species Sample <sup>a</sup>	No. of Reads		SRA Biosample ID
	Before trimming	After trimming	
<i>Tribolium castaneum</i>			
Testes sample 1	18,006,255	17,995,655	SAMN12702873
Ovary sample 1	39,140,493	39,122,050	SAMN12702874
GT-male sample 1	25,630,261	25,609,723	SAMN12702875
GT-female sample 1	41,513,717	41,472,348	SAMN12702876
Testes sample 2	24,795,583	24,787,238	SAMN12702877
Ovary sample 2	22,306,622	22,286,961	SAMN12702878
GT-male sample 2	62,781,001	62,712,242	SAMN12702879
GT-female 2	52,275,340	52,211,149	SAMN12702880
<i>Tribolium freemani</i>			
Testes sample 1	32,222,092	32,203,312	SAMN12702881
Ovary sample 1	40,395,198	40,368,671	SAMN12702882
GT-male sample 1	32,933,858	32,926,509	SAMN12702883
GT-female sample 1	33,163,960	33,147,066	SAMN12702884

<sup>a</sup> Reads were obtained from for two RNA-seq runs of each biological sample.

**Table S2. A.** The number of studied genes (N=7,751) with sex-biased expression in the gonads and GT-soma. **B.** The degree of overlap in sex-biased status on the X-linked genes between the gonads and GT-soma are also shown.

**A**

	<b>N values gonads</b>					<b>N values GT-soma</b>			
	Ovary-biased	Testis-biased	Unbiased <sup>a</sup>	Total		GT-female biased	GT-male biased	GT-unbiased <sup>a</sup>	Total
X-linked	233	9	190	432	X-linked	24	12	396	432
Autosomes	1192	907	5220	7319	Autosomes	208	592	6519	7319
				7751					7751

<sup>a</sup>Includes sexually unbiased genes and those with no expression in Fig. S2.

**B**

<b>Overlap on the X-Chromosome</b>	<b>N overlap in sex-biased status on the X-chromosome</b>
Ovary-biased and GT-female biased	17
Testis-biased and GT-male biased	0
Ovary-biased and GT-unbiased	213
Testis-biased and GT-unbiased	7
GT-male biased and ovary-biased	3

## Text File S1.

### Extracting CDS from *T. freemani*

To extract gene sequences from scaffolds in *T. freemani* we used Web Augustus version 3.3.1 (<http://bioinf.uni-greifswald.de/webaugustus/> (HOFF AND STANKE 2013)) that was trained to the *T. castaneum* genome and set at default parameters with the option to identify full length genes. The Augustus-generated CDS list for *T. freemani* was then assessed in ORF predictor, using its downloadable Perl script (MIN *et al.* 2005) to identify the highest quality reading frame per sequence. In ORF predictor, we employed the option to include the best-hit (lowest e-value) BLASTX alignment (conducted in BLAST+ v2.7.1, <https://blast.ncbi.nlm.nih.gov>) of *T. freemani* CDS versus the reference *T. castaneum* protein database to define reading frames, an approach which yielded 12,432 uninterrupted sequences of full or partial CDS for *T. freemani*. For further stringency in curating the *T. freemani* CDS list, we pooled the identified CDS with all *T. freemani* RNA-seq reads (trimmed reads, Table S1) across all four tissue types (testis, ovaries, male carcass, female carcass) and mapped all sequences to the known and annotated CDS from *T. castaneum* using Geneious read mapper (v11.0.3), which generated consensus CDS. We then extracted CDS wherein all bases had a minimum of 10X coverage, and these were trimmed to the *T. castaneum* reference CDS. In those *T. freemani* CDS (obtained after ORF predictor) wherein the CDS was improved in quality (contained no unknown or ambiguous nucleotides) or in its length and/or the terminal stop codon was added by using the RNA-seq data, which occurred for N=1,249 CDS, we replaced the original Augustus-based CDS (among the 12,432) with the latter RNA-seq-mapped version CDS. Original CDS that were identified in *T. freemani* (and not found in the 12,432 list) only after using the RNA-seq mapping approach (N=196) were also included in the species final CDS list.

### Sample collection and lab procedures

To ensure that all adult animals for both species remained unmated until the time of tissue dissection, all animals were separated as late stage larvae into individual vials containing flour and allowed to pupate into adults. Tissue dissections were then performed on unmated adults within a week after they emerged from the molt. For *T. castaneum* a total of 150 animals per sex per biological replicate were sampled, whereas for *T. freemani*, a total of 50 males and females were collected per sample. For each sample of males and of females, the gonadal and nongonadal tissues were separated and placed into two separate vials containing TRIzol reagent (Ambion Life Technologies, catalog number 15596-018) on dry ice.

As outlined in the main text, the reproductive tissues of males included the testes, accessory glands (mesadenia, ectadenia), vesicular seminalis, vas deferens and ejaculatory duct. The reproductive tissues for females included the ovaries, spermathecal gland, common oviduct, spermathecae, and vagina. All remaining nongonadal tissues of the adult body were collected and defined as GT-males and GT-females. Two biological samples per tissue type (testis, ovary, GT-males, GT-females) were collected for RNA-seq for our main target species for study, *T.*

*castaneum* (eight total samples) while one sample per tissue type was obtained for *T. freemani* (four samples). A total of twelve samples were thus obtained for RNA-seq as shown in Table S1.

The testes, ovaries, GT-males and GT-females were stored in separate vials at -80°C until RNA extraction. RNA-isolation was performed according to the Ambion Life Technologies TRIzol Reagent Protocol, following which the RNA was used for RNA library preparation. Polyadenylated mRNAs were selected from total RNA samples using oligo-dT-conjugated magnetic beads on an Apollo324 automated workstation (PrepX PolyA mRNA isolation kit, Takara Bio USA). Entire poly-adenylated RNA samples were immediately converted into stranded Illumina sequencing libraries using 200 base pair (bp) fragmentation and sequential adapter addition on an Apollo324 automated workstation following manufacturer's specifications (PrepX RNA-seq for Illumina Library kit, Takara Bio USA). Libraries were enriched and indexed using 14 cycles of amplification (LongAmp Taq 2x MasterMix, New England BioLabs Inc.) with PCR primers that included a 6bp index sequence to allow for multiplexing (custom oligo order from Integrated DNA Technologies). Excess PCR reagents were removed using magnetic bead-based cleanup on an Apollo324 automated workstation (PCR Clean DX beads, Aline Biosciences). Resulting libraries were assessed using a 2200 TapeStation (Agilent Technologies) and quantified by QPCR (Kapa Biosystems). Libraries were pooled and sequenced on two Illumina NextSeq 500 high output flow cells using single end 76bp reads.