ABNORMAL CHROMOSOME 10 CAUSES MEIOTIC DRIVE IN A TEOSINTE SUBSPECIES

by

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A Thesis Submitted to the Honors Program of the University of Georgia
in Fulfillment of the Requirements for a 4990H or 5900H course

Background

In a diploid organism, chromosomes are organized into homologous pairs such that one chromosome from each pair is passed on to offspring. Typically, each chromosome follows Mendelian laws and is equally as likely to be inherited by offspring as its homologous partner. Chromosomal meiotic drive is a distortion of this process in which one chromosome has a greater than 50% likelihood of being passed on from parent to offspring. *Zea mays,* or domesticated maize, has ten pairs of chromosomes. In the case of the tenth pair, a plant can either have two copies of "normal" chromosome 10 (N10), or can be hetero- or homozygous for a chromosome called abnormal chromosome 10 (Ab10). When Ab10 is present and heterozygous with N10, it causes the preferential segregation of itself into progeny at rate of 65-80% instead of the Mendelian 50% by positioning itself in the bottom gamete of the linear tetrad during female meiosis. Since the discovery of this phenomenon (Rhoades 1942), characterization of Ab10 and the mechanism for how it causes preferential segregation have been of particular interest.

Ab10 differs structurally from N10. It is much larger, with approximately 20 cM present at the distal end that is not found on N10. Additionally, a series of large inversions in the central euchromatic portion of the chromosome prevents Ab10 and N10 from recombining. Most notably, Ab10 has structures called knobs that are not found on N10, as shown in Figure 1a. These knobs are heterochromatic regions of tandem repeats, and two specific types of knobs are found on Ab10: a 350-bp repeat called TR-1 and a 180-bp repeat called Knob180. These knobs are considered crucial in order for Ab10 to position itself advantageously for meiotic drive to occur. Ab10 encodes genes that cause all knob repeats to gain "neocentromere" activity, in which they are able to move along

spindle microtubules and pull Ab10 and other chromosomes with knobs ahead of those with standard centromeres. As a result, the chromatids with knobs are positioned into the top and bottom gametes of the linear tetrad of female meiosis (Figure 1b). The bottom gamete in the linear tetrad is fertilized, so Ab10 preferentially segregates by positioning itself into this gamete. The presence of Ab10 also activates the neocentromere activity of knobs on other chromosomes, causing the preferential segregation of regions linked to those knobs as well (Longley 1945; Kikudome 1959; Rhoades 1966).

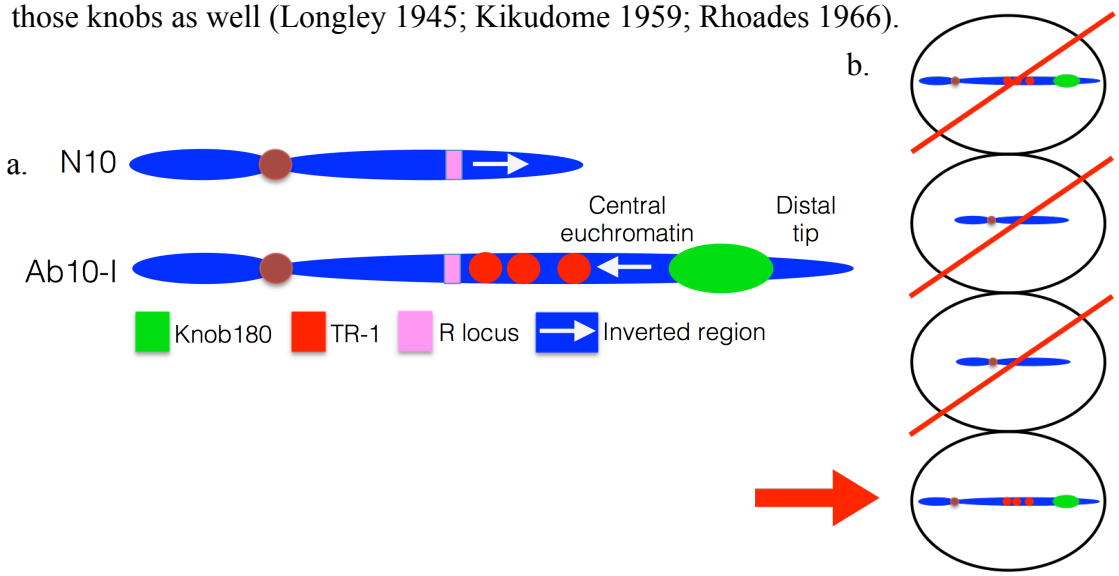

Figure 1. a.) Ab10 differs from N10 by the presence of TR-1 and Knob180 repeats, and it cannot recombine with N10 due to inversions in the central euchromatic portion of the chromosome. Genes on the distal tip are unique to Ab10, but some regions on the chromosome, such as the R locus, are found on both Ab10 and N10. b.) Knobs activate neocentromere activity and position Ab10 in the bottom gamete of the linear tetrad. Other knob-linked regions also preferentially segregate.

Preferential segregation of regions linked to knobs does not occur without the presence of Ab10, so the gene(s) important for meiotic drive are found on this chromosome. However, a deficiency mutant (Df(L)) of the Ab10 chromosome lacking

the end of the chromosome distal to the knobs does not exhibit meiotic drive. Thus, at least one crucial gene is found in this region. Unpublished bioinformatics analyses in the Dawe lab uncovered ten genes in this distal tip region that are candidates for causing meiotic drive. One of these, Kin618, encodes a kinesin, which is a motor protein that can move along microtubules. The other genes do not encode protein but may serve a regulatory purpose. These genes were analyzed in order to shed light on which could be important for causing meiotic drive. An important tool for studying these genes included the use of *suppressor of meiotic drive (smd)* mutants that are defective for meiotic drive. These were identified by taking advantage of the fact that Ab10 is linked to the R kernel pigmentation locus (Figure 1). These heterozygotes also had an active transposon family called *Robertson's Mutator (Mu)* that induces mutations at a high frequency. By screening for ears with close to 1:1 ratio of purple and colorless kernels, *smd* mutants were identified (Dawe and Cande 1996; Hiatt and Dawe 2003). For this study, lines with *smd1*, *smd3*, *smd8*, *smd12*, and *smd13* mutations were utilized.

In addition to being found in domesticated maize, Ab10 is found in the wild relatives of maize, called teosinte. Ab10 is found at comparable frequencies in maize and teosinte, as it is found in 13% of maize landrace individuals and approximately 15% of teosinte individuals (Kanizay 2013). These teosinte individuals are part of populations found in North and South America, with a concentration in Mexico and Central America. Ab10 has been shown to drive in maize, but it has not previously been shown in teosinte. In this study, I demonstrate that Ab10 exhibits drive in a teosinte population.

The study of Ab10 in different maize and teosinte populations has led to the identification of three Ab10 variants, which differ in their knob patterns but all exhibit meiotic drive, and these are shown in Figure 2.

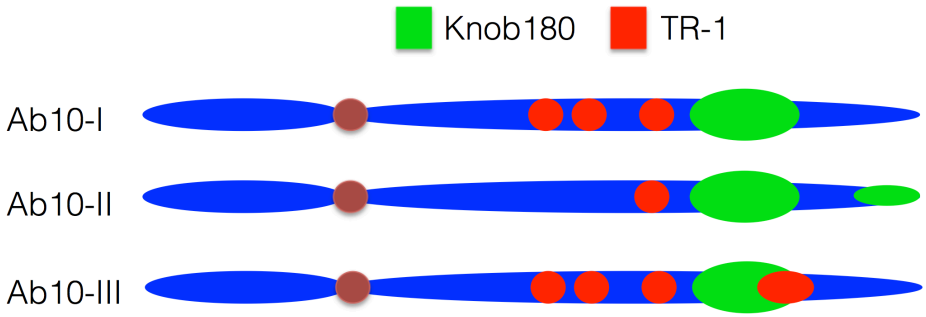

Figure 2. Ab10 variants I, II, and III exhibit different patterns of Knob180 and TR-1, as indicated in the figure. These knobs can be stained using fluorescent *in situ* hybridization and identified by microscopy, but not by PCR.

While Ab10 is not found in any modern maize inbred lines, which are homozygous lines used for breeding purposes, Ab10-I and Ab10-III are found in maize landraces, which are heterozygous and able to pollinate openly in a particular region. All variants of Ab10, however, are found in populations of teosinte. Ab10 cannot recombine with N10 (Kikudome 1959), but it is known that Ab10 types I and II can recombine with each other (Rhoades and Dempsey 1985). It is of interest to determine which populations of teosinte have which variant and which variant is ancestral in order to elucidate the evolutionary history of the chromosome. Additionally, the diversity of Ab10 can aid in determining which genes could be important for meiotic drive. By studying Ab10 in teosinte and maize, we aim to fill some of the existing knowledge gaps about Ab10. This research has two primary aims: 1) to identify genes that are important for meiotic drive

and 2) to determine the Ab10 variant present in a teosinte population and whether it exhibits meiotic drive.

Materials and Methods

These research objectives were filled through extracting DNA and genotyping lines of maize and teosinte to identify plants positive for Ab10. Those positive for Ab10 were used to determine which genes identified on the distal tip of Ab10 were conserved, and expression level analyses were used to elucidate which genes could be of importance for causing meiotic drive. Fluorescent *in situ* hybridization was used to identify knob patterns and determine which variant of Ab10 was present in a particular plant.

DNA Extractions and Genotyping by PCR

Genomic DNA was extracted using leaf tissue from plants using a CTAB method (Clarke 2009) and dissolved in 100 uL TE. This DNA was diluted 1:10 for use in PCR. Primers specific to genes found on the Ab10 distal tip were used for genotyping. Seven primers targeted to the kinesin gene identified, Kin618, were used in different analyses: c.6418 F1/R1, c.6418 F2/R2, and c.6418 F3/R3 were already existing primers in the lab, and Kin618 F1 (CCTGGAGCAGATAGGTCAGC), Kin618 R1 (TGGCATGTTGTCTTTTCGTC), Kin618 F2 (TACTACCCTGTGCGTCATGC), Kin618 R2 (GGGCAAACTGGTTCTGGTAA), Kin618 F3 (CGTTGCGGAATGATCAAATA), Kin618 R3 (GCGGTCTCATATCCAAGGAA), Kin618 F4 (ACCTGTCACGGTCCTGTCTT), and Kin618 R4 (CTGGGCGTGTTAATTTCTGG) were primers designed for this study. Primers for each of the other distal tip genes were used for some analyses: 248 F/R, 348 F/R, 362 F/R, 365

F/R, 405 F/R, 430 F/R, 548 F/R, 613 F/R, and 615 F/R. Phusion MasterMix was used in 20 uL reactions. The PCR cycler protocol used was:

1. 98°C - 30 seconds
2. 98°C – 10 seconds
3. 57-62°C – 15 seconds (Annealing temperature varied by experiment)
4. 72°C – 45 seconds
5. 72°C – 5 minutes

Repeat 35X steps 2-4.

2 uL of TriTrack DNA loading dye was then added to each sample and 10-15 uL of each sample was loaded onto a 1% agarose gel with EtBr added.

Fluorescent in situ hybridization (FISH)

Mitotic chromosome spreads were prepared from root tips that germinated at 30 degrees Celsius for 48 hours as described previously (Kato 2004). Digestion times were shortened to 30-40 minutes for teosinte lines and 50 minutes for maize lines. The centromeric sequence (CentC), knobs (TR-1 and Knob180), and DNA were visualized by utilizing a method previously described in the Dawe lab (Kanizay 2013). Chromosomes were visualized using a fluorescent microscope with magnification levels of 40x and 63x.

Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)

cDNA was previously generated in the Dawe lab from the anthers of plants homozygous for Ab10-I that exhibit meiotic drive along with a series of mutants that have Ab10 but do not exhibit meiotic drive. One of these mutants, Df(L), lacks the distal tip where all ten genes tested are found. This serves as a control and the genes should not be expressed. Other mutants are smd1/1, smd3/3, smd8/8, smd12/12, and smd13/13,

which are deficient in meiotic drive as a result of a transposon mutagenesis screen. Using primers generated for distal tip genes, expression levels for all ten genes were compared to the expression levels of genes in plants with Ab10 that exhibits meiotic drive. The SYBR Green Real-Time PCR Master Mix was used along with primers for each gene and dH2O for 14 uL reactions. Beta-tubulin expression levels were monitored, and cDNA was used undiluted in addition to dilutions of 1:10, 1:100, and 1:1000 as controls to check primer efficiency. Each reaction was performed in triplicate. Data from qRT-PCR was analyzed and graphed using the ddCt method in Excel, and error bars were calculated based on the standard deviation of the three experimental repetitions.

Results

Identifying teosinte lines positive for Ab10

Eleven lines of teosinte were genotyped to determine those that have Ab10. C.6418 primers were used for genotyping to identify plants from line PI 566687 and Ames 8083 as positive, and 548 primers were used for later genotyping of PI 566692 and Ames 21826 once this gene was found to be conserved. Between three and ten plants were genotyped for each line, and four lines had at least one plant with Ab10, as shown in Table 1.

Table 1. Descriptions of each subspecies genotyped and number of plants positive for Ab10 for each.

Line Number	Subspecies	Origin	# Genotyped	# Positive	Percentage positive
Ames 8083	Mexicana	Mexico, Federal District	4	1	25%
PI 566687	Parviglumis	Mexico, Mexico	5	2	40%
PI 615697	Nicaraguensis	Nicaragua, Chinandega	6	0	0%
PI 441934	Huehuetenagensis	Guatemala, Huehuetenango	5	0	0%
PI 566685	Mexicana	Mexico, Mexico	4	0	0%
Ames 21889	Parviglumis	Mexico, Jaliso	10	0	0%
PI 566680	Mexicana	Mexico, Guanajuato	9	0	0%
PI 566692	Parviglumis	Mexico, Michoacan	9	2	22.2%
Ames 21826	Parviglumis	Mexico, Guerrero	8	3	37.5%
PI 566674	Mexicana	Mexico, Durango	9	0	0%
PI 658197	Mexicana	Mexico, Mexico	3	0	0%
Total			72	8	11.1%

The overall percentage of plants positive for Ab10 is 11.1%, consistent with previous study of teosinte populations that found Ab10 was present an average frequency of 15% (Kanizay 2013). The geographic diversity of these plants is represented on the map shown in Figure 3. These plants with Ab10 were then utilized for further study.

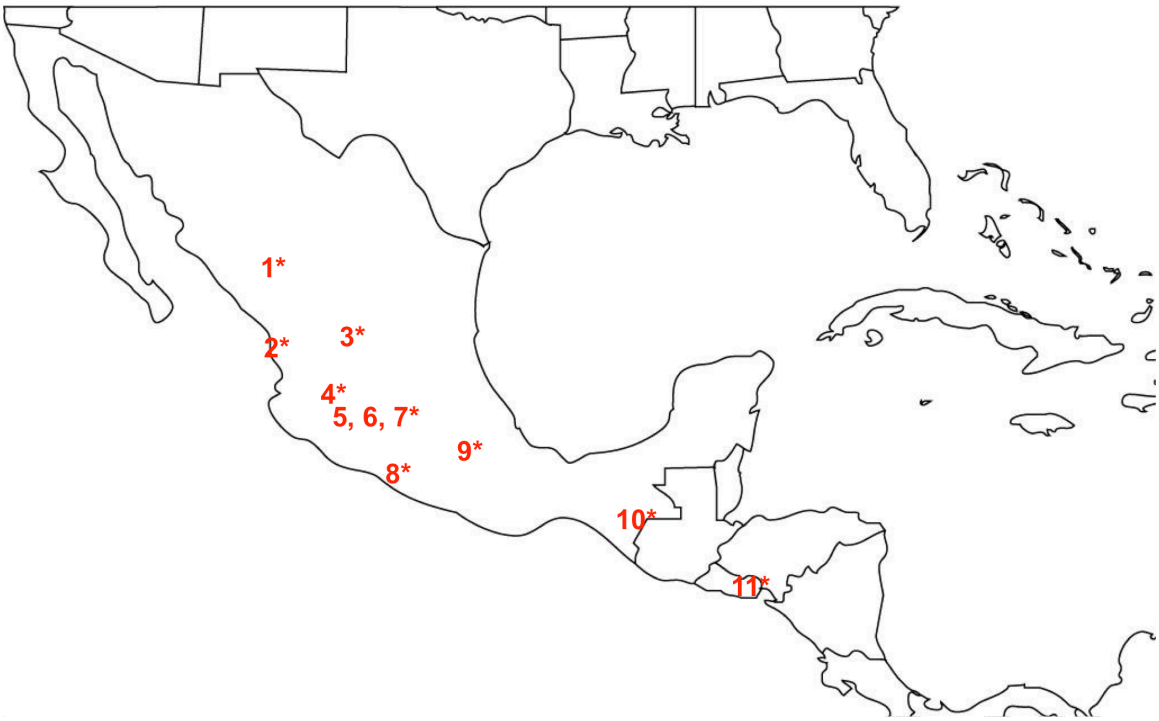

Figure 3. This map shows the geographic location of the eleven teosinte populations genotyped. The asterisks adjacent to each number denote the precise location of the population. The names of these populations, in order on the map, are: 1) PI 566674, 2) Ames 21889, 3) PI 566680, 4) PI 566692, 5) PI 566687, 6) PI 566685, 7) PI 658197, 8) Ames 21826, 9) Ames 8083, 10) PI 441934, and 11) PI 615697.

Lines with plants positive for Ab10 are PI 566692, PI 566687, Ames 21826, and Ames 8083, numbered 4, 5, 8, and 9 on the map, respectively.

Conservation of distal tip genes

After the identification of new genes on the distal tip of Ab10, it was unknown whether these genes were conserved across teosinte and maize lines. Plants from five *Zea mays mays* populations (PI 628445 from Mexico; 490821 from Brazil; PI 444834 from Huila, Columbia; PI 444296 from Caqueta, Columbia; and Ames 19980 from Oaxaca, Mexico), and one *Zea mays parviglumis* population (PI 566687 from Mexico) were

genotyped for ten Ab10-specific genes identified from transcriptome data: C.6418 (also referred to as Kin618), 430, 248, 348, 613, 405, 615, 365, 362, and 548. Of these ten genes, nine were found to be conserved across all lines tested. Gene 365 was not conserved, as shown in Figure 4, meaning it likely is not important for meiotic drive. However, due to the small scale of the conservation experiments, this is not conclusive evidence that all of the other genes play some role. With the exception of C.6418, all other genes lack a protein-coding domain and would likely play some type of regulatory role. In order to test the expression levels in these genes, qRT-PCR was performed.

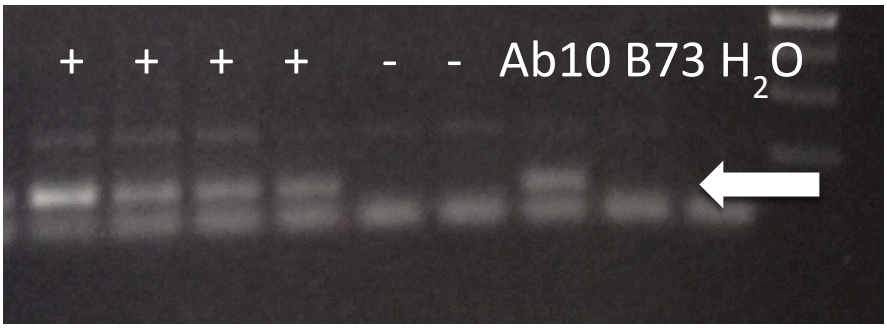

Figure 4. Gene 365 was not found in two Ab10-positive plants, meaning it is not conserved and likely not important for causing meiotic drive to occur.

Expression level analyses

In order to determine if a change in expression of these Ab10-specific genes could be important for causing meiotic drive, qRT-PCR was performed in a series of mutants (smd1, smd3, smd8, smd12, smd13) in which Ab10 is present but does not cause meiotic drive. These levels were compared to the expression levels in a plant with Ab10 that exhibits meiotic drive. The most significant changes were found in Kin618 and 430, as shown in Figures 5 and 7. Kin618 was previously hypothesized to play an important role in meiotic drive because of its function as a kinesin, and the expression levels shown here help confirm this. The Df(L) mutant lacks the distal tip completely, and thus does not

express any of the distal tip genes. In the case of Kin618, the mutant smd12 exhibits a level of expression similar to that of Df(L), making it of interest for further study. Later analysis in the Dawe lab demonstrated that Kin618 in smd12 was highly methylated, while it was not methylated in a line with functioning Ab10. This provides evidence that the methylation of Kin618 could be causing the lack of meiotic drive in smd12.

Of the other genes tested, 430 and 248 also showed considerable expression changes in all mutants, as shown in Figures 5 and 6. The genomic sequence of 430 is absent in Df(L), smd3, and smd8, and it had at least a 10-fold decrease in expression in smd1, smd12, and smd13. While it remains unknown what role 430 may play in causing meiotic drive, these data provide evidence that it likely has some function. Since it is not a protein-coding gene, the role it plays is likely regulatory. 248 primers are also targeted near those for 430, and there was also at least 10-fold reduction in this region in smd1, smd12, and smd13. The genomic sequence of 248 is absent in Df(L), smd3, and smd8, like 430. The reduced expression of these regions targeted by 430 and 248 primers provides further evidence that reduced expression of gene 430 could be important for meiotic drive.

Expression levels of other genes in these mutants did not show large changes in expression, with most staying close to the levels seen in functioning Ab10, but some exhibiting two- to three-fold increase in expression.

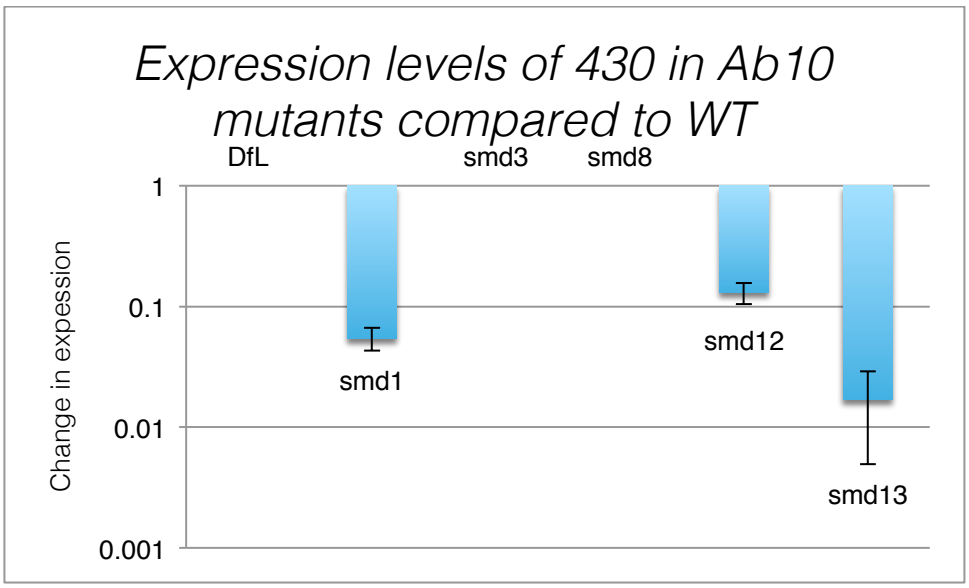

Figure 5. 430 is genomically absent in Df(L), smd3, and smd8, and has at least 10-fold reduced expression in smd1, smd12, and smd13, indicating that it could be a player in causing meiotic drive to occur.

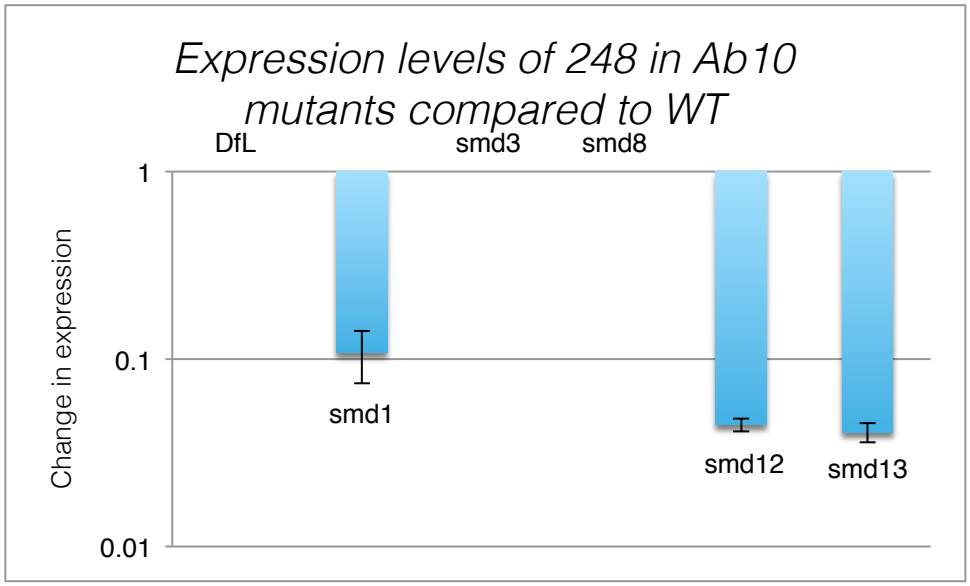

Figure 6. 248 is genomically absent in Df(L), smd3, and smd8 and has at least 10-fold reduced expression in smd1, smd12, and smd13. This gene is in the same region as 430, validating the results shown in Figure 5.

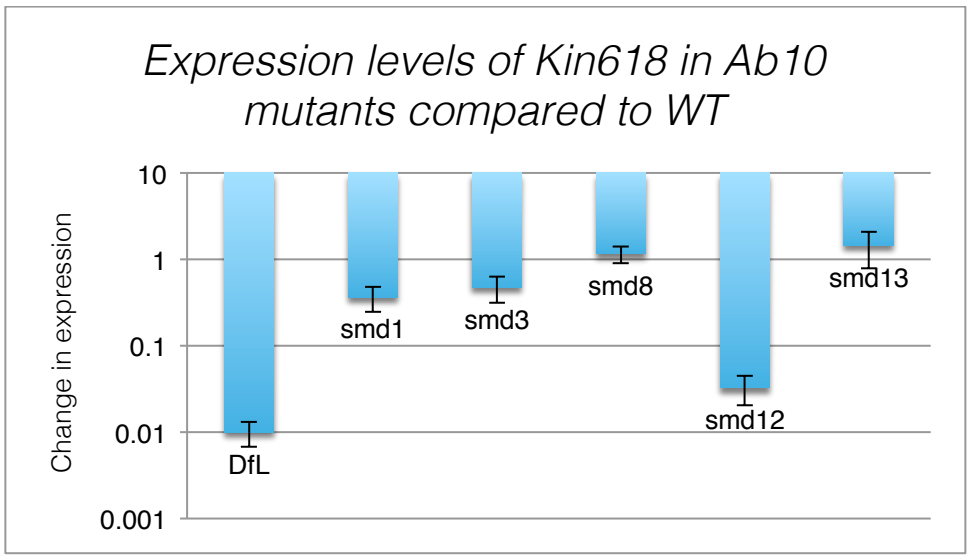

Figure 7. Kin618 has at least 10-fold reduced expression in smd12.

Meiotic drive in teosinte

Despite the wealth of literature demonstrating that Ab10 exhibits meiotic drive in maize (Rhoades and Dempsey 1985, Dawe and Hiatt 2004), it has previously not been shown in teosinte. However, Ab10 is found at a low frequency in a majority of teosinte populations, so it is important to experimentally confirm that drive occurs. Thus, a cross was performed between a maize plant lacking Ab10 and a teosinte plant positive for Ab10 from line Ames 21826. This line is from the subspecies *parviglumis* and is native to Guerrero, Mexico. Importantly, this plant was heterozygous for Ab10 (See below). Since meiotic drive occurs through female meiosis, this plant was used as the female in a cross with a maize plant from the Stock6 line that lacks Ab10. This cross was performed in early summer in Athens, Georgia. The resulting seeds were then used to grow progeny and extract DNA to genotype for the presence of Ab10.

104 progeny of this cross were genotyped using Kin618 F2/R2 primers; of these, 66 were positive, and 38 were negative, resulting in a positive percentage of 63.5% showing meiotic drive. Gels showing the positive Ab10 of the original parent plant and a

sampling of the genotyped progeny are shown in Figure 8. To determine if this is considered significantly different from the Mendelian segregation percentage of 50%, a chi-square test was performed using degrees of freedom (df) equal to 1. This resulted in a probability of $p=0.006 < 0.01$ that these results could have occurred simply by chance. Next, a chi-square test was used to show that the results are not significantly different from 65%, which is the lower threshold of the rates of segregation exhibited by Ab10 during meiotic drive in maize. In this case, the resulting chi-square statistic was 0.10823, again with df=1. This resulted in $p=0.7421$, demonstrating that these data are not significantly different than if the true rate of segregation is 65%, consistent with the rates that occur for meiotic drive in maize.

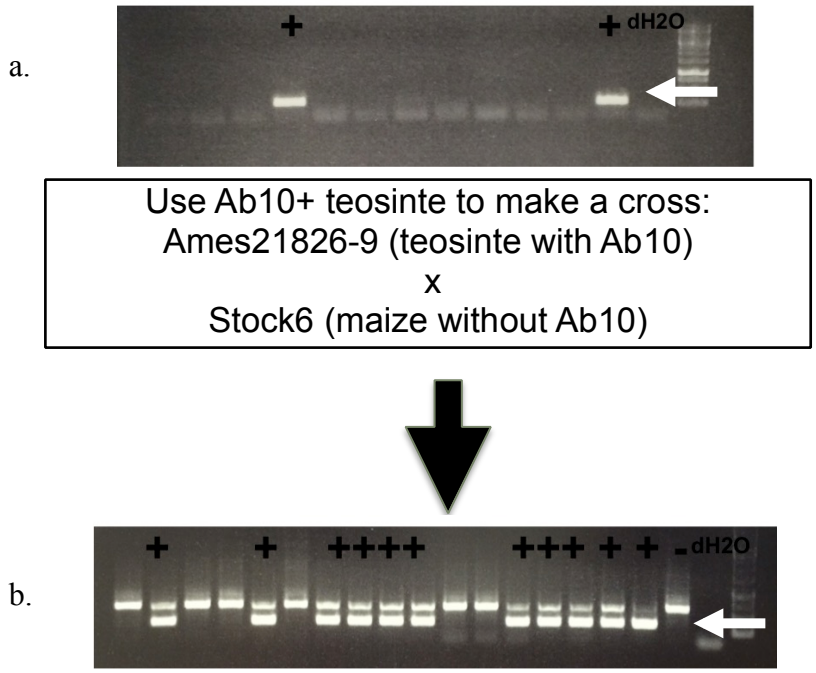

Figure 8. a.) In this gel, Ab10 was identified as positive in a plant from line Ames 21826. The last two lanes of the gel show positive and negative controls. This plant was then crossed with an Ab10-deficient (homozygous N10) Stock6 plant and 104 progeny were

genotyped. b.) This gel shows some of the genotyping results of the progeny from the cross described in 8a. The lower band indicates that Ab10 is present, and the top band was not specific to Ab10 but was found in most of the plants. The last three lanes consist of a positive control and two negative controls.

Table 2. The number of progeny positive and negative for Ab10 are shown, and p-values are calculated to show that this is consistent with ratios seen for meiotic drive.

	Ab10	**Normal 10**	**Total**
Number of plants	66	38	104
Chi-square statistic for 50% segregation rate	3.769	3.769	7.538 p=0.006
Chi-square statistic for 65% segregation rate	0.0379	0.07033	0.10823 p=0.7421

Identifying Ab10 variants in teosinte

As shown in Figure 2, there are three known variants of Ab10 that exhibit meiotic drive, and each of them can be found in teosinte. After determining that Ab10 exhibited meiotic drive in the subspecies *parviglumis* as part of line Ames 21826, the next goal was to determine which variant is present in this line. Fluorescent *in situ* hybridization was performed on the parental Ames21826 plant used in the cross described in Figure 8, and fluorescent microscopy was used to analyze the resulting slides.

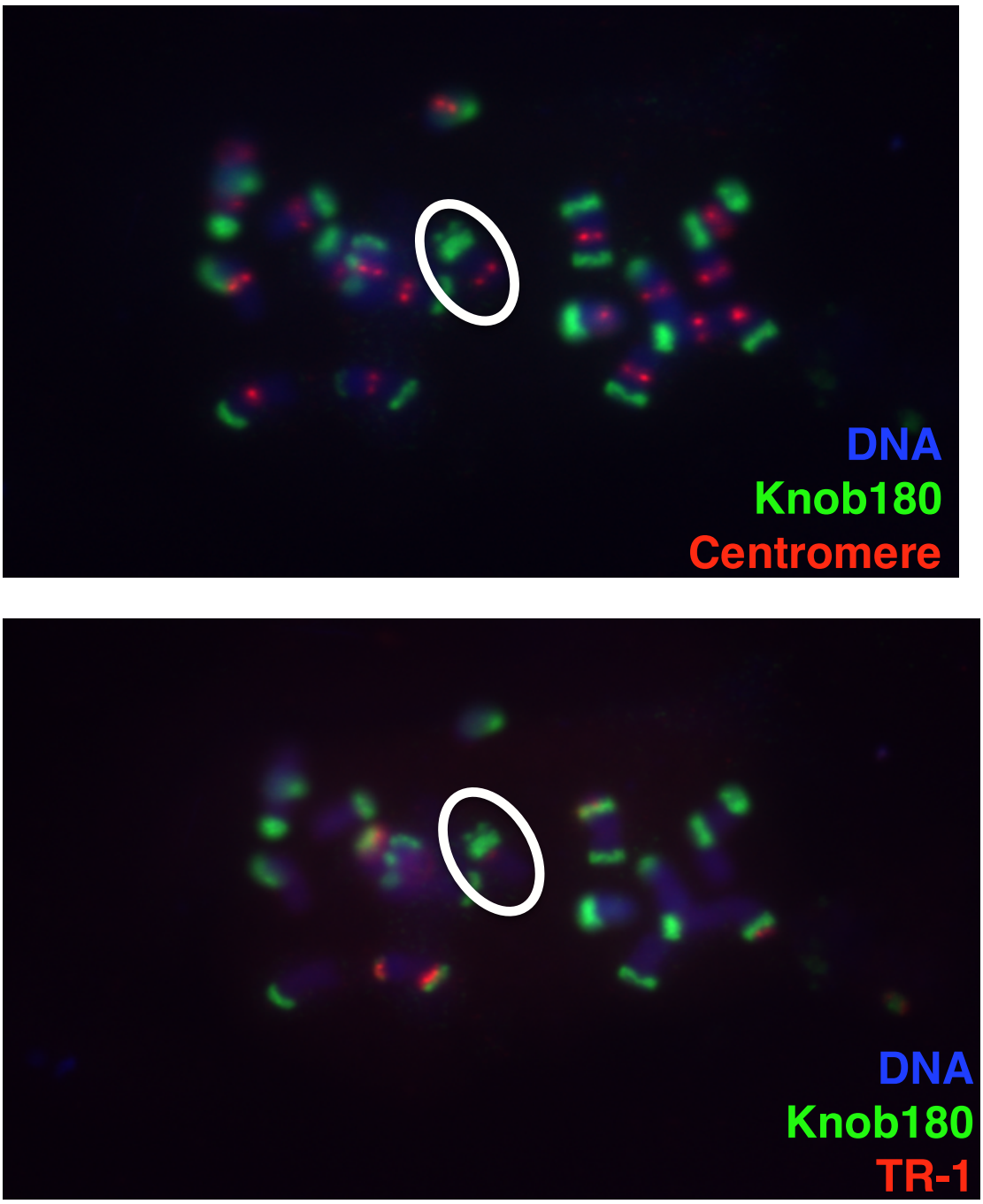

Figure 9. FISH staining of chromosomes in Ames21826-9 was performed and shows Ab10-II is present. The distinctive knob pattern of the chromosome circled in white indicates that Ab10 type 2 is present in *Zea mays parviglumis*. DNA is shown in blue,

Knob180 is shown in green, and the centromere is in red on the top image, while TR-1 is in red in the bottom image.

In both of these images, one Ab10 chromosome is visible and can be clearly identified as Ab10-II. First, the position of the centromere, shown in red in the top image, shows the large difference in size between the long and short arm, which is characteristic of Ab10. All knobs are shown on the end of the long arm, as expected for all Ab10 variants. Shown in green, distinct copies of Knob180 are visible on the distal end of the chromosome, and this is unique to Ab10-II. Additionally, a small TR-1 knob is directly adjacent to the larger Knob180 as shown in the bottom image, providing further evidence that this is Ab10-II.

Discussion

The two primary objectives in performing this research were 1) to identify genes that are important for meiotic drive and 2) to determine the Ab10 variant present in a teosinte population and whether it exhibits meiotic drive. These objectives were fulfilled by analyzing Ab10 in teosinte populations and by utilizing previously identified genes on the distal tip of Ab10.

The first aim of this project was to determine which of the previously identified candidate genes could be important for meiotic drive. This was accomplished primarily through qRT-PCR experiments and also through gene conservation across different teosinte and maize lines. These revealed that Kin618, 430, and 248 genes were likely important for meiotic drive, but 365 was not. Follow-up experiments in the Dawe lab showed that Kin618 is differentially methylated in smd12, the mutant in which it exhibited low expression, compared to a plant in which Ab10 exhibits drive. This

discovery of an "epiallele" has led to this gene becoming the primary focus of further research on Ab10. Still, because of the consistent reduced expression of 430 and 248, these genes are of interest for further study as well.

In addition to determining important genes for meiotic drive, another objective of this research was to determine which variant of Ab10 was present in a teosinte subspecies and whether it exhibited meiotic drive. In Ames 21826, which is part of the teosinte subspecies *parviglumis*, Ab10 was identified as type II using FISH, and was found to preferentially segregate at a rate of approximately 65%. This provides support that Ab10 exhibits drive in teosinte as well as maize. One potential objection to the experimental design of the cross is that the cross was performed between a teosinte and a maize plant, so the resulting progeny are teosinte and maize hybrids. However, meiotic drive of Ab10 occurs specifically in female meiosis, and this cross was performed using teosinte to produce the female gamete and maize to produce the male gamete. Thus, in order to receive Ab10, drive must have occurred in the teosinte plant. As a future experiment, an additional cross could be performed using teosinte to produce the male gamete and maize to produce the female gamete. In this case, Ab10 should segregate at the typical Mendelian ratio since Ab10 drives specifically during female meiosis. Additional experiments could also demonstrate that drive occurs with Ab10-I and Ab10-III in teosinte and across different subspecies, since this experiment addressed Ab10-II in a *parviglumis* population.

Ab10-II was identified in one teosinte population part of the parviglumis subspecies, but three other populations were also identified as having Ab10. Genotyping additional plants from a variety of populations can expand the number of populations

found with Ab10. Then, by identifying the Ab10 variants in each of these, future studies will aim to identify PCR markers for the different variants. Being able to screen for the presence of Ab10 using PCR is a relatively recent development, but currently, the only way to identify Ab10 variants is to use microscopy. Some preliminary experiments involved sequencing some of the ten candidate genes, but there were very few differences in sequence between the Ab10 variants. Thus, the scale of sequencing can be increased to identify more significant differences that could be utilized to facilitate straight-forward identification of the variants. Further, these sequencing efforts could be useful for determining the evolutionary history of Ab10. The age of Ab10 is unknown, and constructing a phylogenetic tree using knowledge of these variants could be one avenue to shed light on this.

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