

Amm_transcriptome_results_update2

We investigated the issue of second cloud of female-biased genes in brain tissues, it turned out that 30% transcripts does not have any BLAST hit (88%) with new XX genome.

We removed the non-frog transcripts, and redone all analysis using the updated transcriptome.

A. Tissue specificity index tau

B. Sex-biased gene expression along developmental stages, across adult tissues

C. Transcription degeneration

D. Coding sequence divergence and Faster-X

A. Tissue specificity index tau

Approach:

1. Define intotal 11 tissues, treating somatic tissues with combined sexes and gonad tissues in separate sexes (see Brown & Bachtrog 2014).
2. Use Kallisto to quantify transcript expression, use the generated output of TPM matrix.
3. Use tissue specificity index Tau formula below (Mank et al. 2008; Brown & Bachtrog 2014):

$$\tau = \frac{\sum_{i=1}^N 1 - \frac{\log E_i}{\log E_{max}}}{N - 1},$$

4. Keep transcripts which the expression are expressed in at least one of the 11 tissues with TPM >=2.

Figure 1. Histogram distribution of Tau for all transcripts in Ammarnas transcriptome.

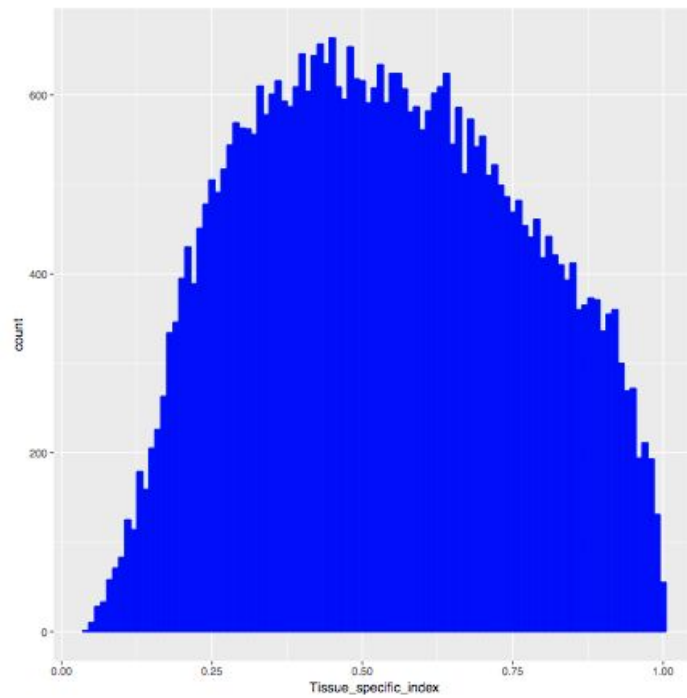


Figure 2. Boxplot of sex-biased genes and the tissue specificity index Tau at G46 stage (sex-biased genes are not including the gene expression from sex-reversed XX male).

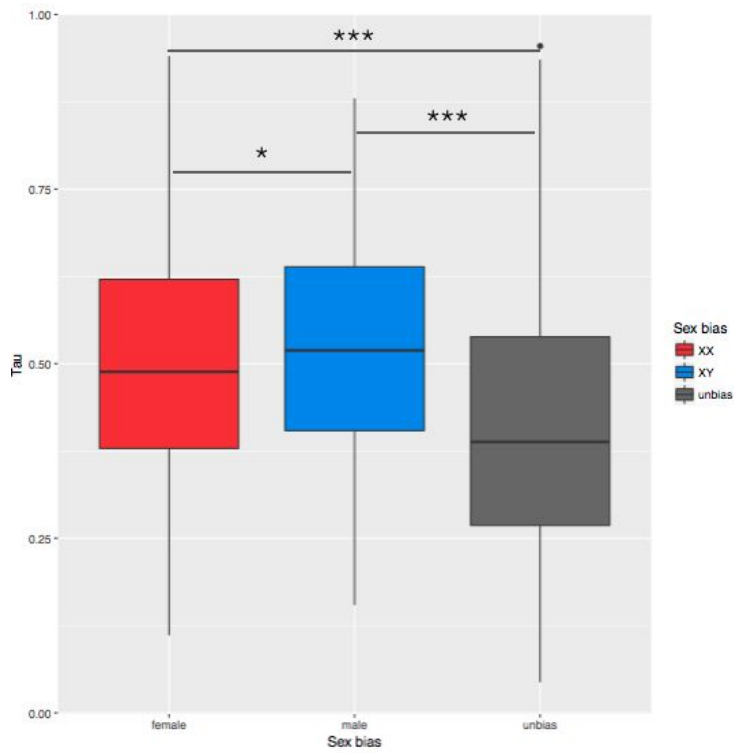


Figure 3. Correlation between absolute values of gene expression ratio $\text{Log}_2(\text{XY46}/\text{XX46})$ (include sex-biased and unbiased genes) and tissue specificity index tau.

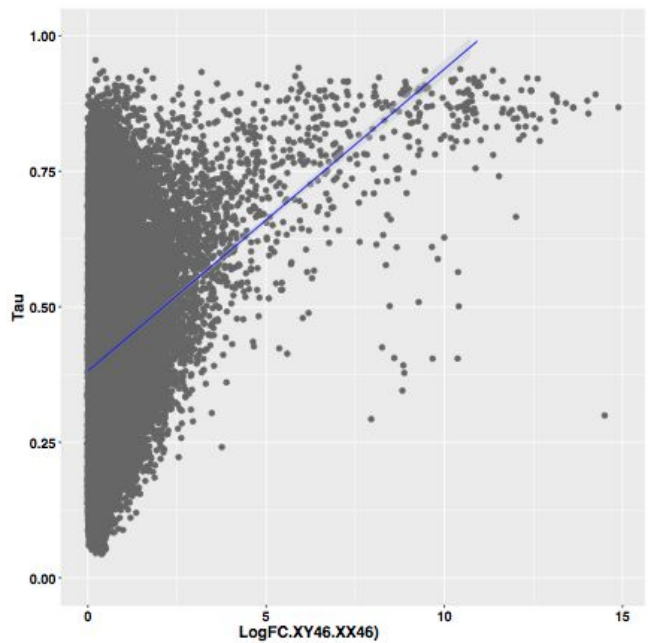


Figure 4. Boxplot of sex-biased genes and the tissue specificity index Tau in gonad tissues.

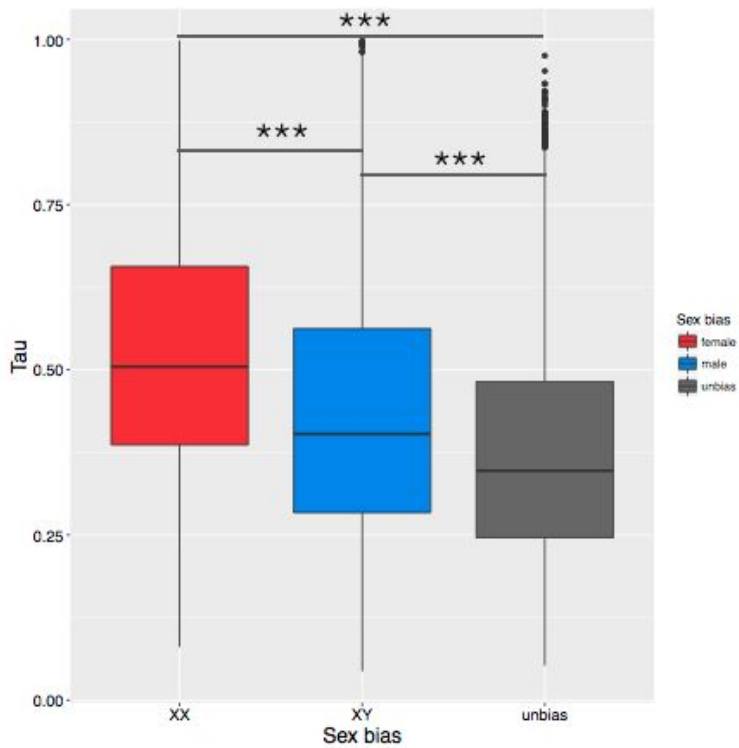
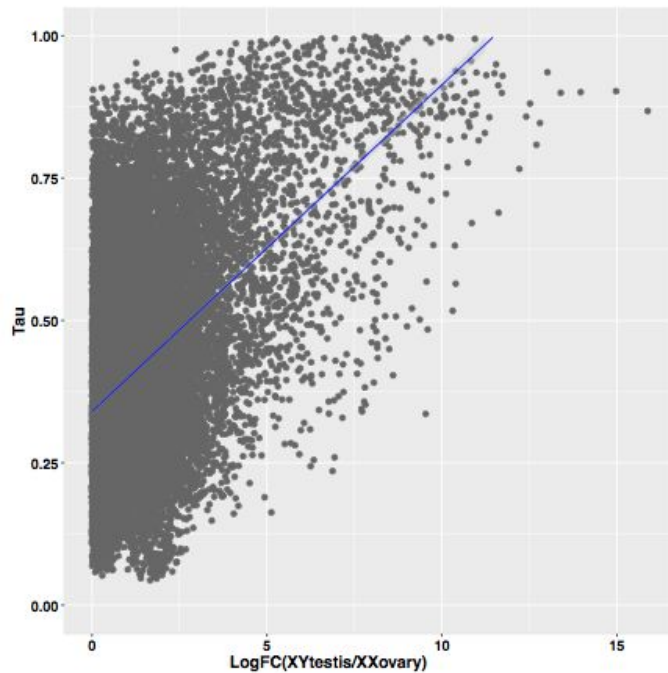


Figure 5. Correlation between absolute values of gene expression ratio $\text{Log}_2(\text{male/female})$ (only for sex-biased and unbiased genes) and tissue specificity index tau.



More analysis will come along the way, now it is just to get some idea on the patterns and trigger further related questions.

B. Sex-biased gene expression along developmental stages, across adult tissues

Approach: the transcriptome with removed-non-frog-transcripts is used, we quantify the transcript abundance with Kallisto.

The EdgeR is applied to analyze differential or sex-biased gene expression across developmental stages, as well as adult tissues.

The selection criteria to remove lowly expressed transcripts are: average $\text{LogCPM} > 0$ and $\text{LogCPM} > 1$ is present in at least half of the tissues per sex.

Figure 6. Number of sex-biased genes throughout development and adult tissues (FDR < 0.05, Log2 >=1).

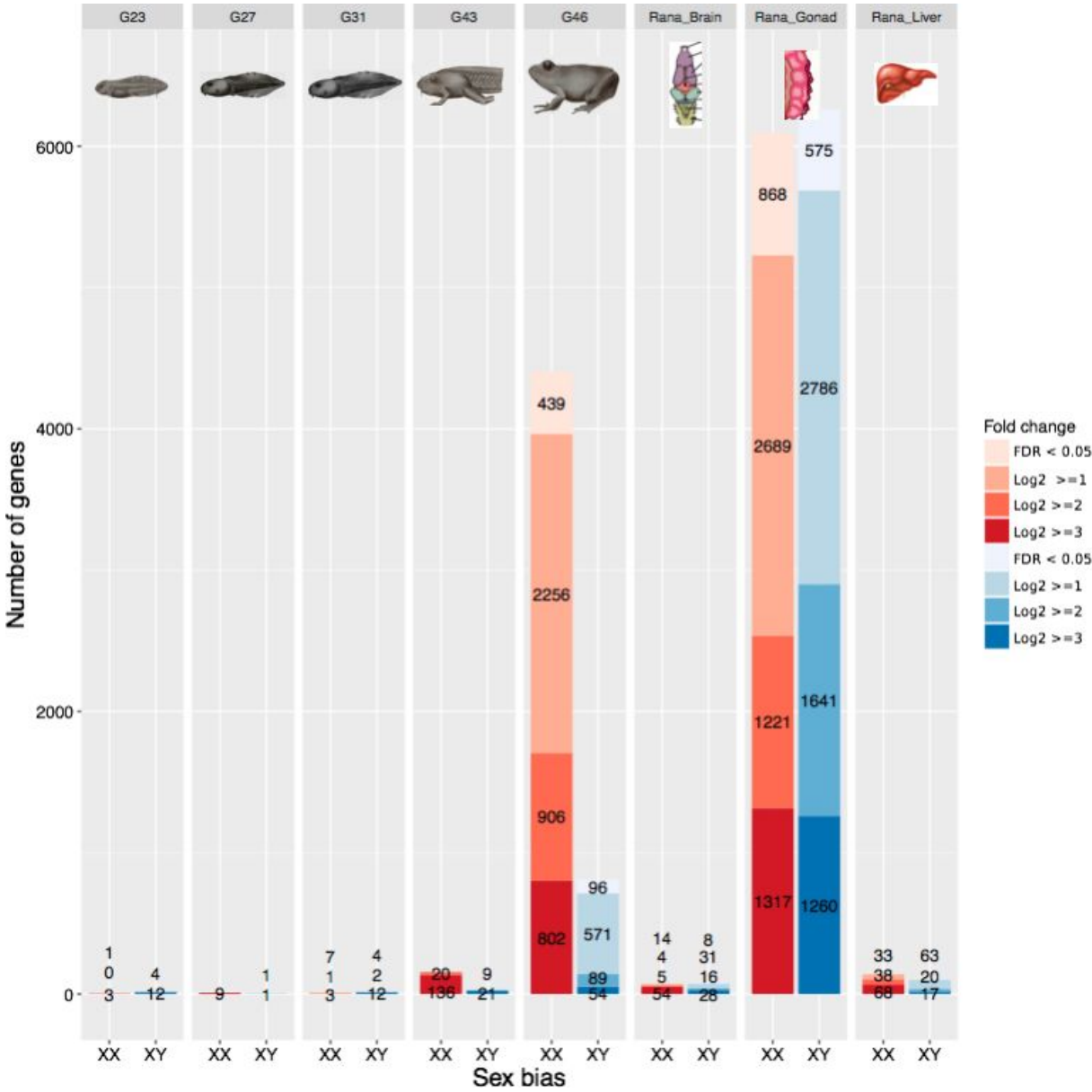


Figure 7. Shared sex-biased genes (a), shared female-biased genes (b) and shared male-biased genes (c) throughout development stages.

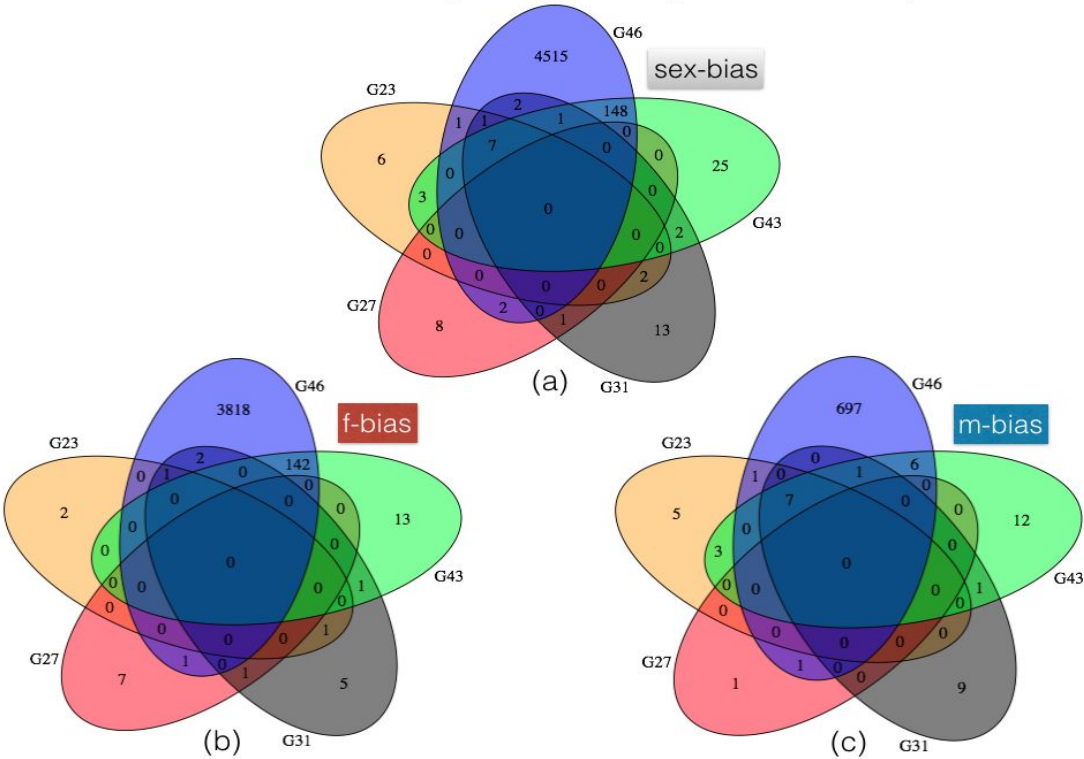


Figure 8. Shared sex-biased genes (a), shared female-biased genes (b) and shared male-biased genes (c) among three adult tissues, brain, gonad and liver.

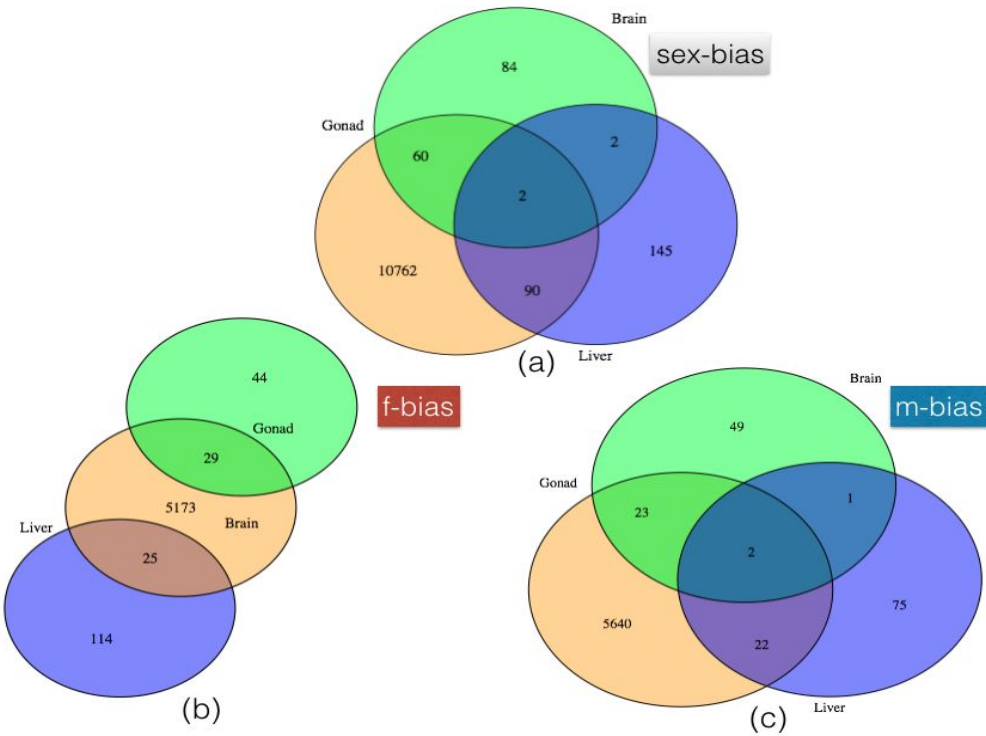


Figure 9. Shared sex-biased genes (a), shared female-biased genes (b) and shared male-biased genes (c) between G43 and gonad tissues which have high sex-biased genes.

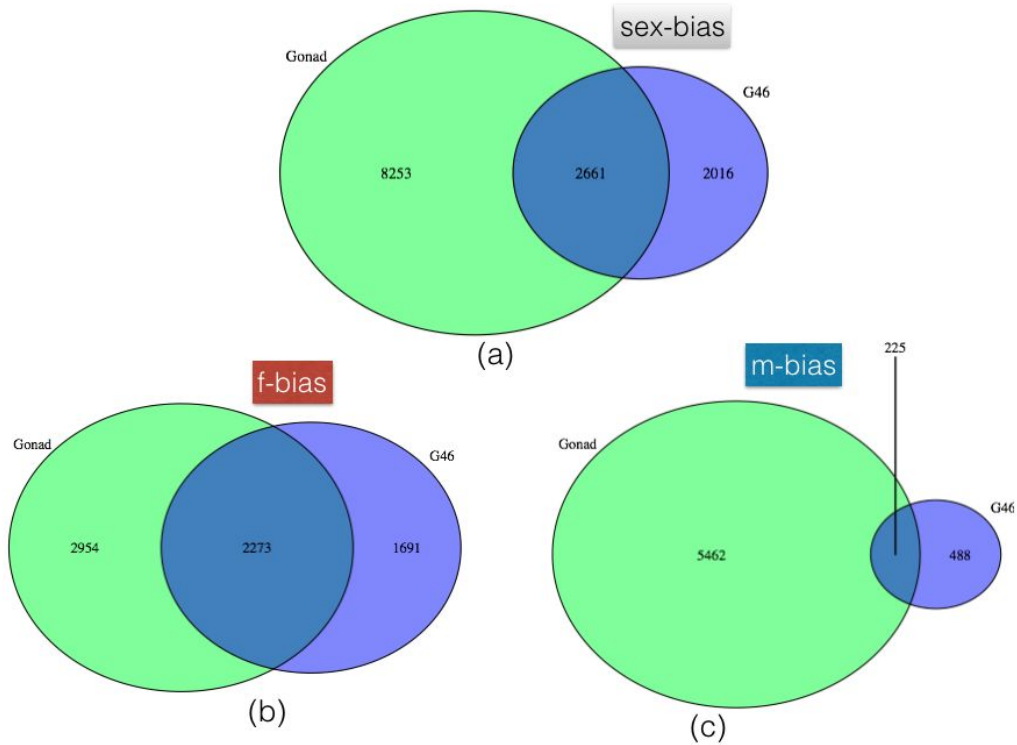
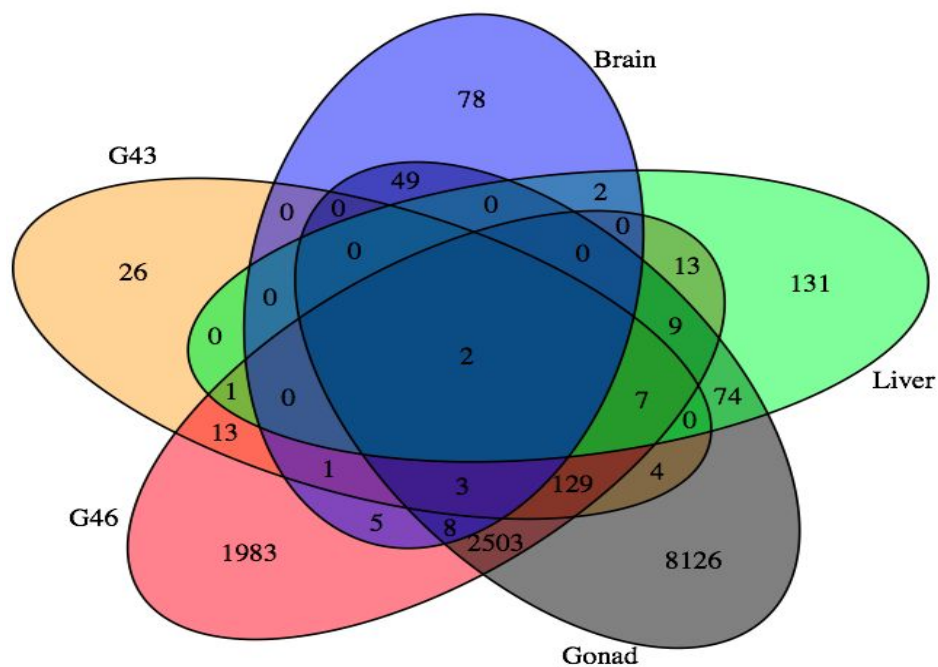


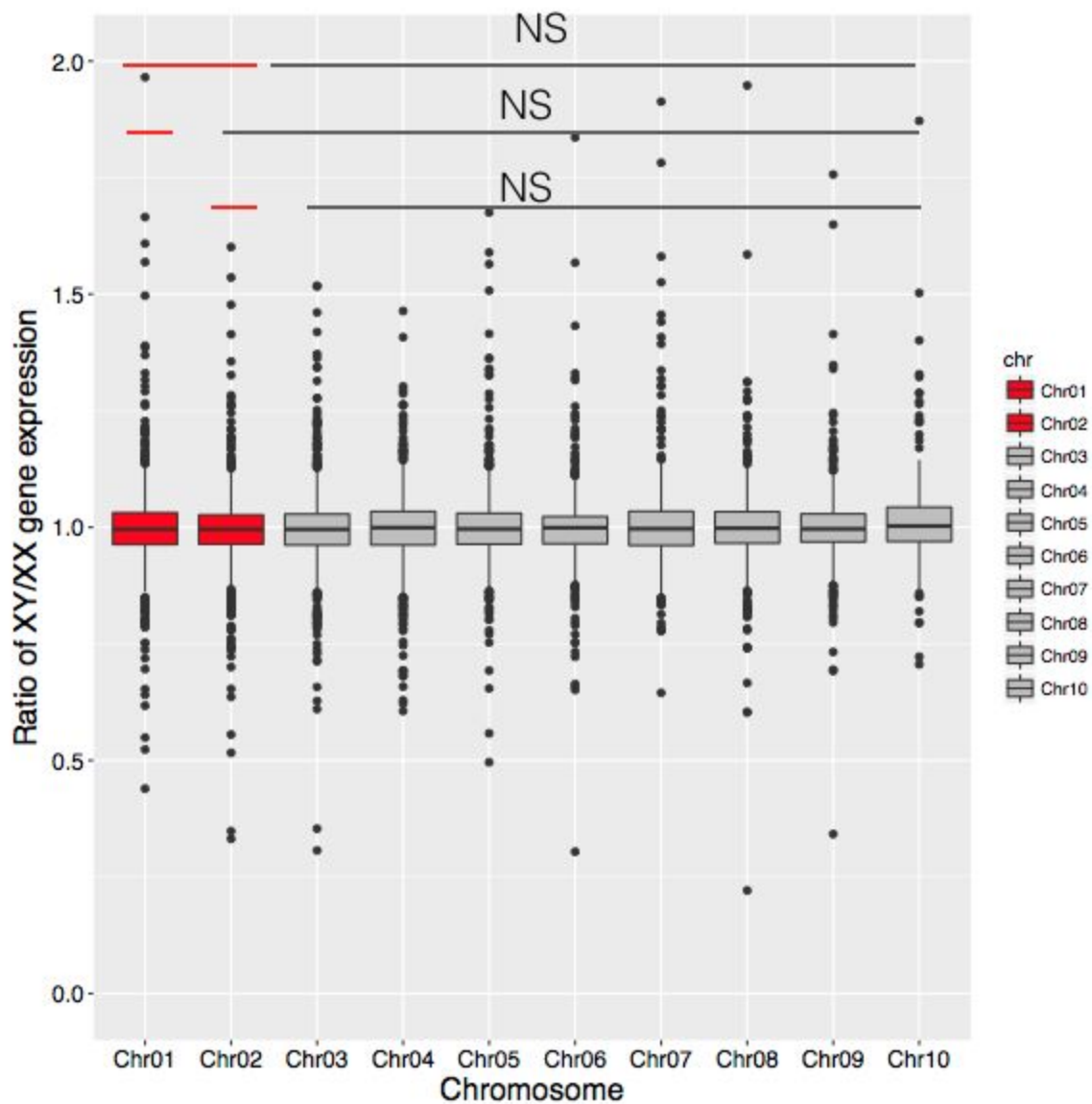
Figure 10. Shared sex-biased genes between tissues of G43, G46, and adult gonad, liver and brain tissues.



C. Transcription degeneration

To investigate possible transcriptional degeneration at early sex chromosome evolution, we compare gene expression ratio of XY individuals with testis and XX individuals with testis, $\text{Log}_2(\text{XY}/\text{XX})$.

Figure 10. BOXPLOT OF Gene expression ratio between individuals of XY with testis and XX with testis. Note, chromosome 1 and 2 are both shown to be linked to sex determination.



D. Coding sequence divergence and Faster-X

Approach: find the longest ORFs per transcript, use 1:1 ortholog with *X.tropicalis* to locate the genome locations for each transcript, finally use PRINK to perform codon alignment and finally calculate dN, dS, dN/dS with Codeml model in PAML.

Figure 11. dN/dS ratio of sex-biased and unbiased genes throughout development and three adult tissues.

*** denotes $P < 0.0005$

** denotes $P < 0.005$

* denotes $P < 0.07$

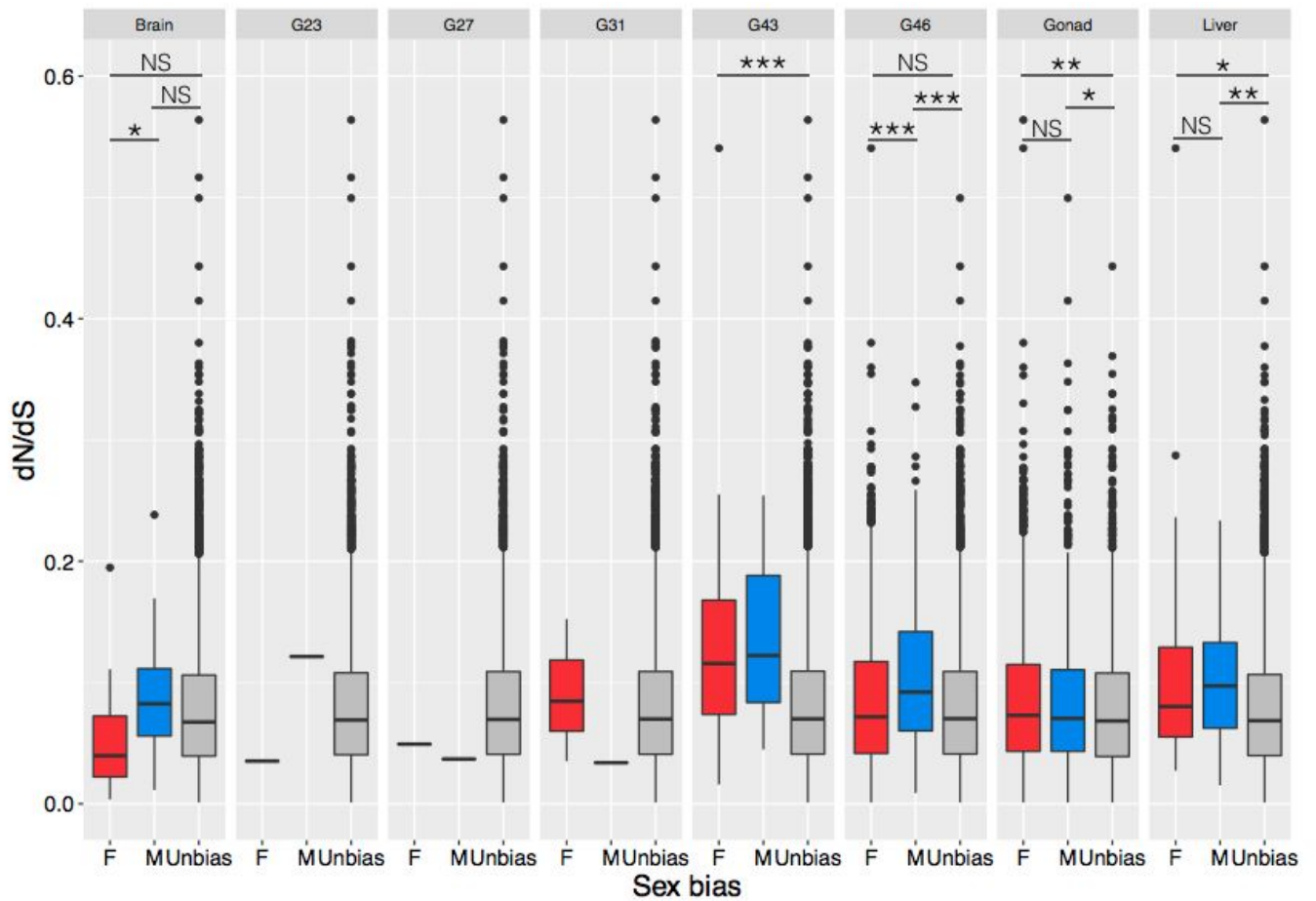


Figure 12. dN (a) and dS (b) values of sex-biased and unbiased genes throughout development and three adult tissues.

*** denotes $P < 0.0005$

** denotes $P < 0.005$

* denotes $P < 0.07$

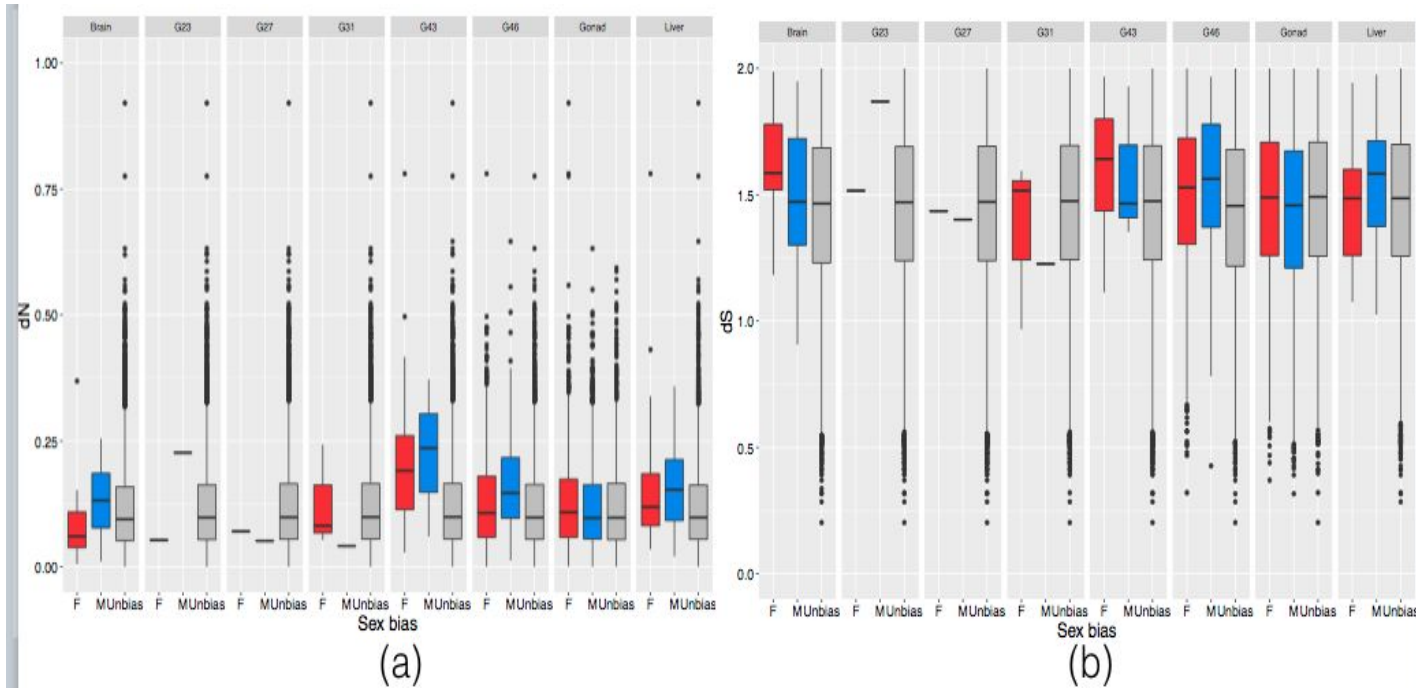


Figure 12. dN/dS ratio of genes on sex chromosomes (chromosome 1 and chromosome 2) and autosomes.

