

Mapping a Suppressor of *dmc1-2* in *Arabidopsis thaliana*

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Senior Honors Thesis  
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Senior Honors Thesis  
April 5, 2015

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**Abstract:**

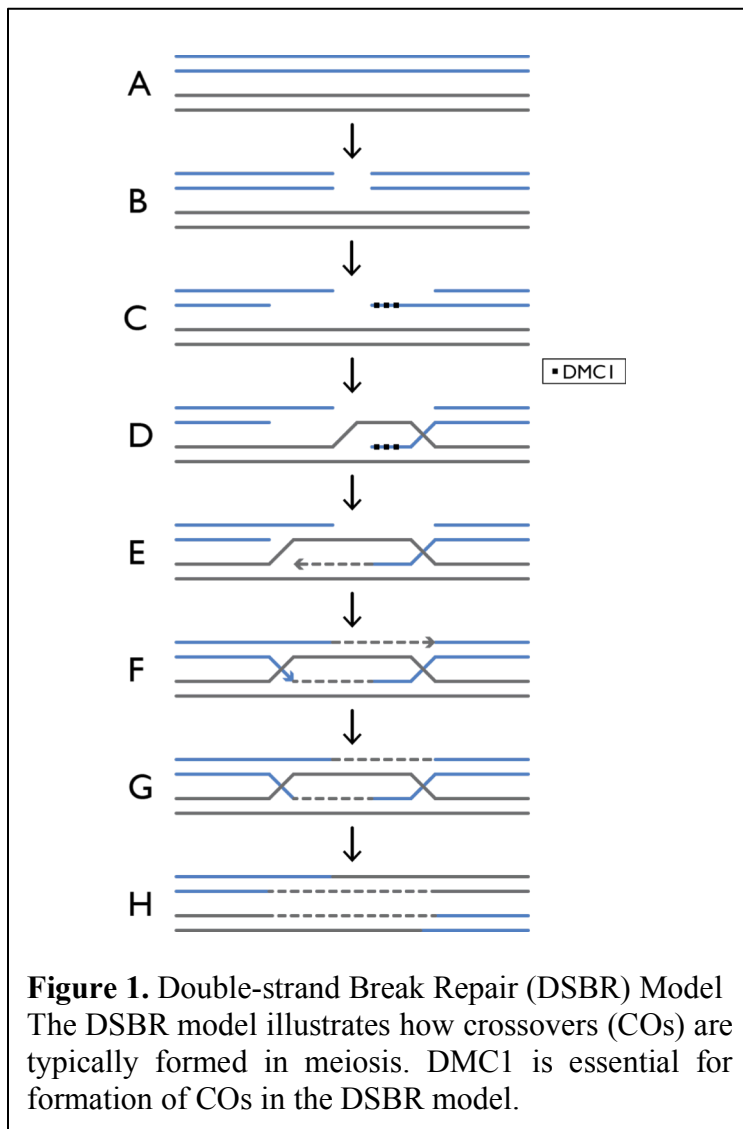
Meiosis is a cellular process in which a diploid cell produces four haploid gamete cells. During Meiosis I, crossovers (COs) between homologous chromosomes result in the reciprocal exchange of genetic material; these COs ensure proper segregation of chromosomes. Without COs, abnormal segregation of unpaired chromosomes in meiosis leaves *Arabidopsis thaliana* sterile. During crossing-over, DMC1 mediates the strand invasion process. Previous studies have shown that *dmc1* null mutants lack COs and are sterile. The Copenhagen laboratory isolated a hypomorphic allele of *DMC1* (*dmc1-2*) and used it to identify suppressor mutants that restore fertility. These were called suppressors of *dmc1-2* mutants (*sdm* lines). Here, we crossed a *sdm* candidate line (*sdm17*) to a polymorphic parent (Landsberg erecta), and screened the resulting F<sub>1</sub>s to identify heterozygotes. Seeds from a single F<sub>1</sub> plant were used to generate a F<sub>2</sub> mapping population. From this population, *dmc1-2<sup>-/-</sup>; sdm17<sup>-/-</sup>* plants were identified by genotyping and phenotypic (fertility) analysis. Fertile F<sub>2</sub>s will be pooled for Illumina sequencing. This will allow us to genetically map the *SDM17* locus. The results obtained from this study will provide novel insight into genes that interact with DMC1 in *Arabidopsis*, which are not well-characterized.

## **Introduction:**

Meiosis is the cellular process in which a diploid progenitor cell produces four haploid daughter cells. During meiosis, genetic variation arises through crossovers (COs) and independent assortment of chromosomes. COs result in the reciprocal exchange of genetic material (DNA). Crossing over between homologous chromosomes is essential for homologous pairing and bivalent formation [1]. The physical connection created between homologs during crossing over acts as an antipoleward force to signal to the cell that homologous pairing has occurred and that meiosis may proceed. In *Arabidopsis*, without homologous pairing each chromosome is left as a univalent and the reductional division of meiosis I results in aberrant chromosomal segregation, leaving the plant sterile [1]. In most organisms, homologous pairing requires at least one CO per chromosome for bivalent formation in order to undergo proper chromosomal segregation [1,2].

COs are thought to result from the double-strand break repair (DSBR) pathway (Figure 1) [1,3]. In the DSBR model, SPO11, a topoisomerase-like DNA transesterase, initiates the formation of double-strand break (DSBs) along chromosomes (Figure 1B) (see Kohl & Sekelsky 2013 for a recent review of recombination) [4,5]. Following a DSB, the 5' ends of the strands exposed by the DSB are resected, leaving two single-stranded 3' tails (Figure 1C). In strand invasion one of the 3' tails "invades" a non-sister chromatid of its homologous chromosome, forming a displacement loop (D-loop) (Figure 1D). The invading strand primes DNA synthesis using the homolog as a template and extending the D-loop (Figure 1E). After D-loop extension, the non-invading 3' tail of the DSB captures the D-loop (Figure 1F). After additional DNA synthesis and ligation a

double-Holliday junction (dHJ) intermediate forms (Figure 1G). Finally, the dHJ is resolved into a CO product (Figure 1H). Theoretically dHJs can be resolved to form either COs or non-crossovers (NCOs), but data from yeast indicate that COs are the primary product [6]. Most, if not all, NCOs are thought to result from a pathway called synthesis-dependent strand annealing (SDSA) [6,7]. In the SDSA pathway, after some DNA synthesis, the invading 3' strand disassociates from its homolog. The other free 3' strand, whose 5' end was resected, is able to anneal back onto this recently elongated strand. Gap-filling synthesis and ligation results in a NCO.



**Figure 1.** Double-strand Break Repair (DSBR) Model  
The DSBR model illustrates how crossovers (COs) are typically formed in meiosis. DMC1 is essential for formation of COs in the DSBR model.

The Copenhagen lab investigates meiosis and how it is regulated, particularly in *Arabidopsis thaliana*. *Arabidopsis* is a small flowering annual plant in the *Brassicaceae* family with five pairs of chromosomes and a small sequenced genome (125Mb), making it an ideal model organism to study plant genetics. My research project is focused on the meiosis-specific protein DMC1. In the DSBR model, DMC1 is loaded onto the 3' strand (Figure 1C), enabling it to invade a non-sister chromatid (Figure 1D) [1, 8]. Thus, DMC1 is vital in meiosis because its role in strand invasion facilitates the formation of COs [9,10]. COs, in turn, result in bivalent formation among homologs and allow for the proper segregation of homologs that is required in meiosis. Studies on *dmc1* null mutants in *Arabidopsis* show the effects of the inability to form crossovers [4]. RAD51, another recombinase present in DSBR, is not meiosis specific and repairs DSBs in mitosis as well. In the absence of DMC1, RAD51 repairs meiotic DSBs using the sister chromatid as a template [4]. This prevents both the formation of crossovers between homologous chromosomes as well as chromosome pairing, resulting in unpaired univalents and aberrant chromosomal segregation, which leads to sterility [1, 4].

Previously, a forward genetic screen using ethyl methanesulfonate (EMS) mutagenesis to search for reduced recombination rates found plants that displayed a 25% reduced recombination rate. The cause was found to be a single nucleotide polymorphism (SNP) in *DMC1*, resulting in a hypomorphic allele, *dmc1-2*. Instead of the complete infertility phenotype seen in *dmc1* null plants, *dmc1-2* plants have a reduced fertility phenotype, suggesting that *dmc1-2* plants retain some DMC1 functionality in strand invasion.

A second forward genetic screen in a *dmc1-2* background yielded plants with higher fertility than *dmc1-2* homozygotes but lower fertility than wild type *DMC1*. Mutants with this rescued fertility phenotype are named suppressor of dmc1 mutant, or *sdm*, lines. The objective of this research project is to genetically map the *SDM17* locus using a combination of marker-based analyses and bulked-segregant mapping with Illumina whole-genome sequencing.

## **Methods:**

### *Plant Growth and DNA Extraction*

A reciprocal cross was made between a *dmc1-2<sup>-/-</sup>; sdm17<sup>-/-</sup>* line in the Columbia ecotype genetic background and second accession, Landsberg erecta (*DMC1<sup>+/+</sup>; SDM17<sup>+/+</sup>*). F<sub>1</sub> seed from this cross was plated on MS (Murashige and Skoog) plates, and the plates were then stratified for 5 days at 4°C. The plants were then moved to a 22°C growth chamber with 18h days. After seedlings had developed 2-4 true leaves, they were transplanted to MetroMix 360 potting soil in 2.5" pots. F<sub>2</sub> seeds were sown directly onto potting soil in 2.5" pots, and stratified for 10 days at 4°C. All plants were grown in a 21°C growth room under 18h days. The plants were allowed to bolt and once the first true leaves emerged plant tissue was collected in 15 mL micro centrifuge tubes containing 400 uL of Edward's buffer (200 mM Tris, pH 8.0, 200mM NaCl, 25 mM EDTA, 0.5% SDS). The tissue was ground with a sterile pestle and then centrifuged for 5 minutes at 14,000 rpm. 300 uL of supernatant was transferred to a fresh tube followed by the addition of 300 uL of isopropanol. The tubes were then inverted until well mixed. After incubation at room temp for 2 minutes the tubes were centrifuged for 5 minutes at 14,000

rpm. Supernatant was discarded and the DNA pellets were washed with 700 uL of 70% ethanol. Tubes were centrifuged for 2 minutes at 14,000 rpm. Supernatant was discarded and the pellets were allowed to dry for 30 minutes. DNA was resuspended in 100 uL of sterile water and the tubes were stored at 4°C. Seeds from a single, heterozygous (*dmc1-2*<sup>+/-</sup>) F<sub>1</sub> were used to create an F<sub>2</sub> mapping population.

### *DMC1* genotyping

*DMC1*-locus specific dCAPS [11] primers were used to amplify the *DMC1* locus using PCR (See Table 1). PCR conditions used are as follows: 13 minutes at 95°C, 2) 45 seconds at 94°C, 3) 45 seconds at 50°C, 4) 1 minute at 72°C, 5) Repeat steps 2-4 34 more times, 6) 10 minutes at 70°C, 7) held at 10°C. Using a *PmeI* digest, the *DMC1* and *dmc1-2* alleles were analyzed by gel electrophoresis to genotype the F<sub>1</sub>s and F<sub>2</sub>s. *PmeI* cleaves 27 base pairs off of the *dmc1-2* PCR product, leaving a 321 bp product, but does not cleave the *DMC1* product (348 bp) (Figure 2). The digest conditions used are as follows: 1) 2 hours at 37°C, 2) 20 minutes at 65°C. Digested PCR products were run on a 3% agarose gel in TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) buffer solution and the digest products were allowed to migrate at 141 V for approximately one hour, or until separation of the two bands was distinguishable enough for genotyping.

	Primer Sequence (5'-3'):
Forward Primer	GCAAGTTGTAAGTACTGATGCTCATAGGTTTAA
Reverse Primer	CATGAAAGGAGGGAATGGAA

**Table 1.** Derived cleaved amplified polymorphic sequence (dCaps) Primers  
The primers used for amplifying the *DMC1* locus in preparation for *PmeI* digest. The forward primer incorporates an A → T polymorphism that allows *PmeI* to cleave the *dmc1-2* allele only.

### *Fertility analysis*

Three different methods were used to gauge the fertility of the *dmc1-2<sup>-/-</sup>* F<sub>2</sub> plants: seed count, silique length, and a visual assessment of fertility (qualitative fertility assessment). Once fully elongated, siliques 5-9 of the primary stem were collected for imaging to measure seed count and silique length. These five siliques were mounted onto a laminate sheet and scanned using an Epson GT-1500 scanner set to scan the image as a transparency, thus allowing the seeds in each silique to be digitally visualized. The number of seeds in each silique was then counted manually. Silique length was obtained by measuring the length of each silique using the program ImageJ. A third, quantitative, assessment was made based on the visual fertility of each plant and ranking it on a score of one to three where one represented mostly sterile, two represented intermediate fertility, and three represented fully fertile or nearly fully fertile plants.

### *SSLP Marker Analysis*

Simple Sequence Length Polymorphism (SSLP) markers were amplified by PCR using genomic DNA and marker-specific pairs of primers (Table 2). PCR and gel electrophoresis conditions were identical to those used for genotyping F<sub>1</sub> and F<sub>2</sub> plants. One-way ANOVAs and Tukey-Kramer tests were used to assess the significance of Columbia/ Landsberg erecta allelic frequency on the plants fertility for each marker.



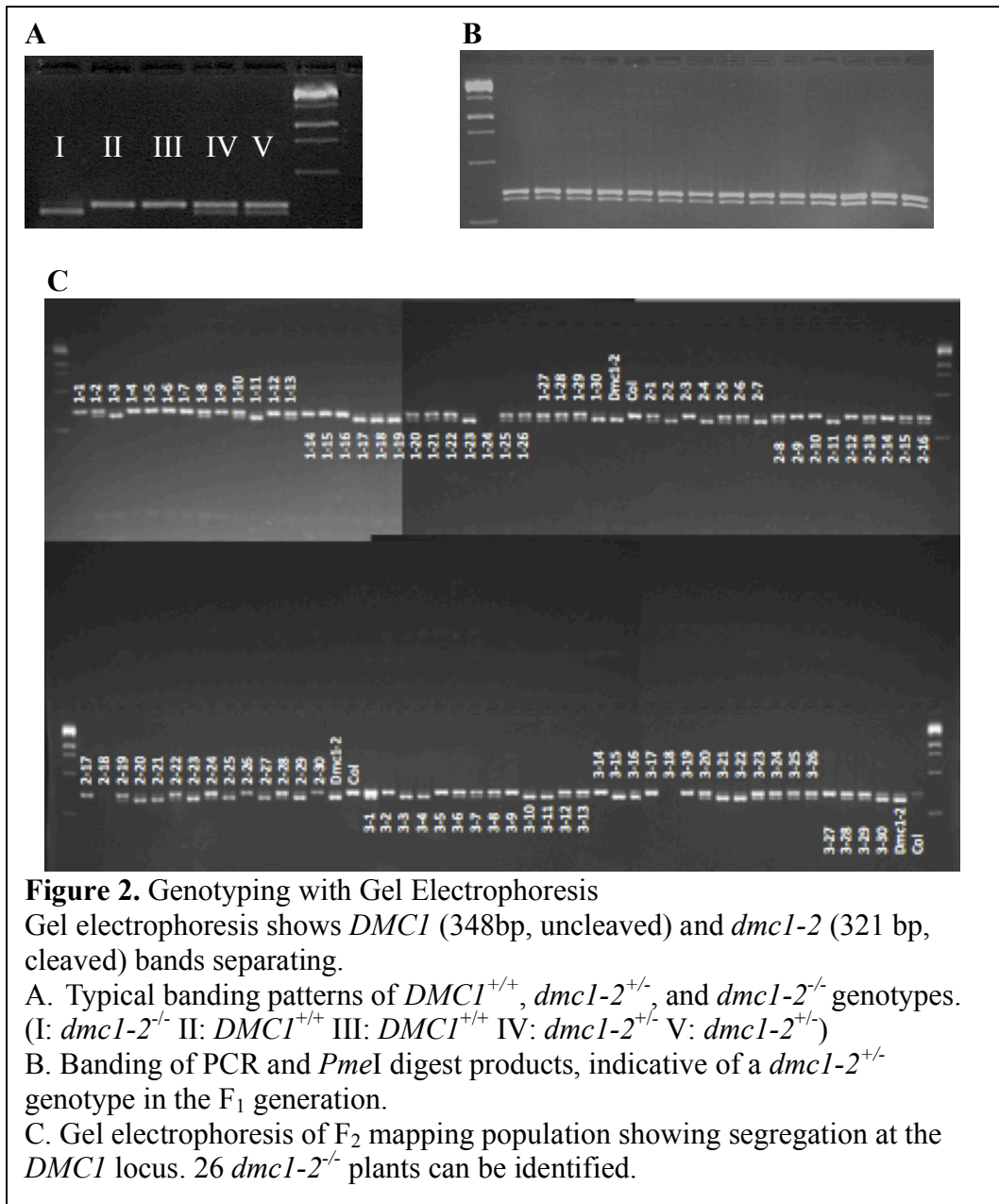
Marker:	Chrom.:	Chrom. Arm:	Forward Primer Sequence (5'-3'):	Reverse Primer Sequence (5'-3'):	Col. Length (bp) :	Ler Length (bp) :
8002_3	1	Top	ACCAACACCACAA CAAACGAC	CTTTTTCTGTTCT TCCGCTATTC	168	205
8014_15		Bottom	GATACGTTCAAAA TTAGGGACTTC	TGTATTTTGCTAA TTGAGGTTATGG	218	180
8018_19	2	Top	CTGGAGATCATCC AACAAAG	TGCAATGGAATG GGCTGGTC	336	293
8028_29		Bottom	CGGGCTGTCCATG AACTATT	AAACCAAATCGA ACCAACCA	226	200
8036_37	3	Top	CCCTGGCAAGACA TAACCAA	CTTCTTGTTTGC CTCTGTGG	227	186
8044_45		Bottom	TGGACCCAAGTCT TTGGATT	CCCTCGTTTCTCT TTCTCGTT	232	206
8052_53	4	Top	TGAAGCGGTTTCA AGATTGTT	TGAAGCGGTTTC AAGATTGTT	217	192
8060_61		Bottom	AGAGAGCACGATG CCTGATAG	AATGCTTCAGCG ATTGAGAAC	180	205
8070_71	5	Top	CGTTATGTAATAG TCATCACGTTTTG	TTCACAATTAGA ACGCTGAATCAT	100	135
8080_81		Bottom	TGAACTGGATCAG CTTACTTGA	AGGTGCACGAGA TGGTTCAT	197	158

**Table 2.** SSLP Marker Primers

Information on the ten markers used includes each set of forward and reverse primers, the chromosome and arm the marker is located on, and the sequence length polymorphisms between the Columbia and Landsberg erecta ecotypes. The SSLP primer sequences were provided by Hong Ma (Fudan University, Shanghai, China).

## Results:

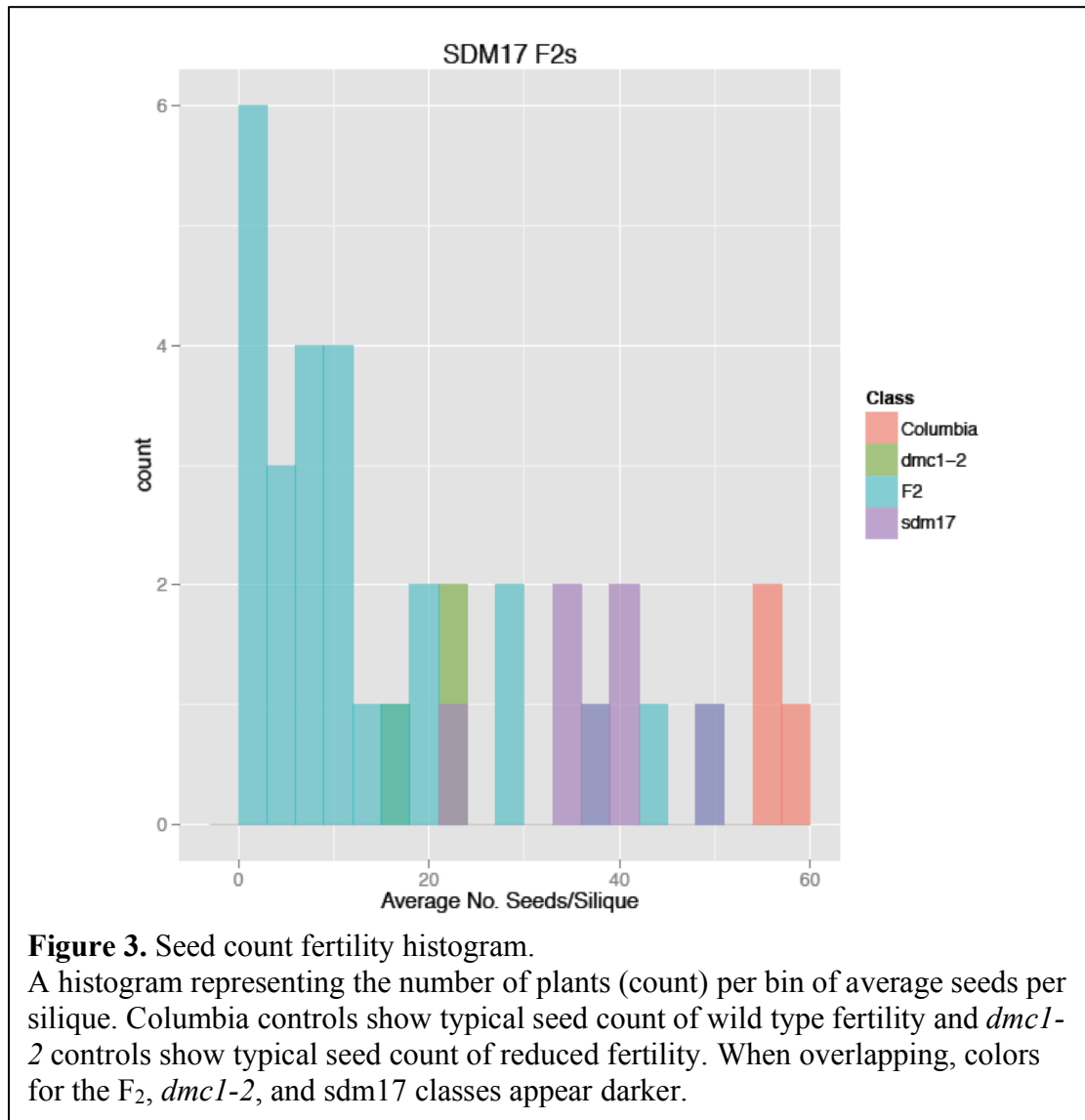
To create a mapping population, *dmc1-2 sdm17* plants in the Columbia background were crossed to a polymorphic parent in the Landsberg background (*DMC1 SDM17*). From the resulting F<sub>1</sub> generation, twenty-six *dmc1-2<sup>+/-</sup>; sdm17* plants were identified through genotyping using PCR analysis with primers capable of distinguishing the *DMC1* and *dmc1-2* alleles. A single F<sub>1</sub> was allowed to set self-fertilized seeds to generate the F<sub>2</sub>s (Figure 2B). From the F<sub>2</sub> mapping population, 26 *dmc1-2<sup>-/-</sup>* F<sub>2</sub> plants were identified through PCR-based genotyping (Figure 2C).



### Fertility Analysis

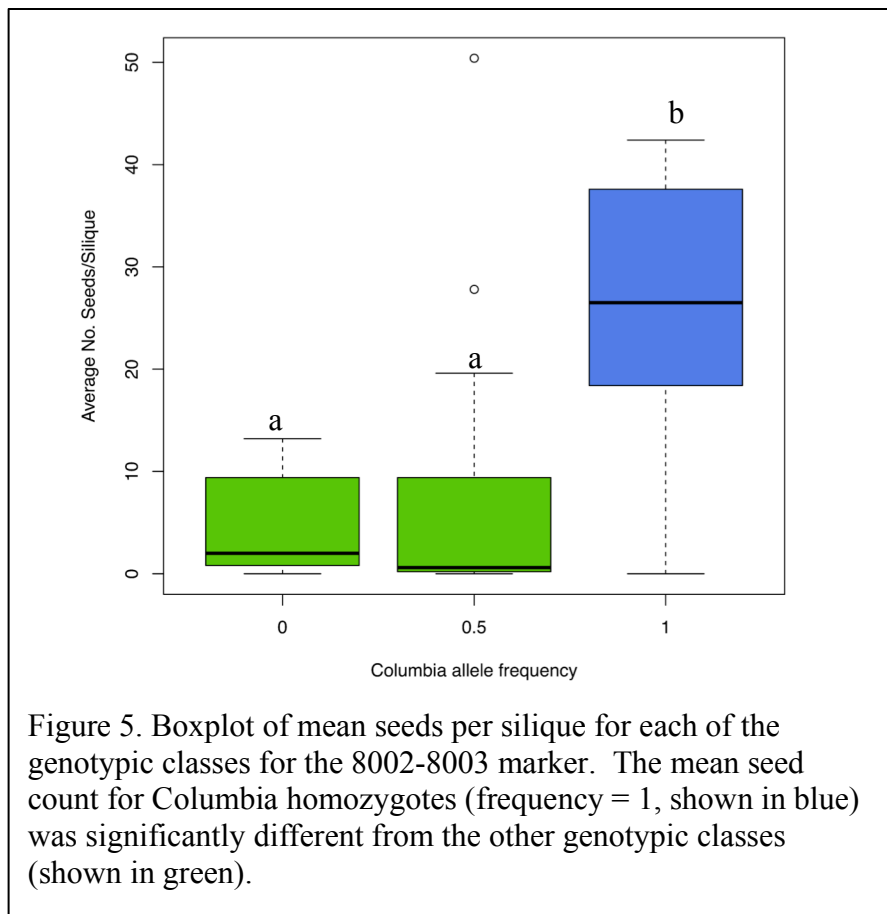
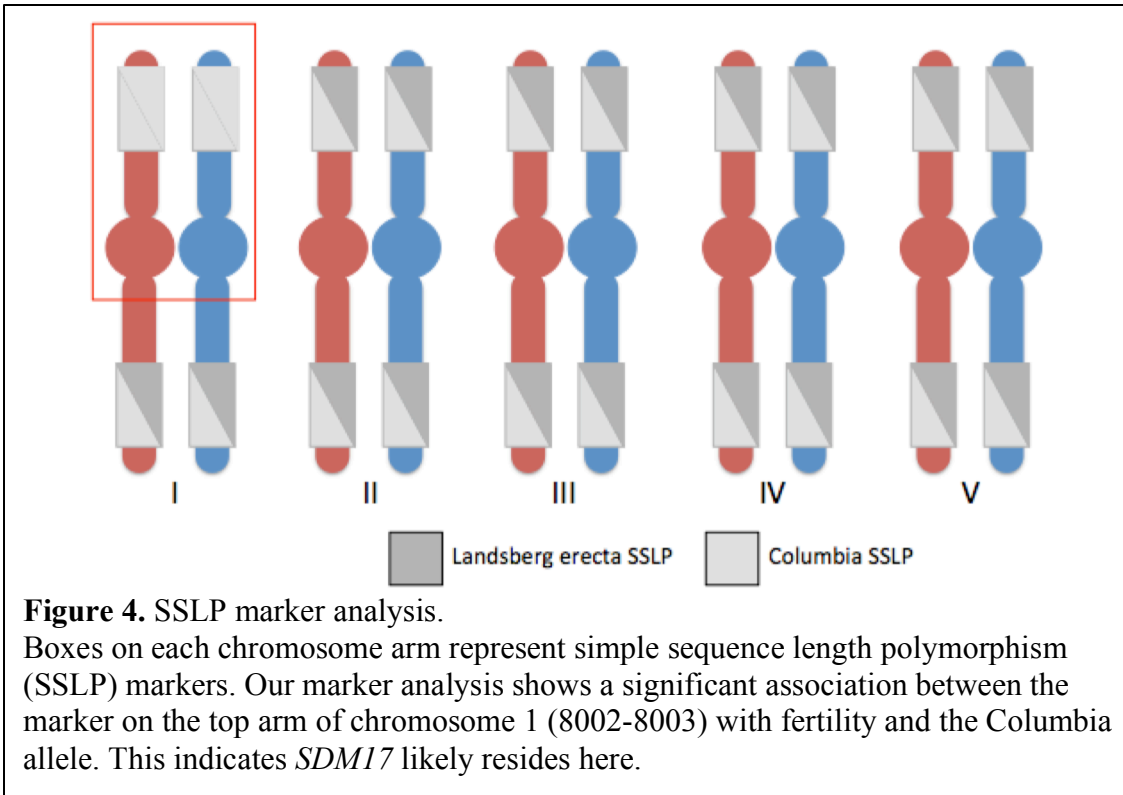
In order to assess the fertility of the 26 *dmc1-2*<sup>-/-</sup> F<sub>2</sub> plants we used three analyses. Comparing the average seeds per silique, we found five F<sub>2</sub> plants with higher fertility than the *dmc1-2*<sup>-/-</sup> controls (Figure 3). A second qualitative analysis of the F<sub>2</sub>s resulted in six

plants that were scored as “Most Fertile,” or ranked a three on a scale of one to three for fertility.



### *SSLP Marker Analysis*

In order to identify the approximate location of the *SDM17* locus we used SSLP markers. SSLP markers differentiate between the Columbia and Landsberg erecta ecotypes. Because the *sdm17* allele was discovered using a forward genetic screen in a Columbia background we expect its locus to be linked to a Columbia marker. Two markers were used for each chromosome in Arabidopsis (one for each chromosome arm). Using a total of ten SSLP markers (Table 2), PCR and gel electrophoresis were used to genotype 14 of the 26 *dmc1-2<sup>-/-</sup>*; *sdm17* F<sub>2</sub> plants (7 of the least and greatest fertility based on seed count, respectively). Individual one-way ANOVAs were used to test for an association between the Columbia allele frequency (CAF) at each marker and average seed count. The data suggests that the *SDM17* locus lies on the top arm of chromosome 1 (Marker 8002-8003,  $F_{1,26} = 7.872$ , p-value: 0.009381, Figure 4). We found that a Tukey-Kramer HSD test for difference among means of the average seed count per silique between the genotypic classes (Columbia and Landsberg erecta) at the 8002-8003 marker revealed a significant difference between Columbia homozygotes (mean = 21.005) and both Ler homozygotes (mean = 5.080) and heterozygotes (mean = 8.677) (Figure 5).



## Discussion:

Five out of the 26 F<sub>2</sub> *dmc1-2<sup>-/-</sup>* plants showed a higher fertility than the *dmc1-2<sup>-/-</sup>* controls. We believe that the rescued fertility displayed in these plants is due to a *sdm17<sup>-/-</sup>* genotype. Using SSLP markers, we found a significant association between this rescued fertility and the top arm of chromosome 1 in *Arabidopsis*. In order to map the *SDM17* locus more precisely we can conduct further SSLP marker analysis using more markers along the top arm of chromosome 1.

In addition to SSLP marker analysis, we plan to use Illumina Sequencing on the fertile and sterile pools we created from the F<sub>2</sub> mapping population. Using the sequencing data we can analyze the SNP distribution of the Landsberg erecta and Columbia alleles across the genome. Because *sdm17* was discovered in a Columbia background its propagation through both of my crosses will be identifiable because it is linked to Columbia specific SNPs. Therefore, we expect to identify a Landsberg erecta SNP desert, or a portion of the genome that is absent of any Landsberg erecta specific SNPs. To continue mapping using either of these methods we will produce more F<sub>2</sub> plants to increase our resolution to find the causal mutation underlying *SDM17*.

A better understanding of DMC1's role in strand invasion, CO formation, and homologous chromosome pairing and segregation are of agricultural and economic significance, as these underlying mechanisms of meiosis allow for and maintain fertility in plants that rely on sexual reproduction to propagate. Agriculturally, we are dependent on many crops, particularly crops whose seed is harvested for food, such as barley, rye, and wheat. In any crop, an understanding of the contributing factors to sterility is vital for our continued use of that crop. Once *SDM17* has been genetically mapped we will be

able to conduct additional research investigating *SDM17*'s molecular role in meiosis and specifically how it might interact with DMC1. Because the accessory proteins that assist DMC1 loading and homology search are not well characterized, the research on mapping *SDM17* will provide insight into these poorly understood molecular interactions.

## References:

1. Osman K, Higgins JD, Sanchez-moran E, Armstrong SJ, Franklin FC. *Pathways to meiotic recombination in Arabidopsis thaliana*. *New Phytol.* 2011;190(3):523-44.
2. Nicklas, R.B., How cells get the right chromosomes. *Science*, 1997. **275**(5300): p. 632-637
3. Bishop DK, Park D, Xu L, Kleckner N. *DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression*. *Cell*. 1992;69(3):439-56.
4. Pradillo M, Varas J, Oliver C, Santos JL. *On the role of AtDMC1, AtRAD51 and its paralogs during Arabidopsis meiosis*. *Front Plant Sci.* 2014;5:23.
5. Keeney, S., Giroux, C. N.; and Kleckner, N. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of widely conserved protein family. *Cell*, 1997. **88**(3):375-84.
6. McMahon, M. S.; Sham, C. W.; and Bishop, D. K. Synthesis-dependent strand annealing in meiosis. *PLoS Biol*, 2007. **5**(11): e299
7. Allers, T. and M. Lichten, Differential timing and control of noncrossover and crossover recombination during meiosis. *Cell*, 2001. **106**(1): p. 47-57.
8. Vignard J, Siwec T, Chelysheva L, et al. *The interplay of RecA-related proteins and the MND1-HOP2 complex during meiosis in Arabidopsis thaliana*. *PLoS Genet.* 2007;3(10):1894-906.
9. Klimyuk VI, Jones JD. *AtDMC1, the Arabidopsis homologue of the yeast DMC1 gene: characterization, transposon-induced allelic variation and meiosis-associated expression*. *Plant J.* 1997;11(1):1-14.
10. Couteau F, Belzile F, Horlow C, Grandjean O, Vezon D, Doutriaux MP. *Random chromosome segregation without meiotic arrest in both male and female meiocytes of a dmc1 mutant of Arabidopsis*. *Plant Cell.* 1999;11(9):1623-34.
11. Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. *Plant J* 14: 387–392