A first look at the cultivated sunflower (*Helianthus annuus* [L.]) methylome

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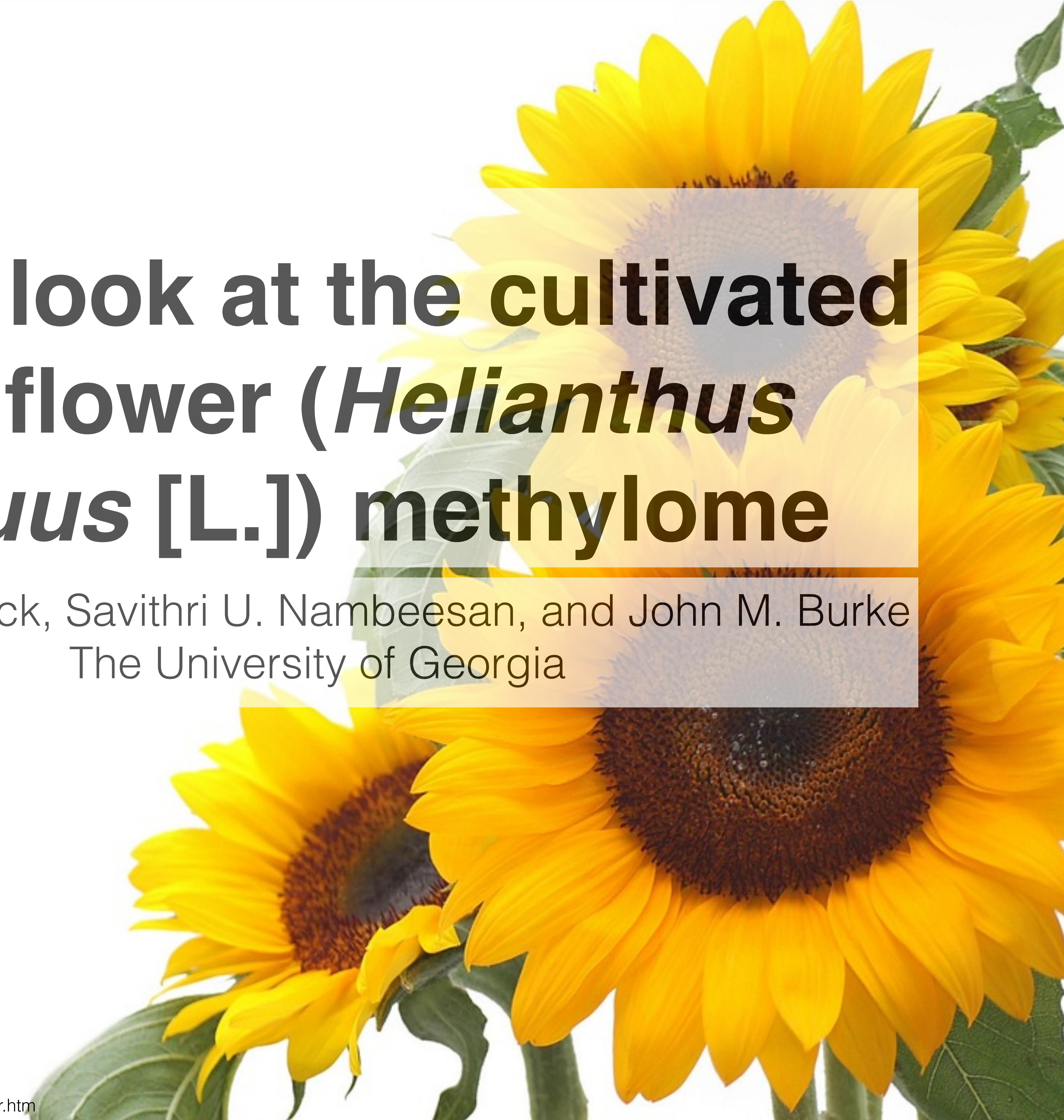
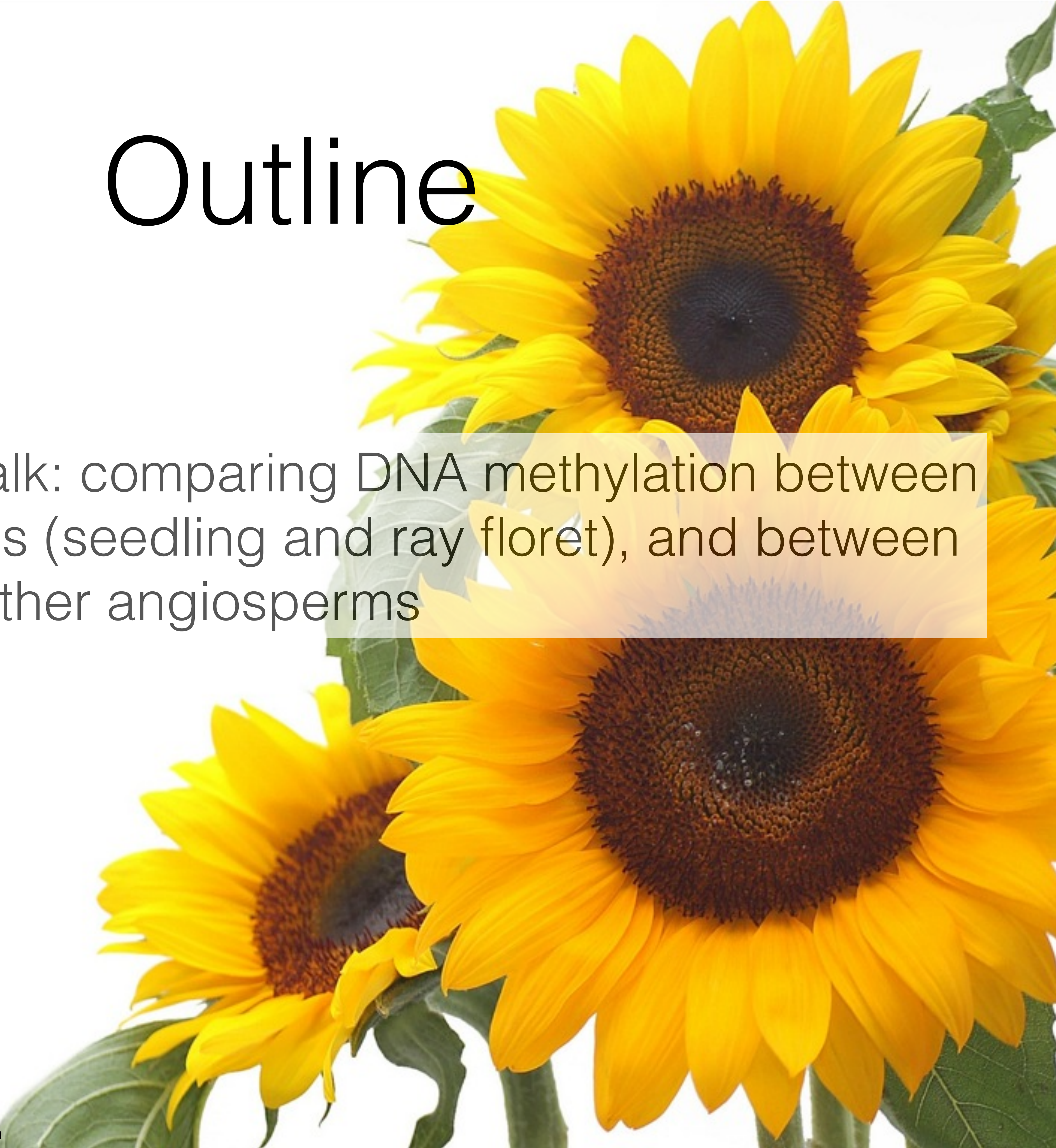
The University of Georgia



http://wallpaper-kid.com/sunflower-iphone-5-wallpaper.htm

Outline

A comparative talk: comparing DNA methylation between *H. annuus* tissues (seedling and ray floret), and between *H. annuus* and other angiosperms



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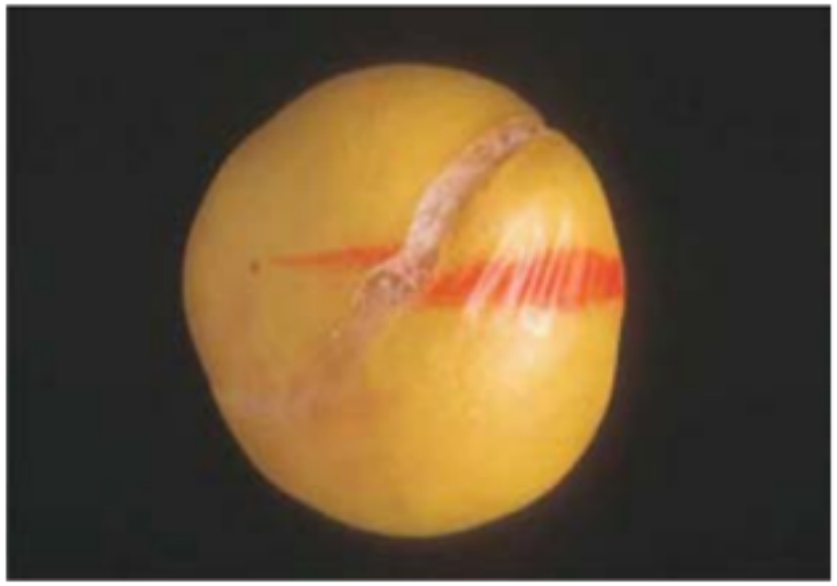
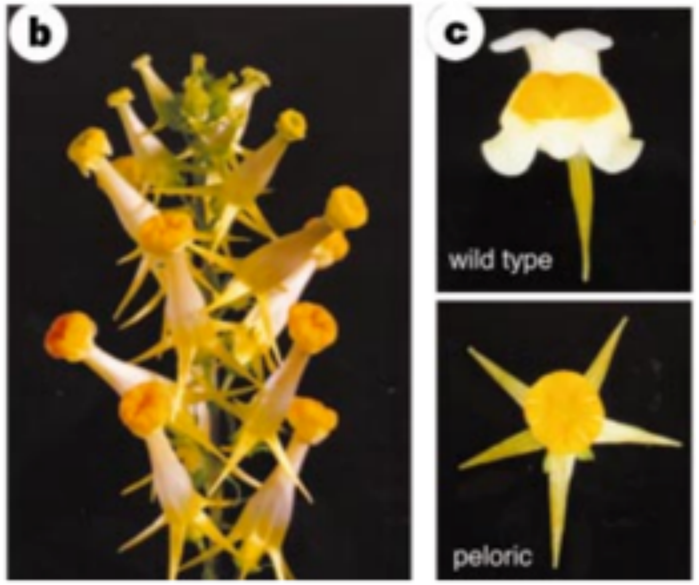
DNA methylation can play a role in phenotypic changes/variation

- The addition of a methyl group to the 5’ carbon of cytosine
- Important in genome stability, and gene expression
- Rare “*epivariants*” or “*epialleles*” with substantial phenotypic divergence

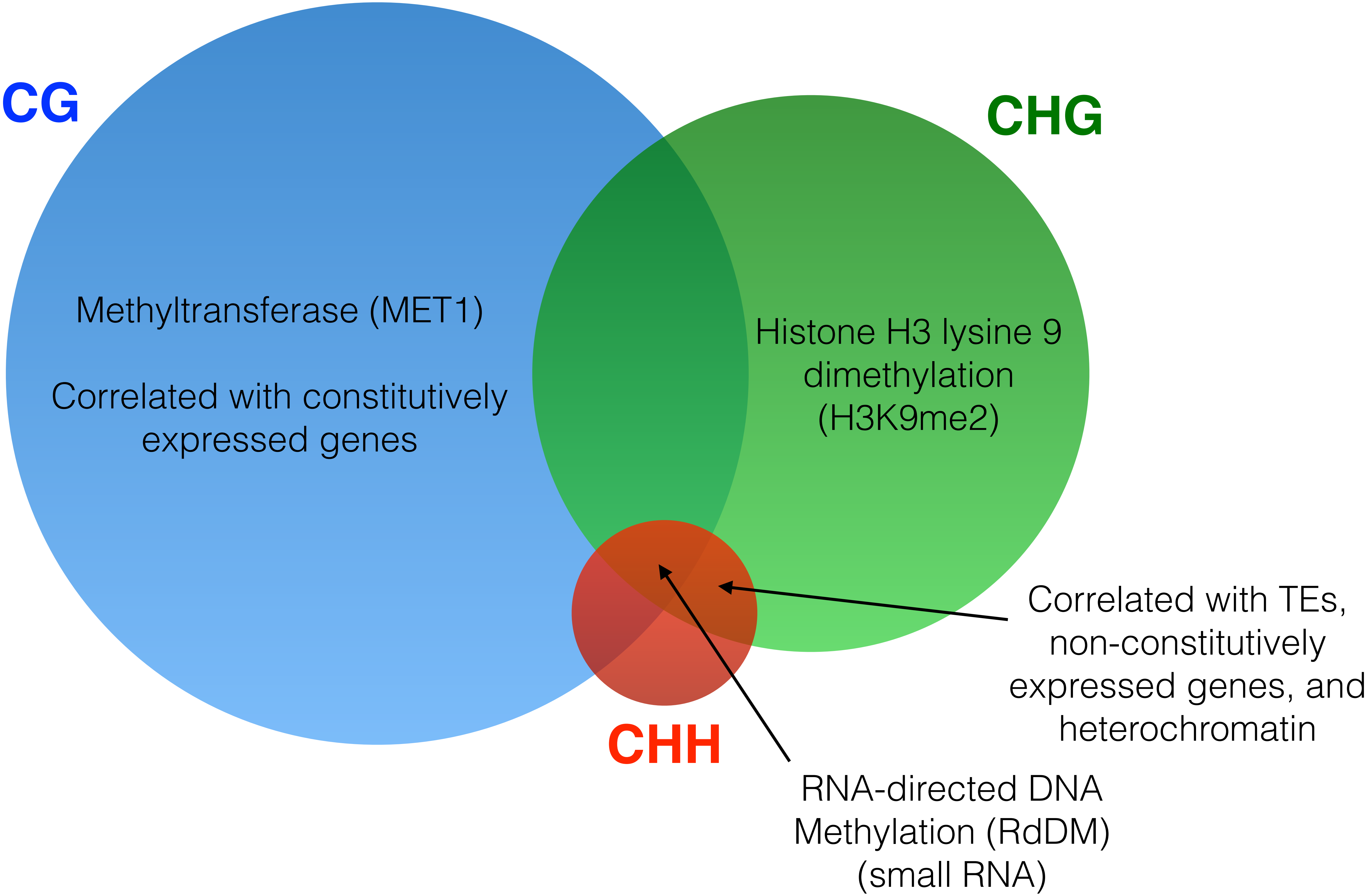


Fruit ripening in tomato



Yellow toadflax, or butter-and-eggs

3 flavours of DNA methylation



CG

Methyltransferase (MET1)

Correlated with constitutively expressed genes

CHG

Histone H3 lysine 9 dimethylation (H3K9me2)

Correlated with TEs, non-constitutively expressed genes, and heterochromatin

CHH

RNA-directed DNA Methylation (RdDM) (small RNA)

Assaying DNA methylation: bisulfite sequencing (BS-seq)

Template DNA	GA**C**TGA**C**TG**CG**
	↓
Bisulfite conversion	GA**U**TGA**U**TG**CG**
	↓
PCR	GA**T**TGA**T**TG**CG**
	↓
	GA**T**TGA**T**TG**CG**
Genome alignment	and
	GA**C**TGA**C**TG**CG**

Contributing a Composite methylome

- Several plant methylomes have been published (e.g., *A. thaliana, O. sativa*, and *Z. mays*)

- Many novel (29 *sp*.) methylomes will soon be available (Chad E. Niederhuth, Adam J. Bewick, Bob J. Schmitz*, *et al*.)

- A Composite is missing from the list – How does the DNA methylation landscape compare to other species, and between tissues within a Composite?

Shameless self-promotion…

http://schmitzlab.genetics.uga.edu/plantmethylomes

Barebones workflow

- **HA412 seedling** (4 true leaves), and **ray** floret gDNA
- Illumina **NextSeq** PE 75bp; alignment to *bronze* assembly using **methylpy** (bowtie2)
- **TE**: RepeatMasker using sunflower LTR library (Staton *et al*., 2012); **Coding**: annotation v1.1 (INRA) vs. CDS set (Rieseberg) reciprocal best BLAST
- Assessing patterns of, and associations with DNA methylation between tissues, and species

Tissue collection

↓

Sequencing and alignment

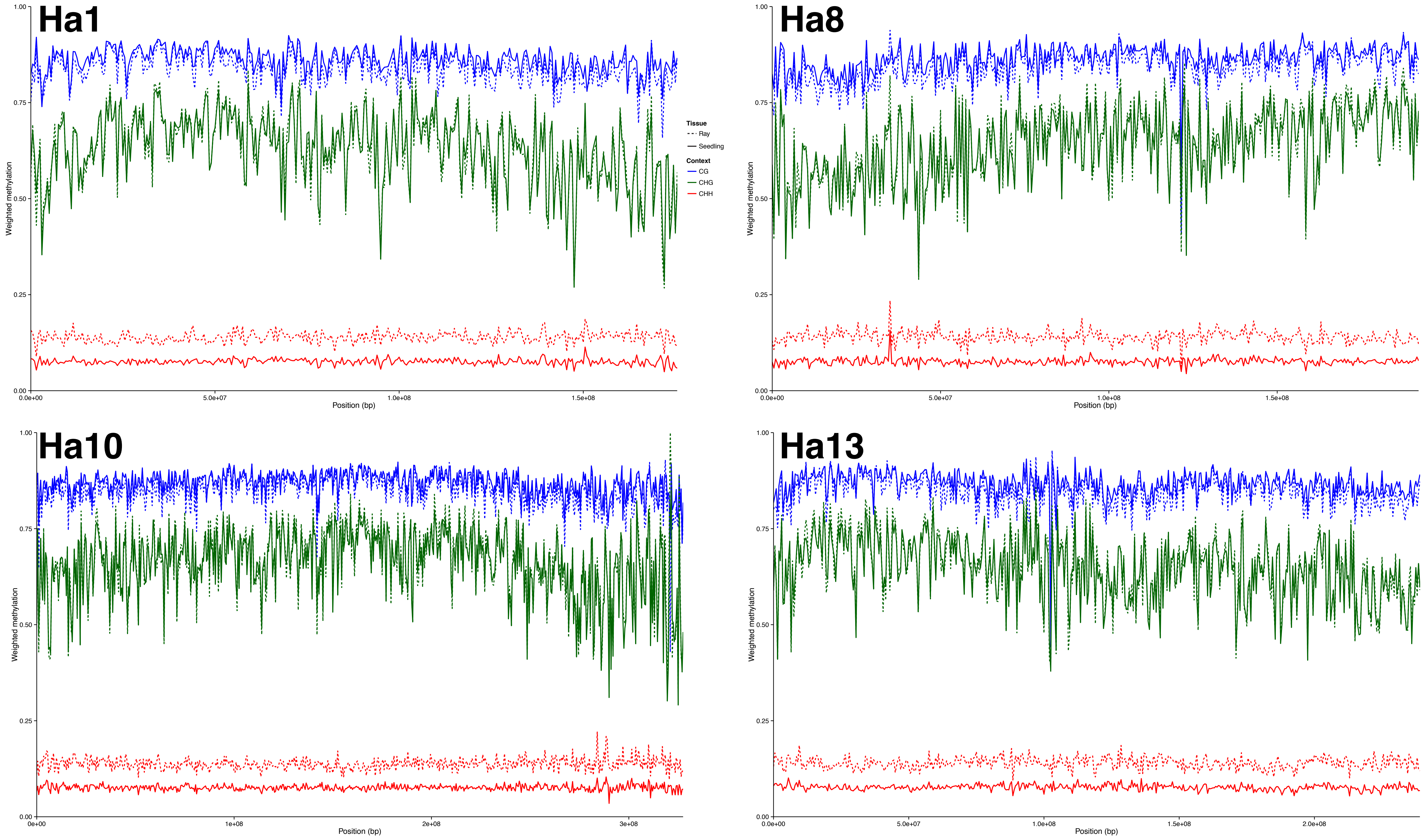
↓

Extract genomic features

↓

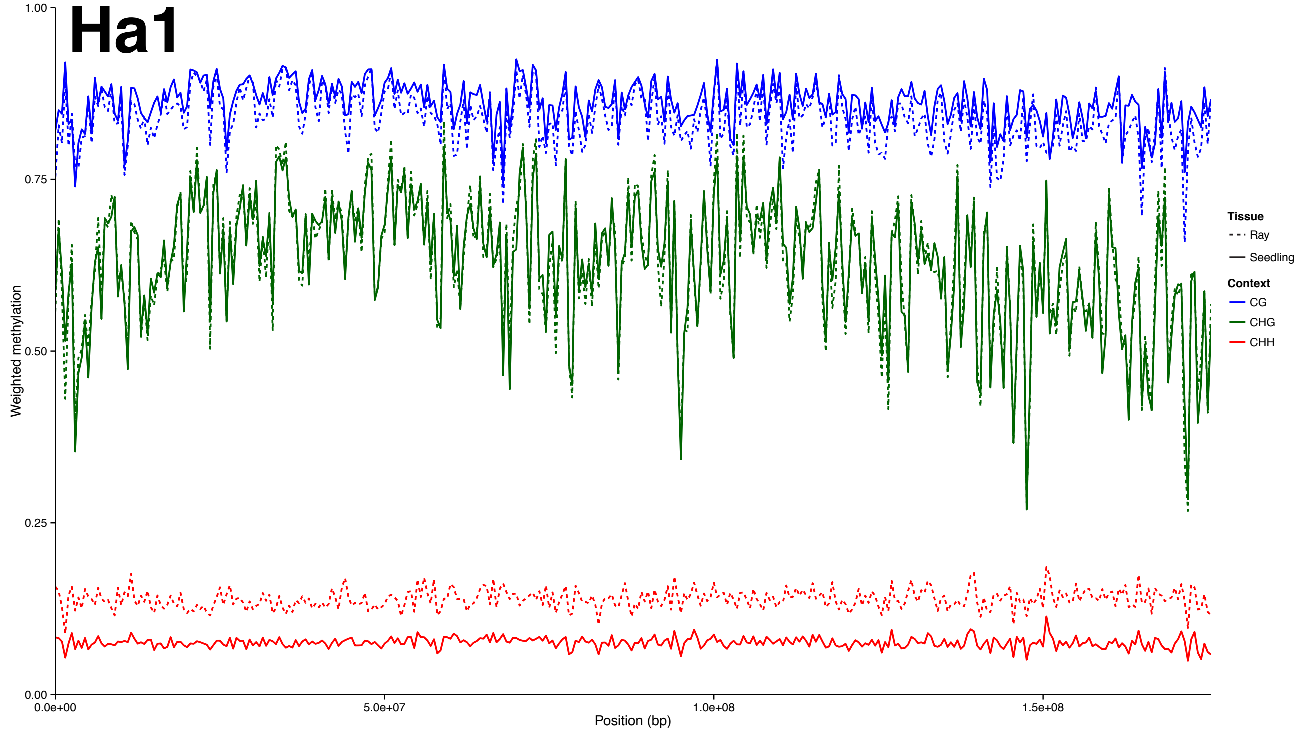
Analyses

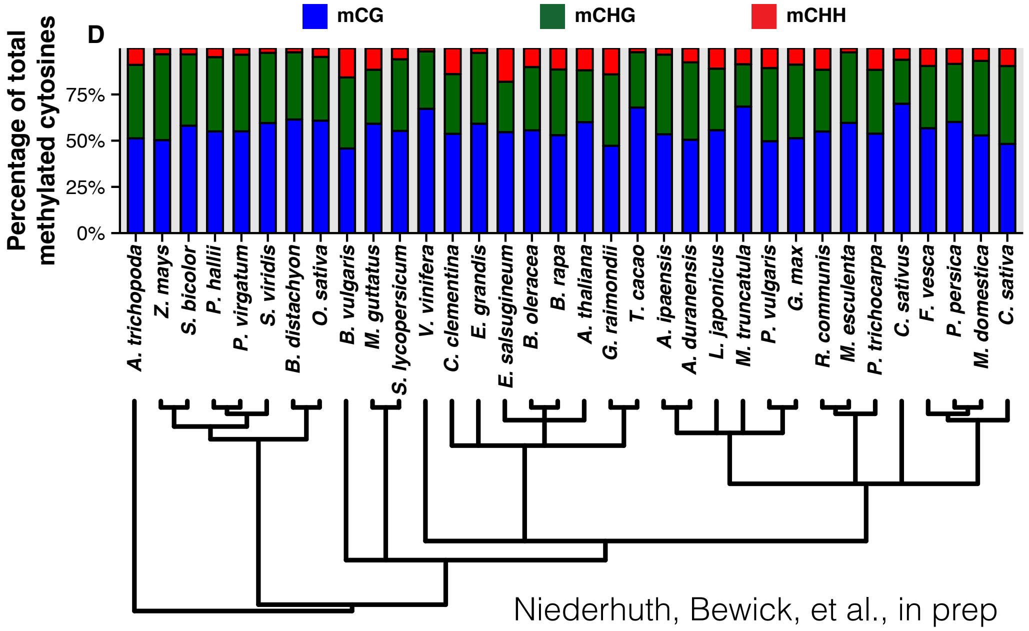
Ray CHH < seedling CHH across chromosomes



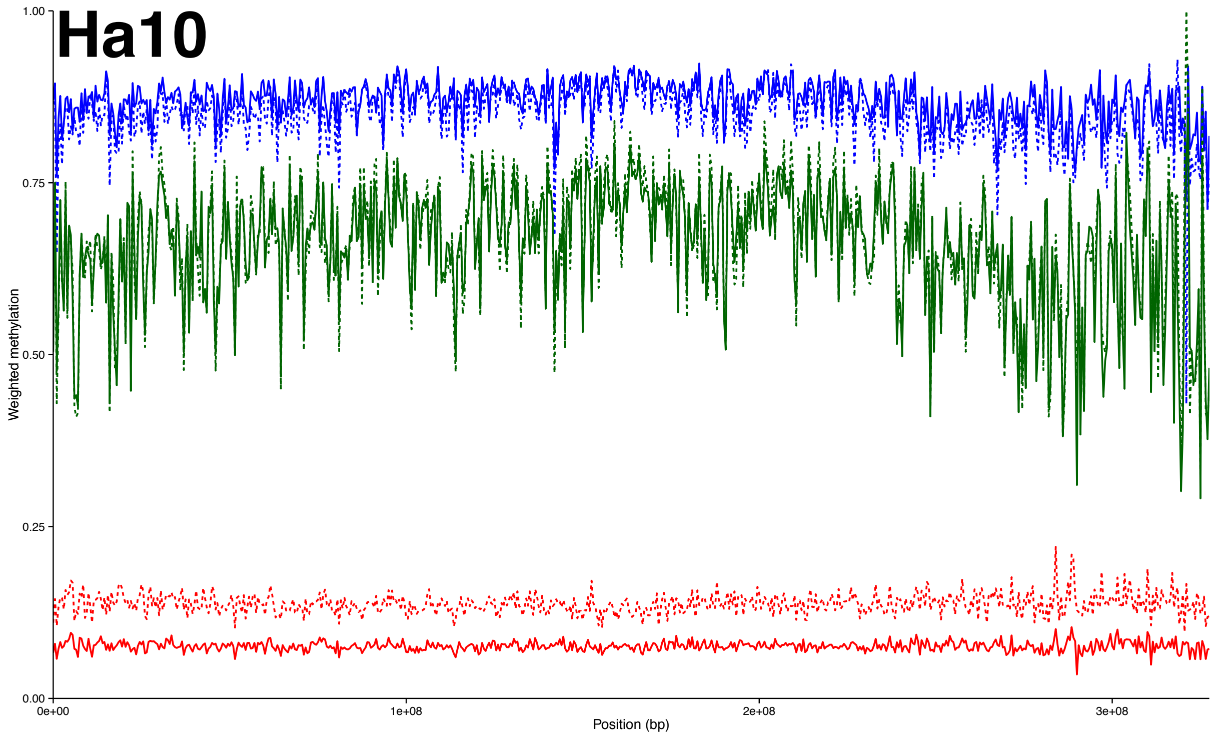
Weighted methylation = $\text{Reads}_{\text{methylated}}$ / $\text{Reads}_{\text{total}}$

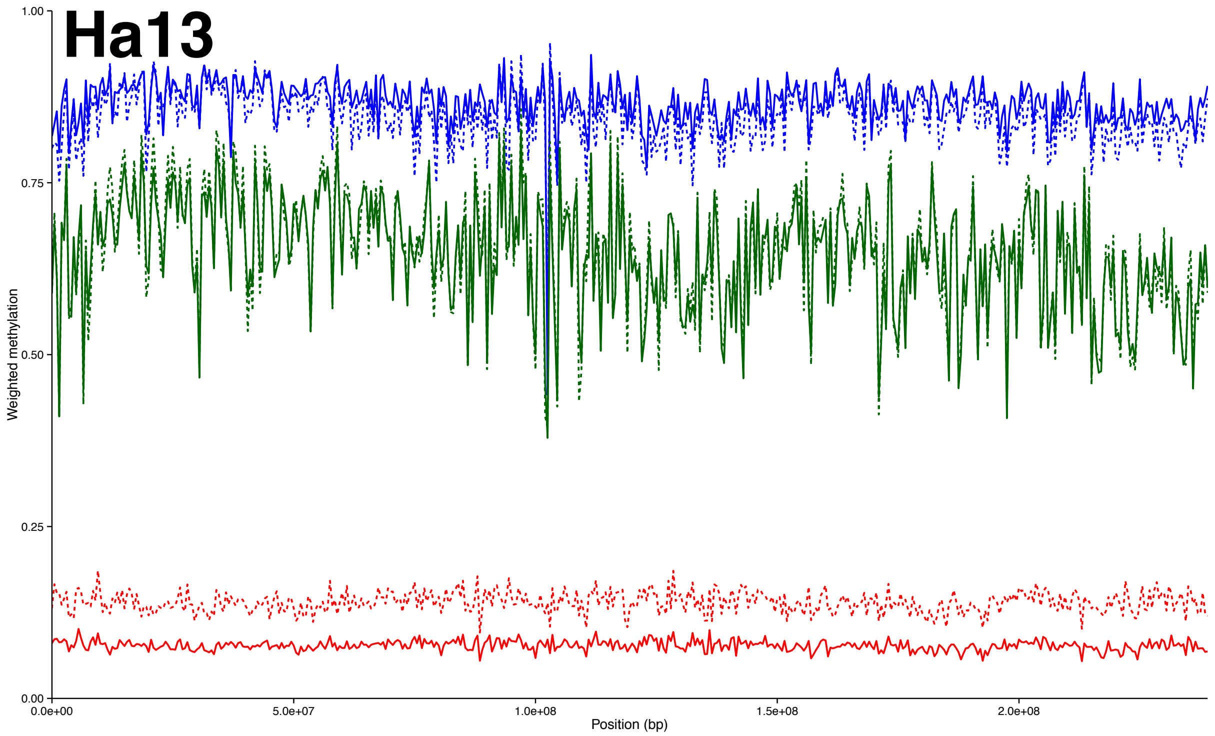
Ray CHH < seedling CHH across chromosomes





Niederhuth, Bewick, et al., in prep





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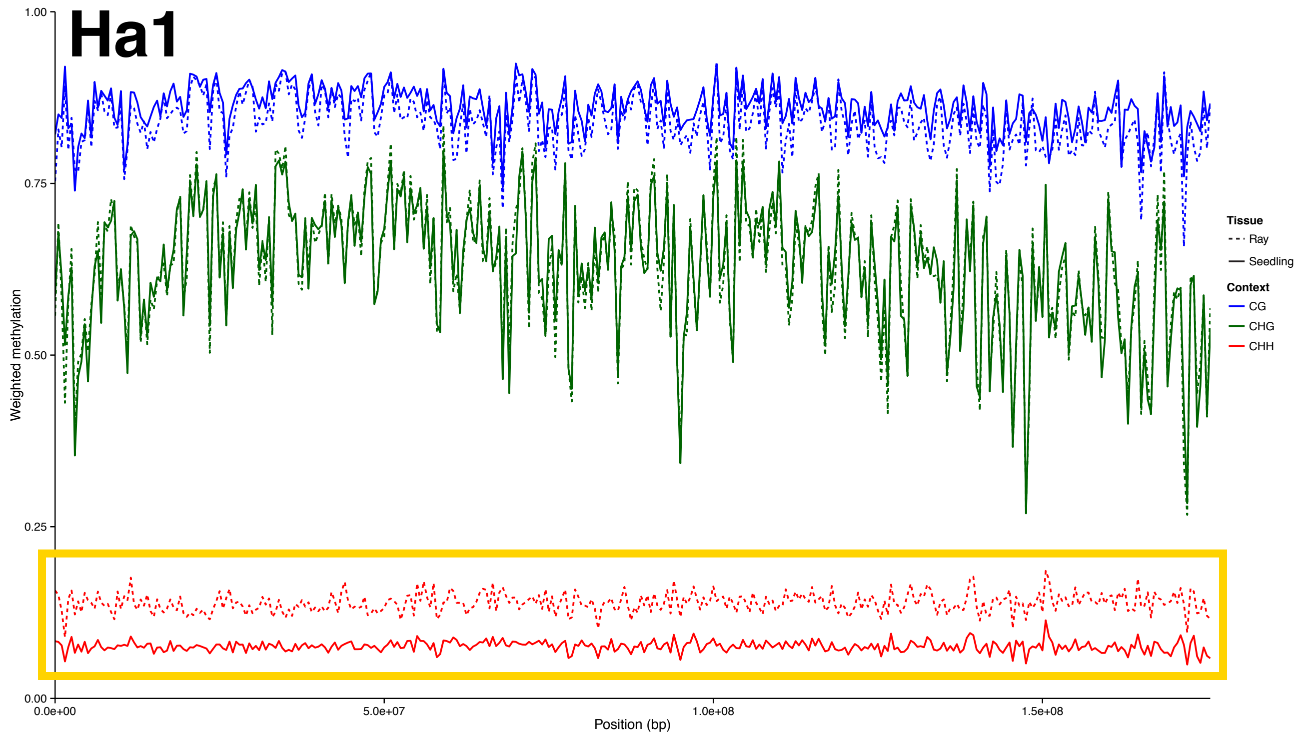

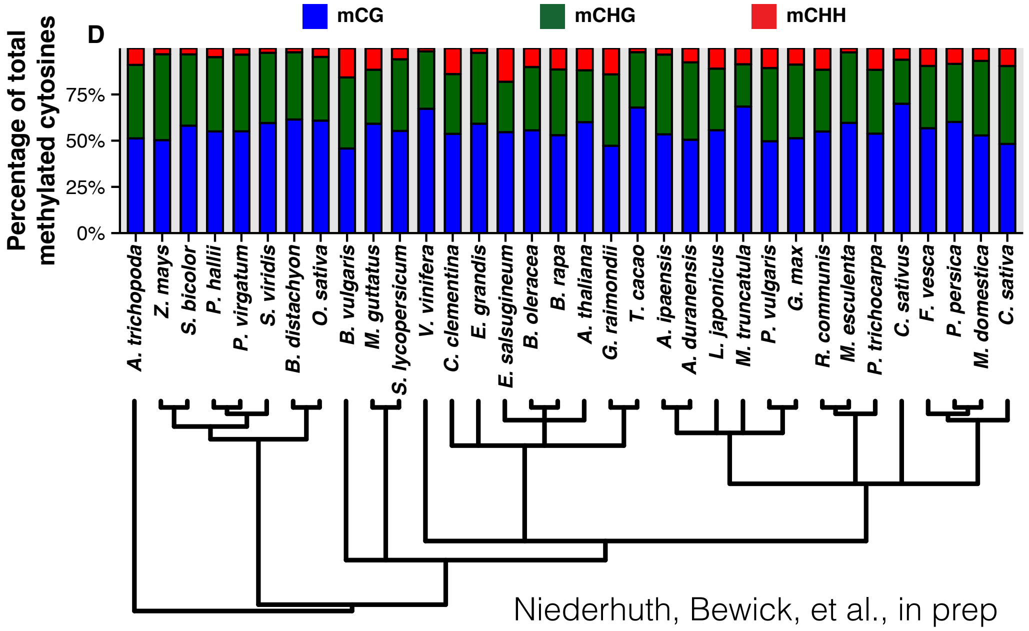

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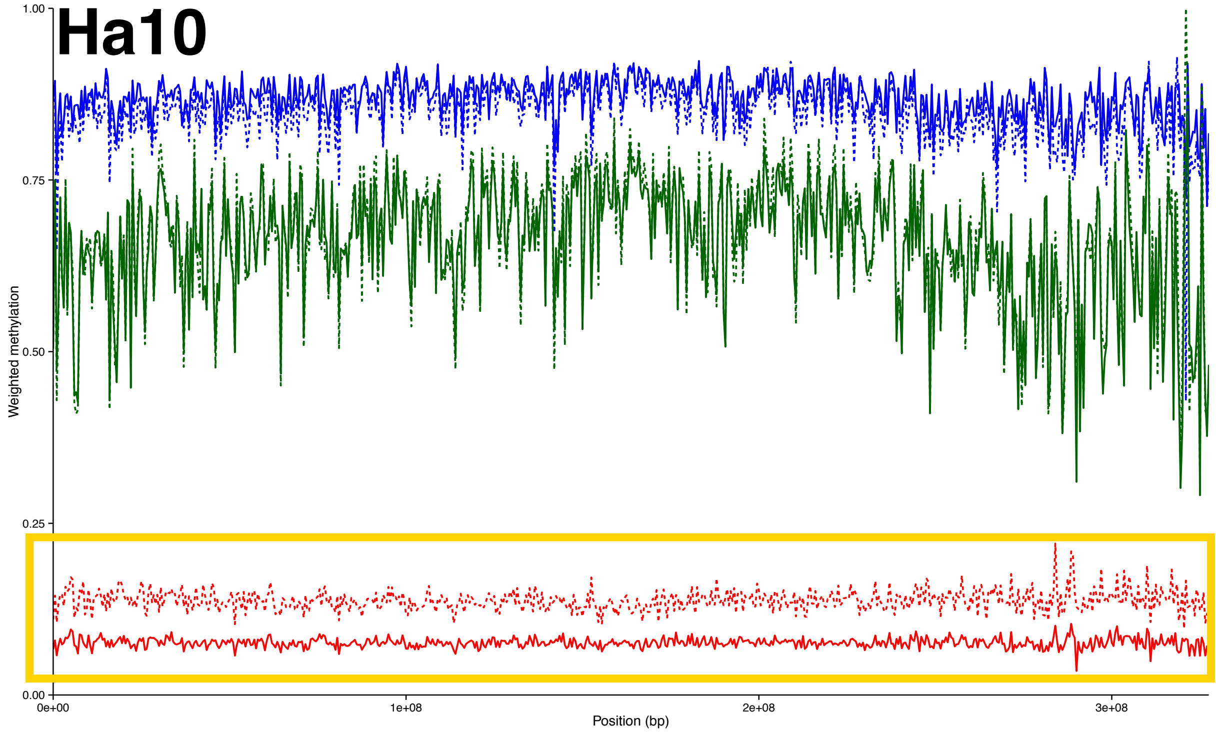
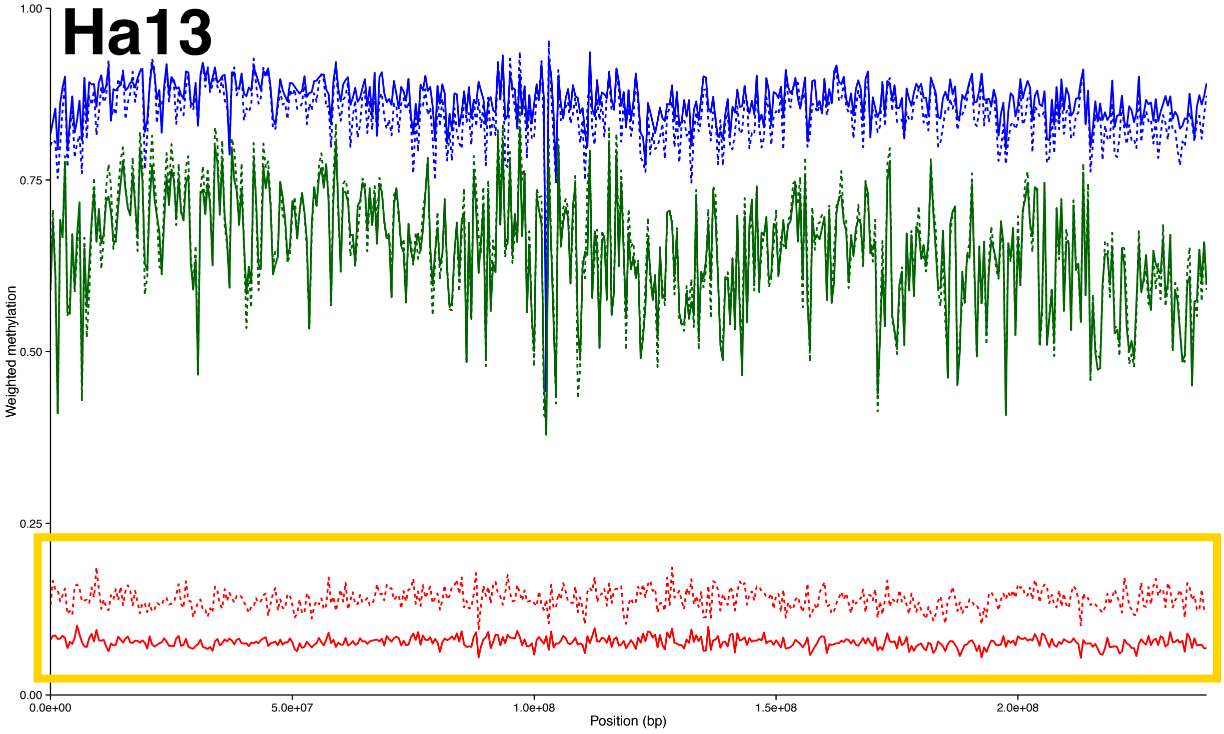
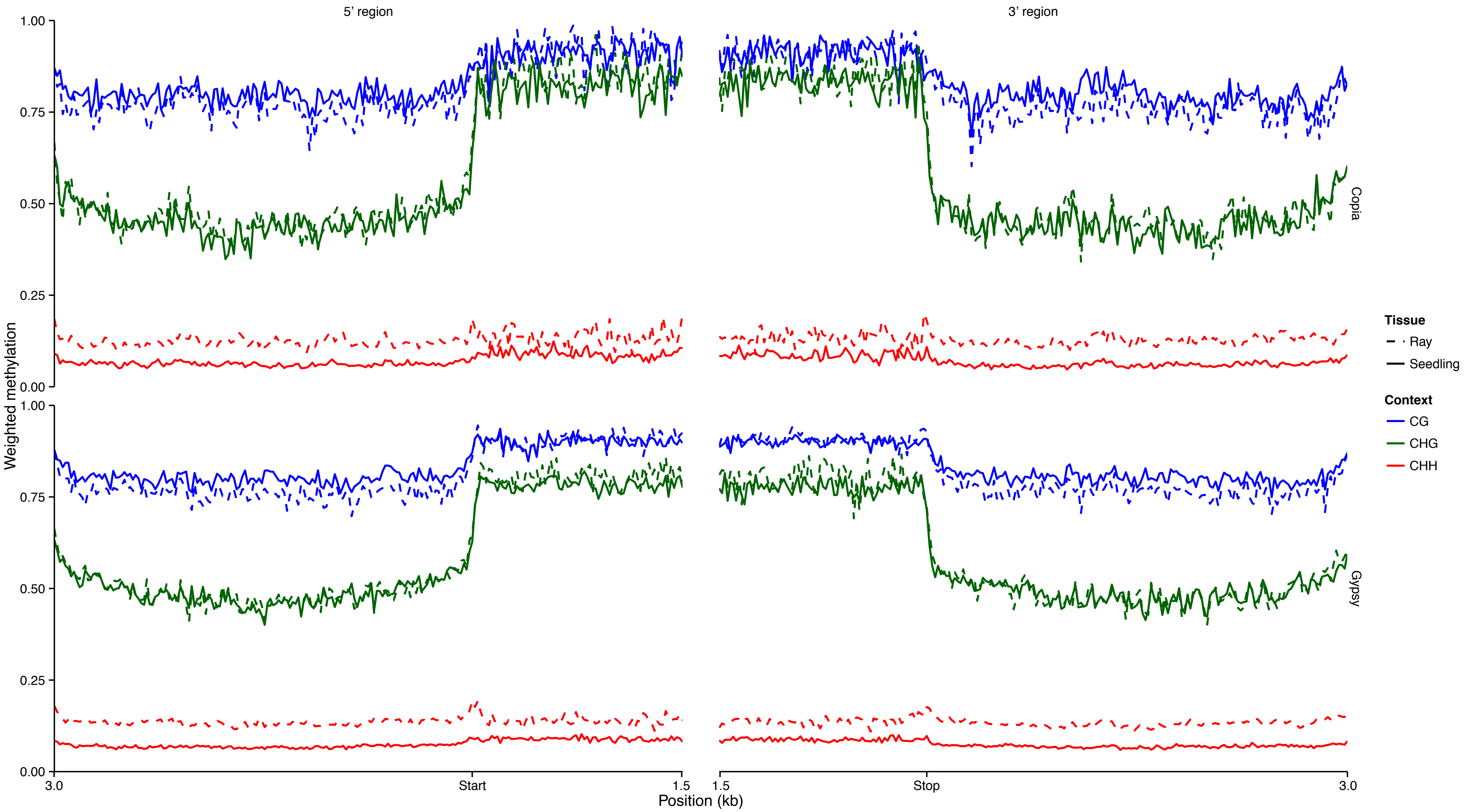


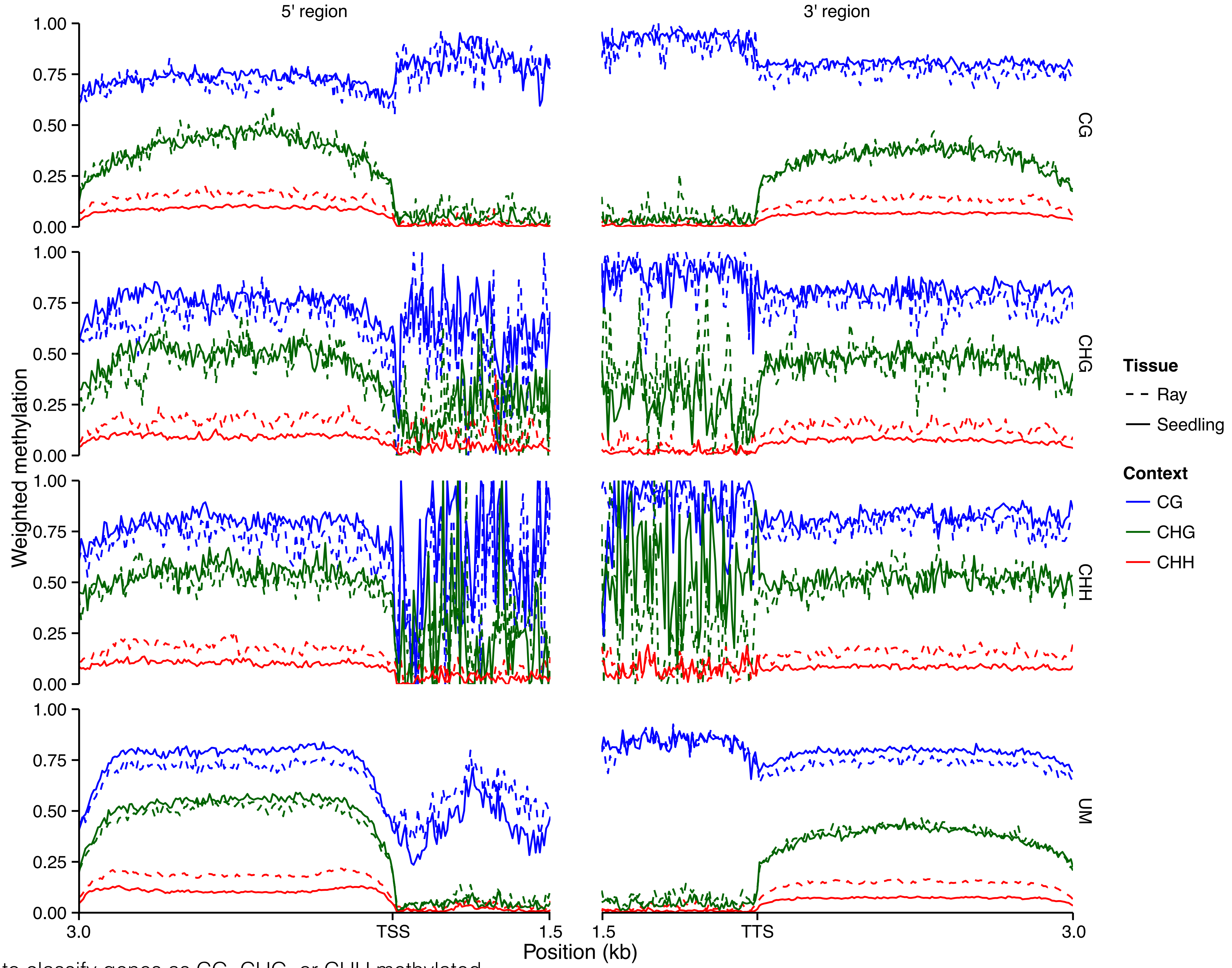


Weighted methylation = $\text{Reads}_{\text{methylated}}$ / $\text{Reads}_{\text{total}}$

CHH gap is present within, and flanking Copia and Gypsy LTRs

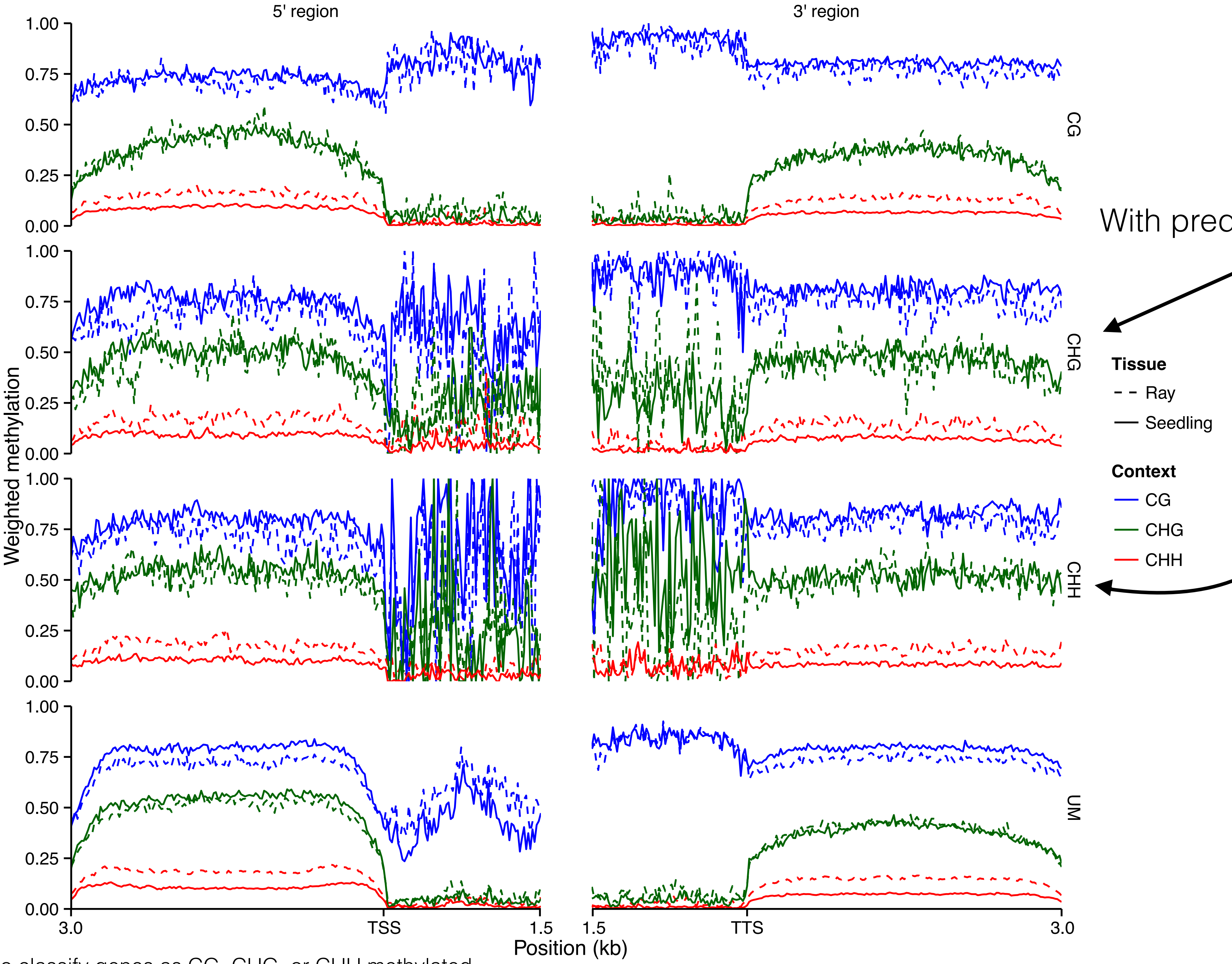


CHH gap qualitatively diminishes within genes



Binomial statistics were used to classify genes as CG, CHG, or CHH methylated

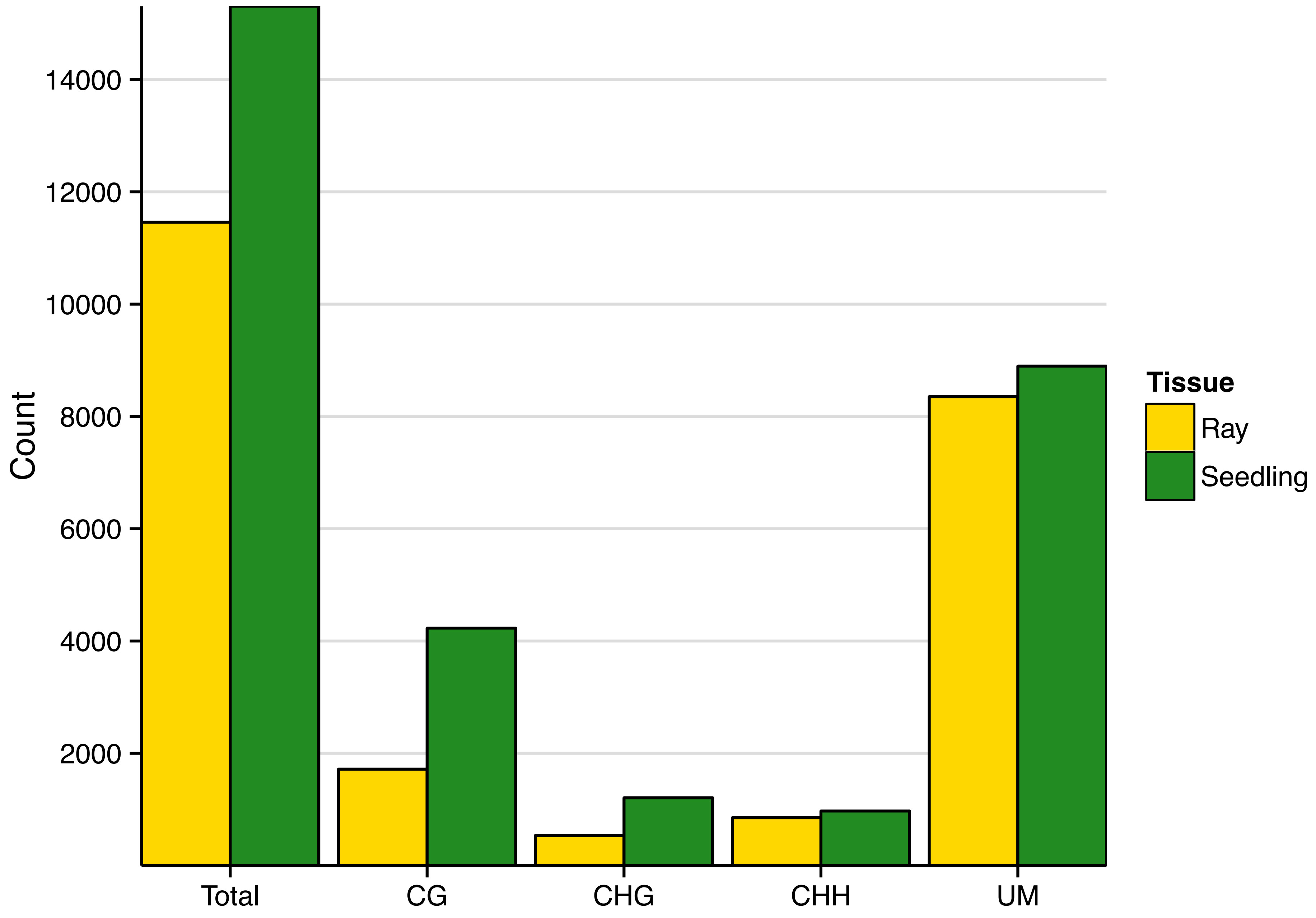
CHH gap qualitatively diminishes within genes



With predicted exceptions…

Binomial statistics were used to classify genes as CG, CHG, or CHH methylated

Similar number of methylated and unmethylated genes between tissues



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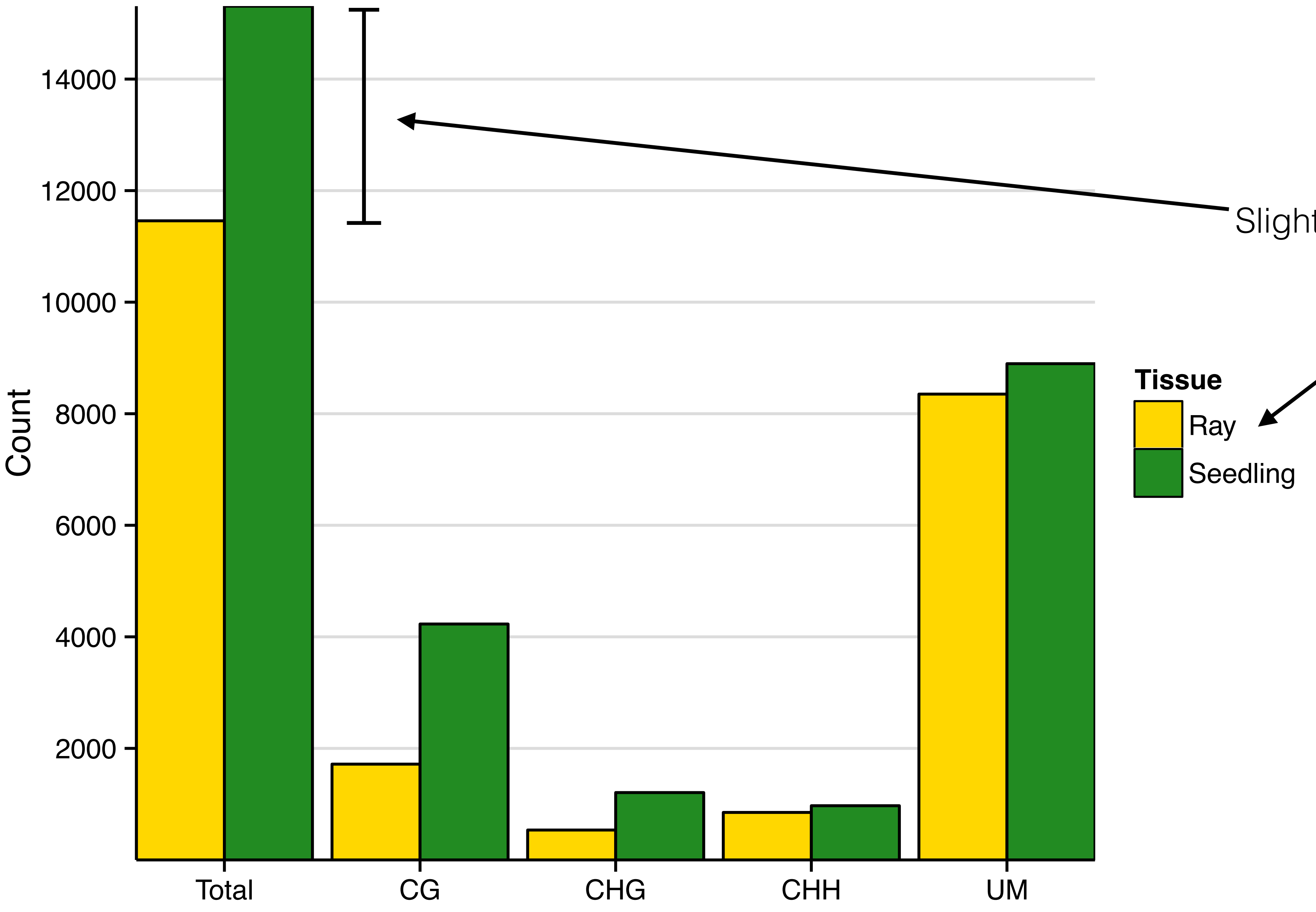
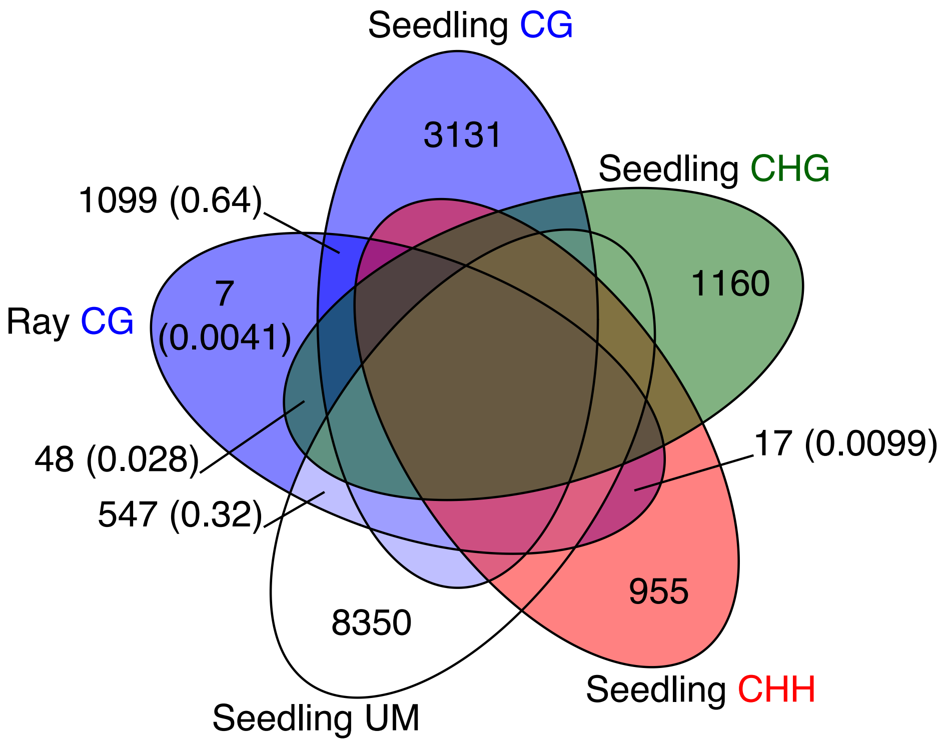
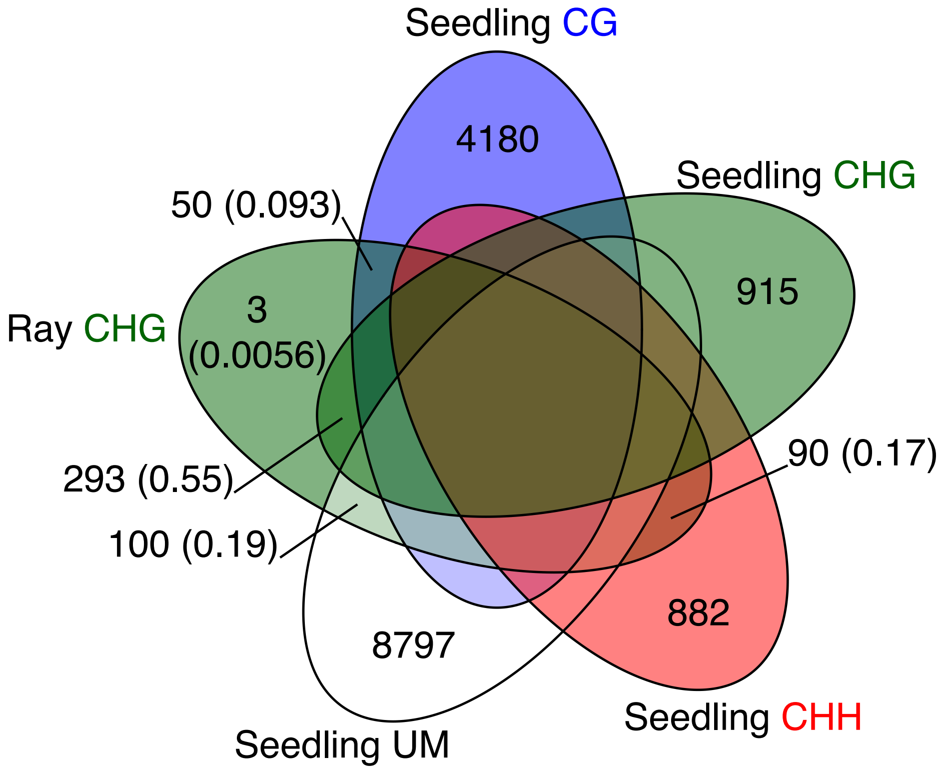


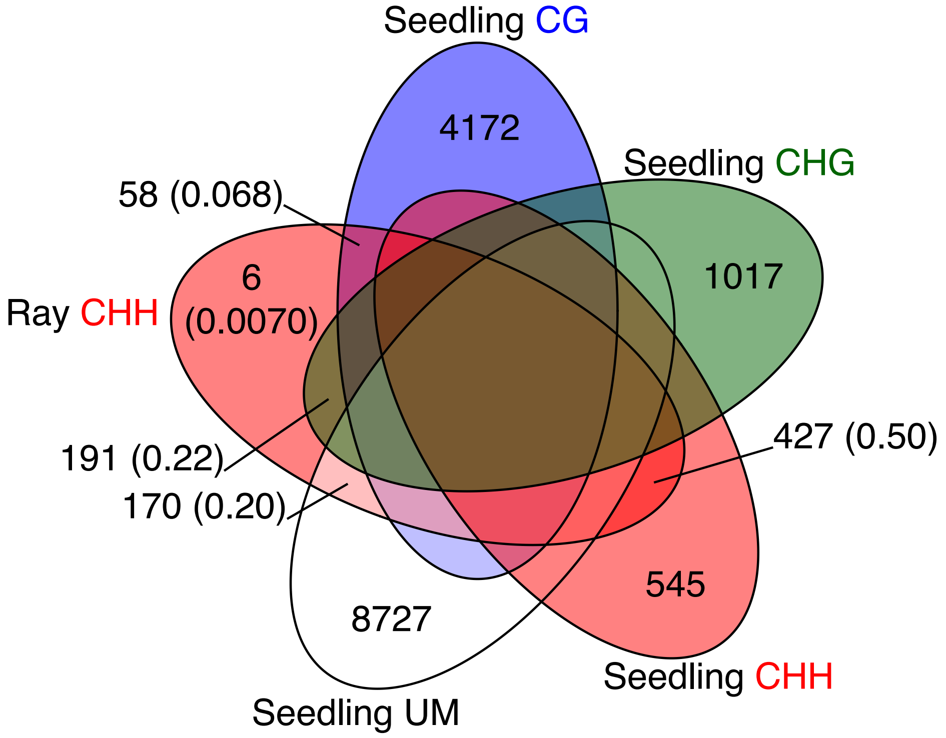
Slightly lower coverage
3.6 vs 5.2X

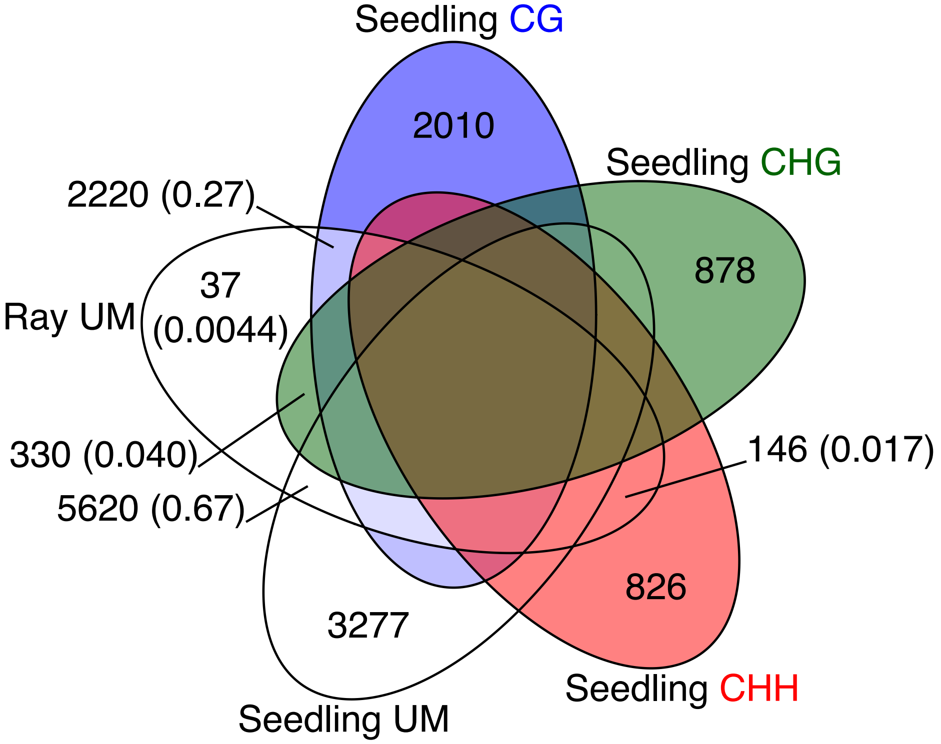
Binomial statistics were used to classify genes as CG, CHG, or CHH methylated

Genes switch methylation context between tissues









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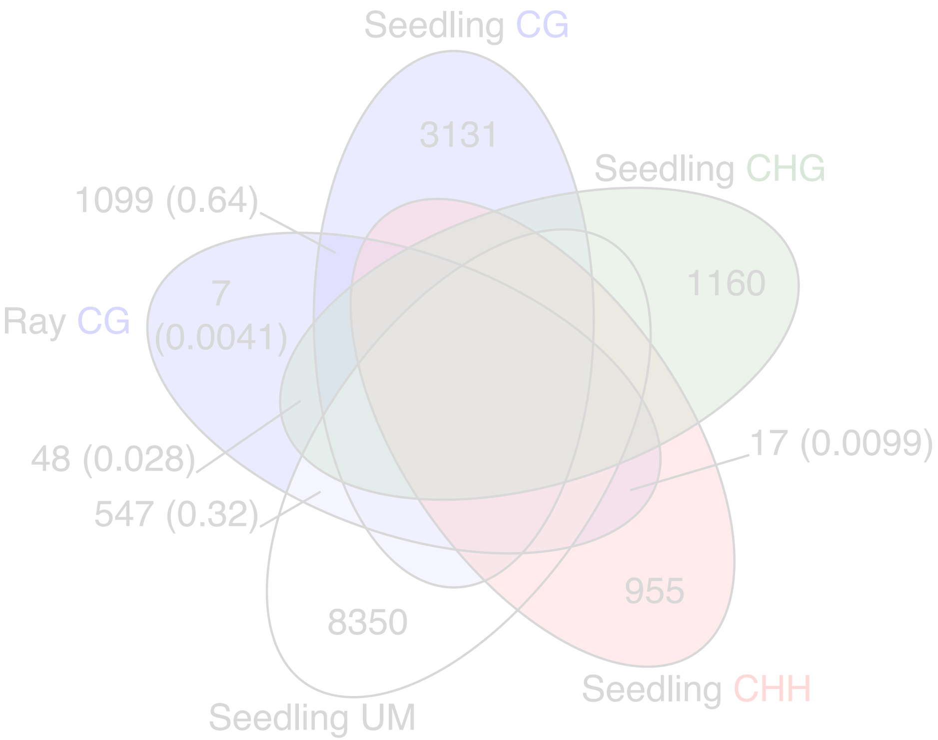
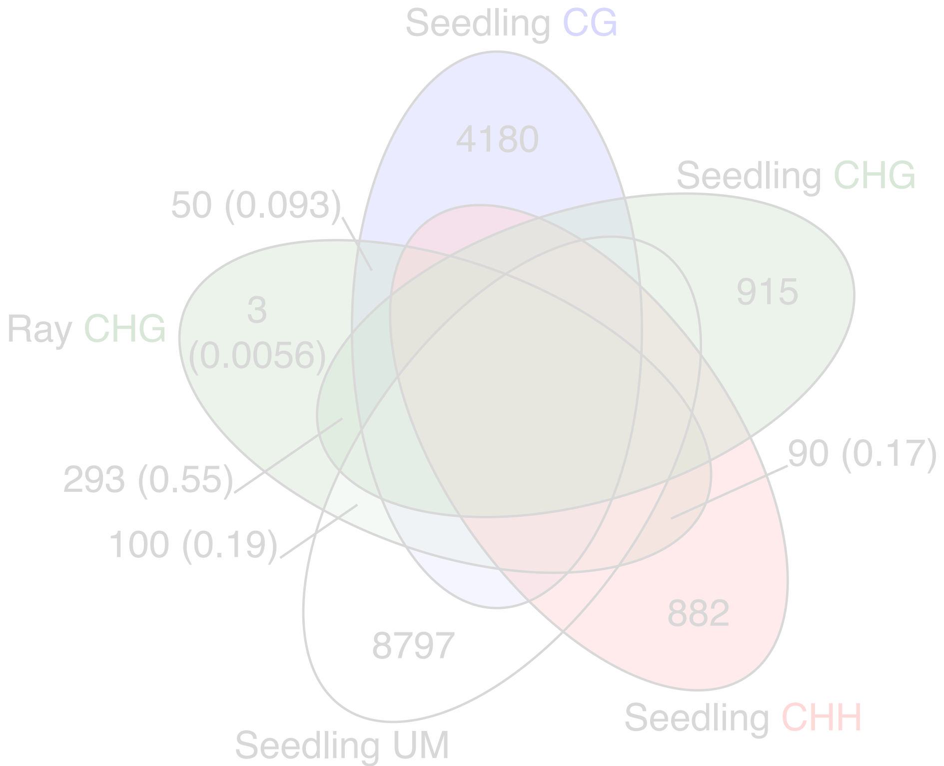
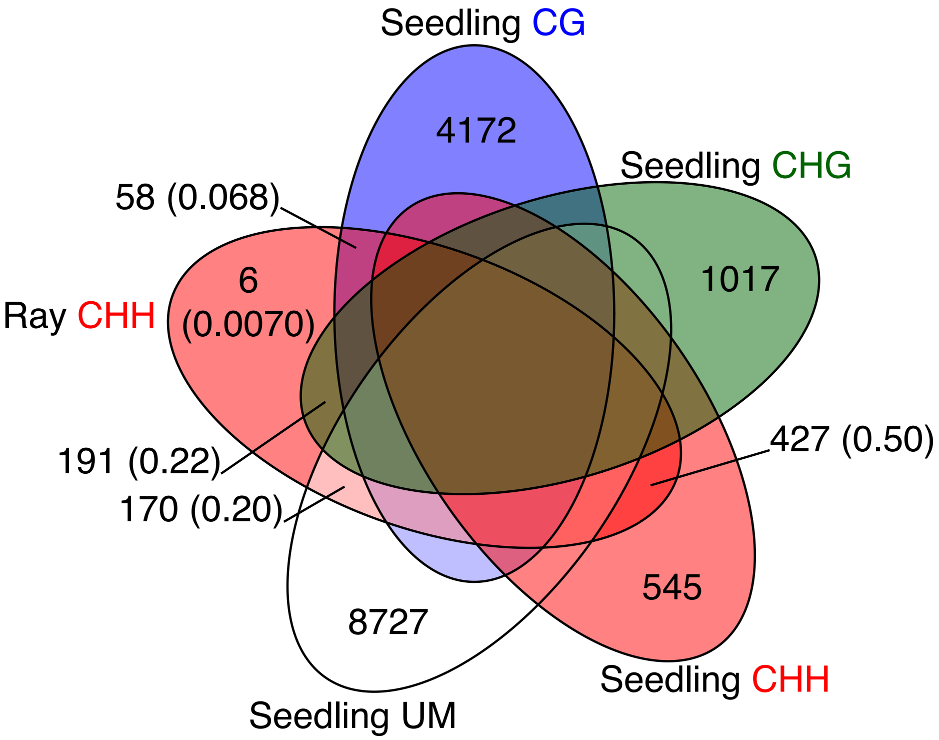
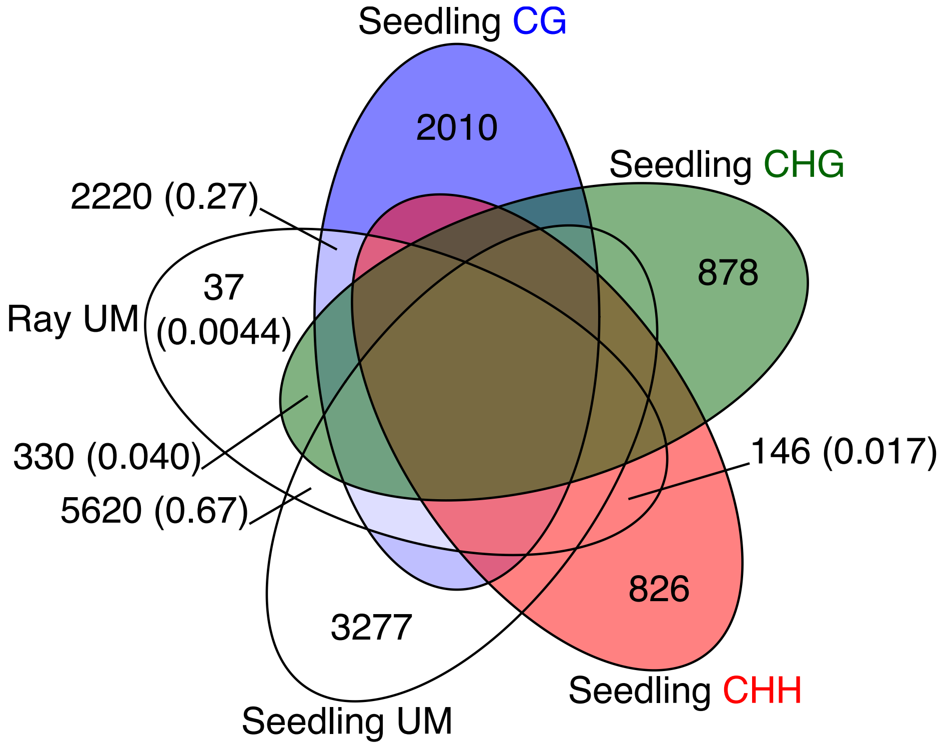
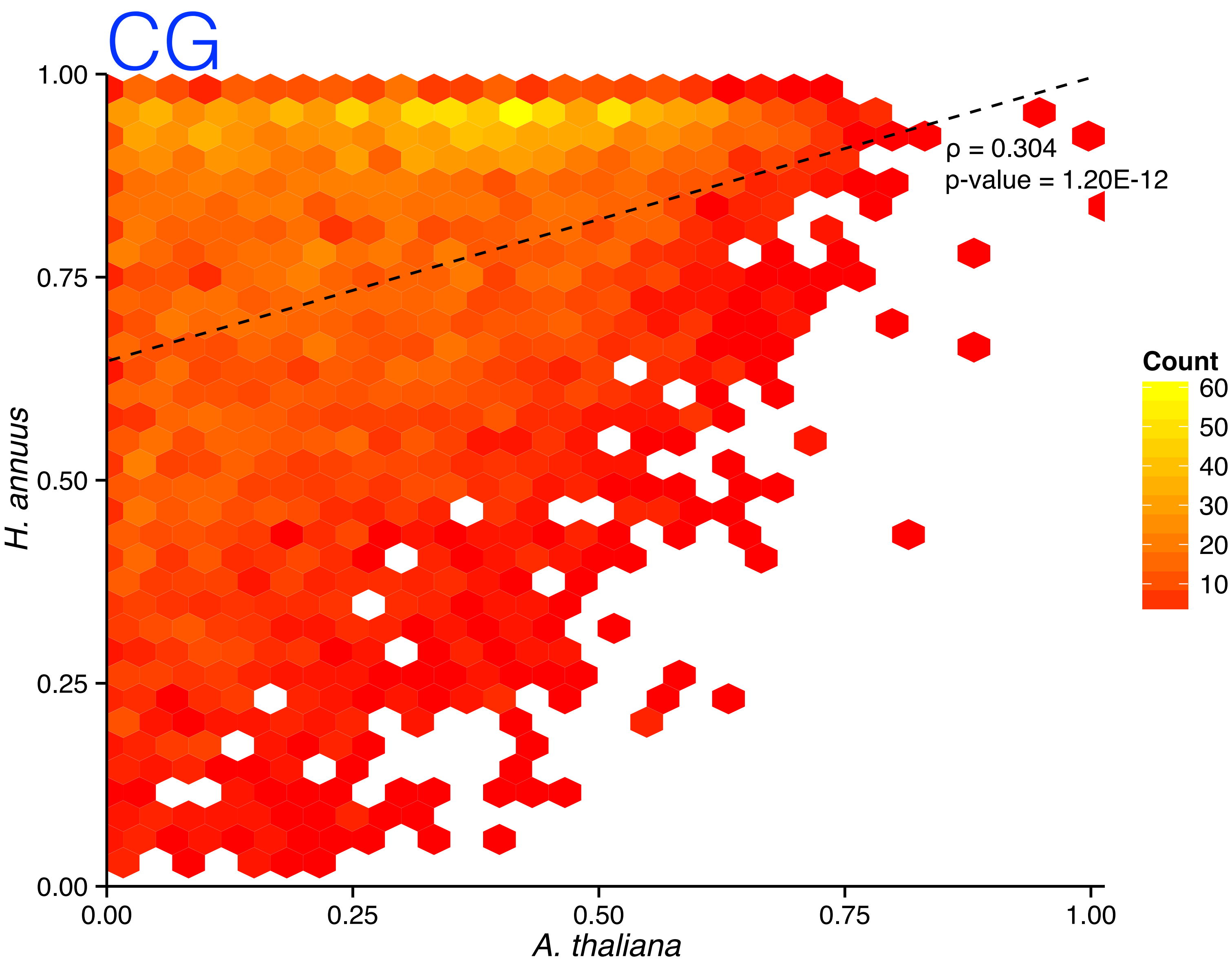


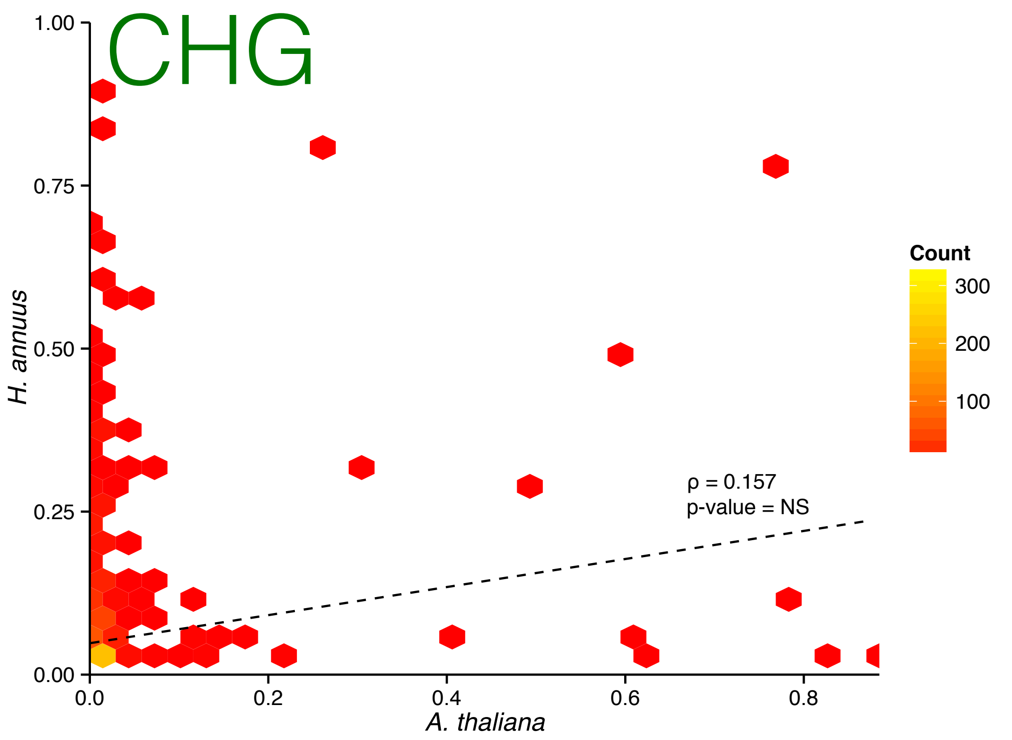


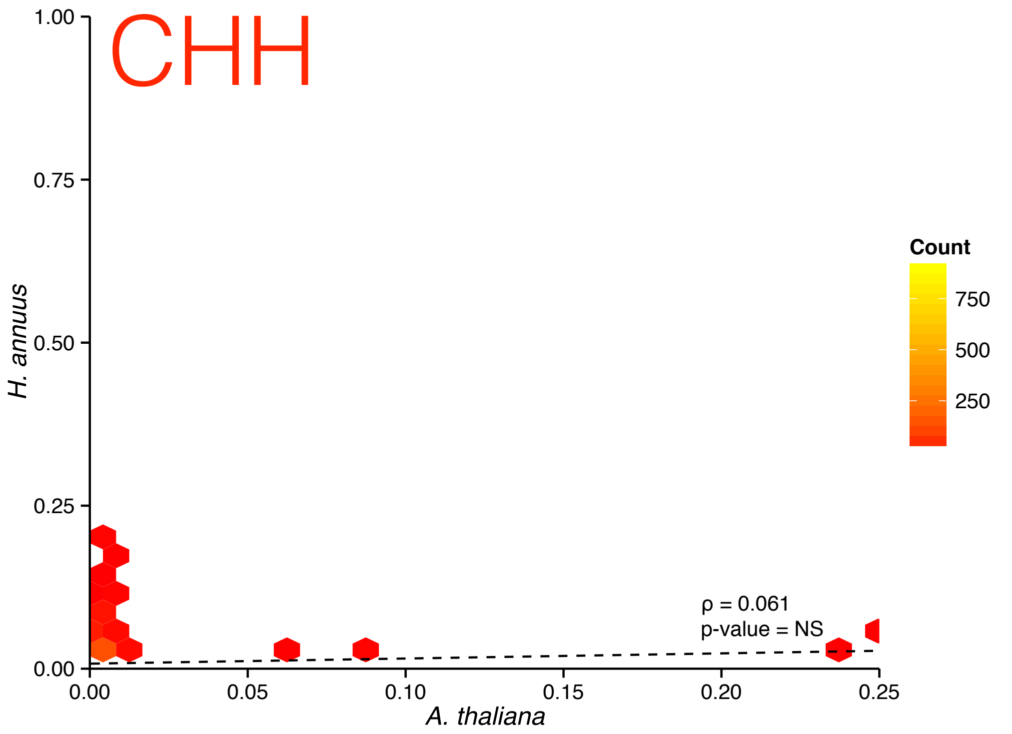




CG methylation is conserved (seedling)

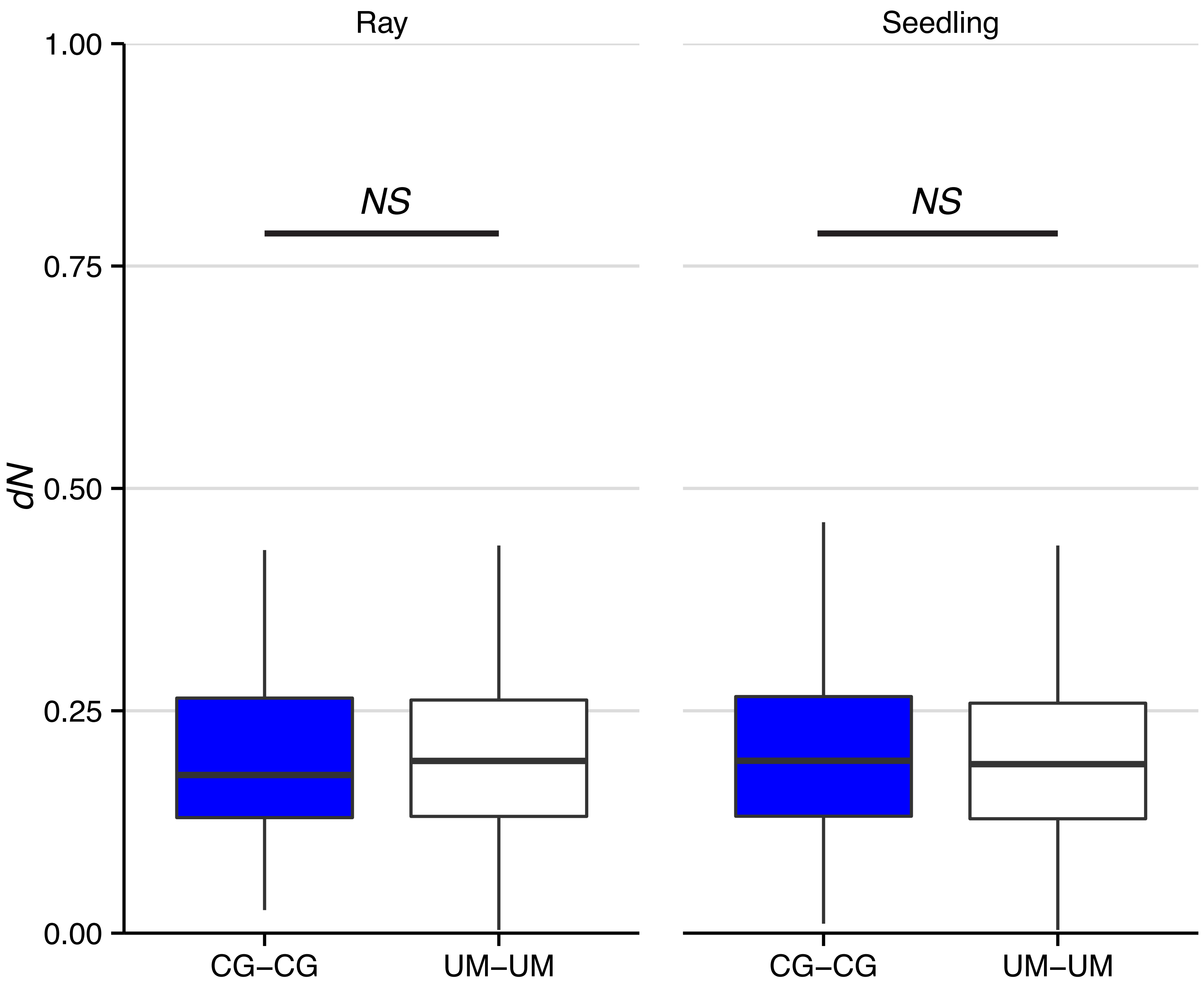






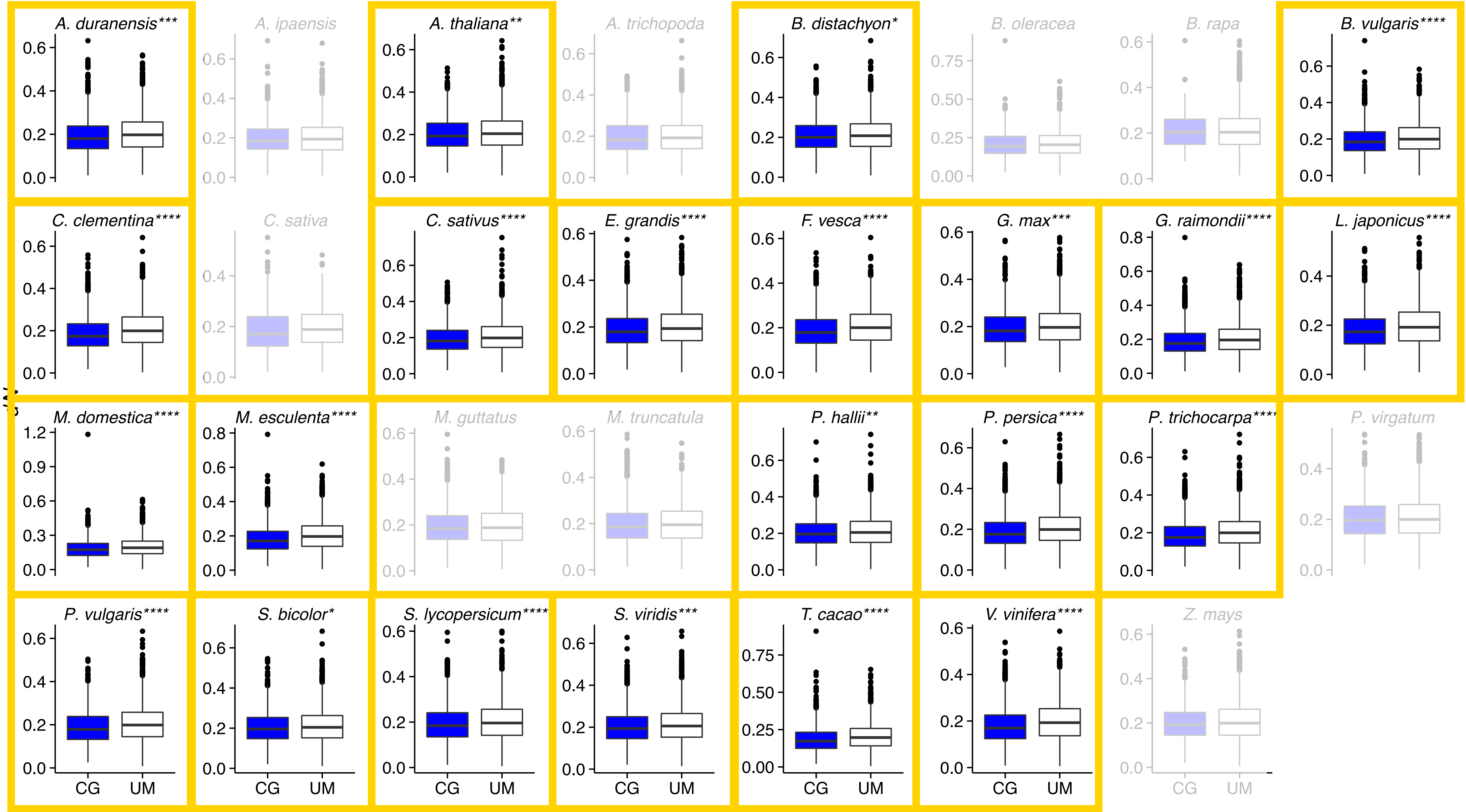
P-values were Bonferroni corrected

CG and UM genes have approximately the same *dN* (seedling)



Permutation test for 100,000 generations using *A. thaliana* as the outgroup

However, in general CG genes have lower *dN* than UM

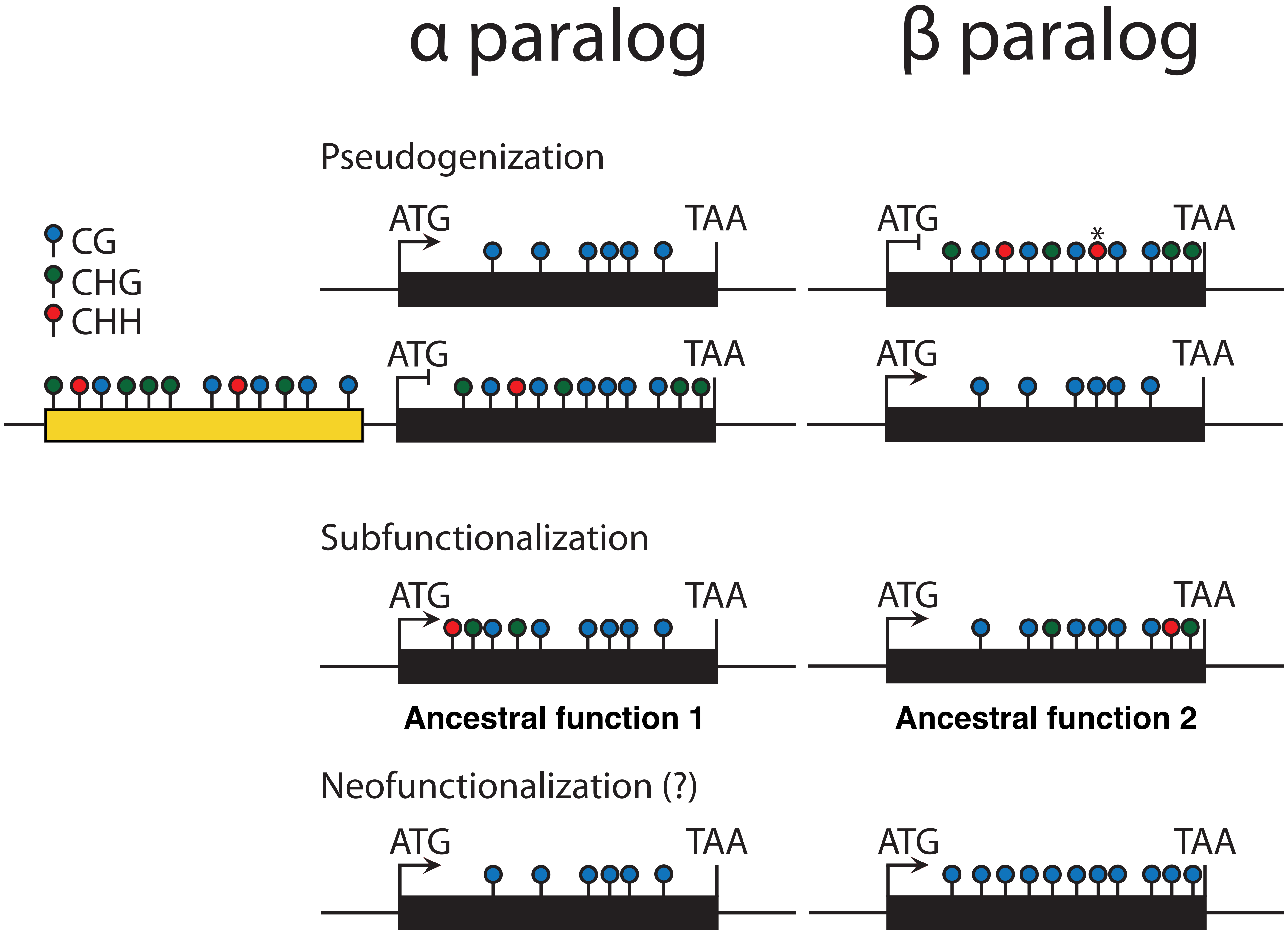


Niederhuth, Bewick, et al., in prep

Overview

- DNA methylation is similar/typical in sunflower compared to other plants
- Differences in CHH methylation, and switching of methylation context between tissues, concrete explanations for which remain elusive for the time being – non-genic features may be a factor for the former
- **Future directions**: the effects of whole genome duplication (WGD), and its consequences on the methylome

Hypothesized outcomes of WGD, and DNA methylation



α paralog

β paralog

Pseudogenization

Subfunctionalization

Ancestral function 1

Ancestral function 2

Neofunctionalization (?)

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Georgia Advanced Computing Resource Center (GACRC)