The *Arabidopsis* chromatin remodeling ATPase, *CHR23*, demonstrates a novel parental effect on seed size

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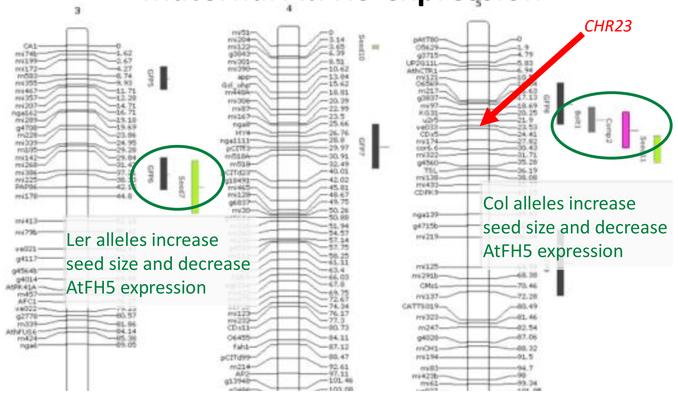
Is Parental Genomic Imprinting Adaptive?

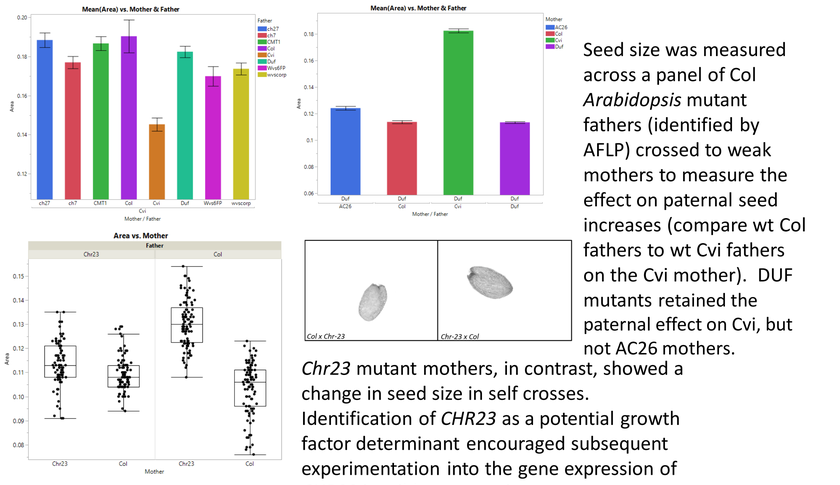
The genetics of seed size is confounded by the strong parental contributions to seed growth and development. For example, using Columbia (Col) ecotype pollen on C24 ovules produces an atypically large seed whereas the reverse cross produces a normal seed. This is partly explained by strong parental genomic imprinting of genes expressed in the endosperm, like *AtFH5*. Although Histone and DNA methylation pathways are both involved in seed size, specific realizators of the parental programs are not known. Using DNA methylation-sensitive AFLP, a screen was conducted for cis and trans-regulators of differential DNA methylation between Col and C24. A locus was identified, *CHR23,*that shares homology with SWI/SNF2-type chromatin remodeling ATPases. *CHR23* has been implicated in cell growth and the regulation of gene expression, though most of its function remains uncharacterized. Crossing *chr23-1* mutants and wild-type Col *Arabidopsis* revealed larger seeds when *chr23-1* was the maternal factor, larger seeds were seen. Surprisingly, homozygous *chr23-1* seeds appeared in all ways normal. Analysis continued by isolating mRNA from crosses and using reverse transcriptase (RT) and PCR to amplify expression and identify genes. Next, we will verify *CHR23* expression and further identify parental contribution to *Arabidopsis* seed size.

Paternal QTL influence seed size and maternal *AtFH5* expression



ColxLer RI lines were crossed as fathers to C24 KS117 which reports expression of the maternally expressed *AtFH5*. Size and GFP expression of the resulting seeds were scored independently. Resulting trait averages were used to generate the QTL map shown. Two indicated loci provide increased seed size in combination with reduced expression.

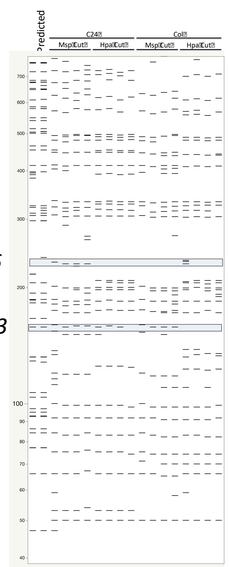
Parental effect on seed size screened among reciprocally-crossed *Arabidopsis* species



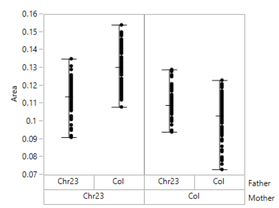
Seed size was measured across a panel of Col *Arabidopsis* mutant fathers (identified by AFLP) crossed to weak mothers to measure the effect on paternal seed increases (compare wt Col fathers to wt Cvi fathers on the Cvi mother). DUF mutants retained the paternal effect on Cvi, but not AC26 mothers. *Chr23* mutant mothers, in contrast, showed a change in seed size in self crosses. Identification of *CHR23* as a potential growth factor determinant encouraged subsequent experimentation into the gene expression of *CHR23* in wild-type species.

A methylation sensitive AFLP strategy to track *cis* and *trans* regulations identified *CHR23*

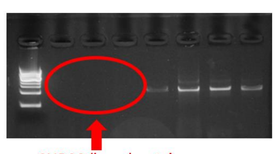
In our preliminary analysis we identified targets that demonstrate differential patterns of regulation. The figure to the left represent AFLP selective PCRs using EcoTG-MspTTC and EcoAA-MspTGG primer pairs, respectively. In a few cases, there are easily discernable bands based on predicted sequence polymorphisms from genomic data (TAIR, 1001genomes.org). Interestingly, PRT6 is involved in seed after-ripening and sugar sensitivity (Holman et al., PNAS **106**(11)p.4549) and a C24 polymorphism introduces a N->I missense mutation in the DNA methylated background. *CHR23* does not display a sequence polymorphism, but is differentially methylated in the Col background. Consistent with the repressive Col background, overexpression of *CHR23* has been shown to increase gene expression variability (Folta et al. BMC Plant Bio **14**:76.

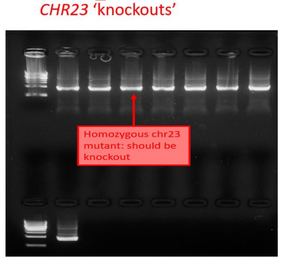


Determining *CHR23* parental influence and verifying 'knockout' expression



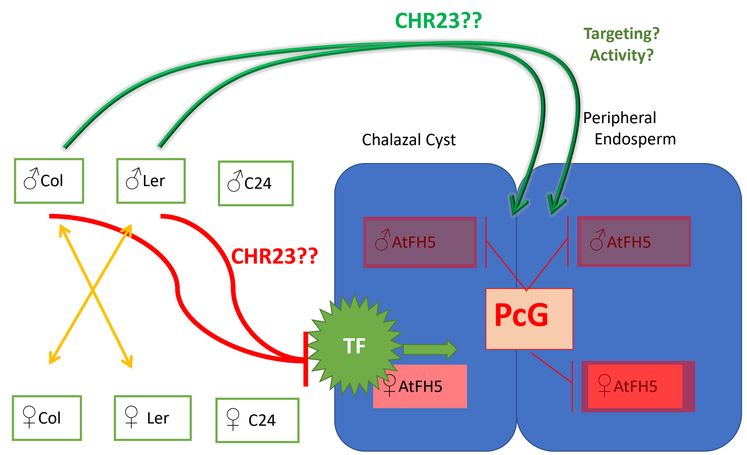
Component	Var Component	% of Total
Mother	0.00003445	20.8
Father	0.00001181	7.1
Mother*Father	0.00002904	17.5
Within	0.00009051	54.6
Total	0.000016581	100.0





The differences in seeds sizes between chr23-1 mutants and wild type were considered significant by two-way ANOVA ($p<0.0001$ for either). Computed variance components suggests that maternal effects contributed most to the size of the seed (20.8% of overall variation) while the paternal genotype was responsible for only 7% of total size variance, supporting the theory that *CHR23* is a significant player in seed size development. It was verified using Chr23-RTF2 primer pairs that *CHR23* was not expressed in the mutants, but was, however, expressed in wild-type Col and Duf, as expected. Continued efforts to understand *CHR23* expression involves the amplification of the *CHR23* coding domain in knockouts and wild-type *Arabidopsis* crosses using Chr23-RTF2 and Chr23-MM primers.

Our hypothesis: Imprinted and Positional Regulation of *AtFH5* expression



The phenotype of CUT4 expression in the segregating Col/Ler and C24 backgrounds le our current model. Considering C24 as our "wild type". PcG enforces expression limited to maternal alleles in the chalazal cyst. Euchromatin is still subject to normal transcriptional regulation and can be repressed in trans by paternal Col/Ler inhibitors. F2 segregants that fail to target AtFH5 to the chalazal pole (not shown) suggest that regulation in Col/Ler maybe more complicated, involving possible cis and trans regulation to ensure proper PcG targeting. But which pathway is *CHR23*?