THE VARIANTS OF MAIZE CHROMOSOME 10 AND THEIR ROLES IN MEIOTIC DRIVE

by

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Under the Direction of R. Kelly Dawe

ABSTRACT

Meiotic drive of knobbed chromatids in maize occurs when abnormal chromosome 10 (Ab10) substitutes for the canonical chromosome 10 (N10). Ab10 is defined as containing extra chromatin on the end of its long arm (the Ab10 haplotype) that includes euchromatic regions and knobs (large heterochromatic arrays of tandem repeats). Knobs are also found on the arms of all other chromosomes. They are composed of an 180 bp repeat (knob 180), a 350 bp repeat (TR-1), or a mixture of both. When Ab10 is present, the normally inert knobs transform into neocentromeres that dramatically move themselves along the meiotic spindle toward the spindle poles. Poleward movement of chromosomes is typically mediated by a highly conserved protein complex (the kinetochore) that binds the centromeric DNA to the microtubules. Neocentromere movement is distinctive in that it uses unknown, non-kinetochore proteins. The outcome of neocentromere activity during female meiosis is that knobbed chromatids end in the upper and lower cells of a naturally linear tetrad. The lower cell develops into the egg. Thus, neocentromere activity leads to meiotic drive.

Through creating an Ab10 BAC library and sequencing 11 BAC clones, three dominant molecular markers for the Ab10 haplotype were produced and used to screen wild populations of maize and teosinte, its progenitor. This work facilitated the discovery of new molecular and structural variants of Ab10 and showed that the majority of teosinte populations are segregating Ab10. One structural variant of Ab10, Ab10-L2, contains only TR-1 and exhibits very strong TR-1 neocentromeres, however it does not show strong drive. Rather, Ab10-L2 down regulates the strong meiotic drive the other Ab10

variants confer. These data combined with the cytological characterization of four mutants indicate that knob 180 is essential for meiotic drive, but TR-1 is not. In sum, the data presented here shed light not only on the composition of Ab10 but also provide fresh insights into the functional and population dynamics of the different variants of the haplotype. It is evident that the selfish and competitive Ab10 haplotype continuously plays into shaping the genetic and cytological landscape of modern maize.

INDEX WORDS: meiotic drive, meiosis, abnormal chromosome 10, knobs

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Meiosis and Meiotic Drive

The advent of sexual reproduction is responsible for the vast diversity and beauty held in the natural world. Higher eukaryotes, including plants, animals, and fungi, achieve sexual reproduction through the elaborate process of meiosis. Meiosis is a very specialized form of cell division in which a diploid parent cell is reduced to four haploid daughter cells (meiocytes) that go on to develop into gametes (functional sex cells). While the mechanics of meiosis are the same in males and females, the male and female meiocytes develop differently. In the males of plants and animals, all four meiocytes develop into functional gametes (pollen or sperm) and these gametes have an equal chance of being transmitted to the progeny. In females only one meiocyte develops into a functional gamete (the egg).

Meiosis acts to create genetic diversity in two steps. In the first meiotic division (meiosis I) homologous chromosomes are precisely paired, recombined, and carefully separated, thus reducing the number of chromosomes. During the second meiotic division (meiosis II) the two sister chromatids (which are no longer exact replicates due to the recombination that occurred in meiosis I) are separated, yielding four genetically distinct haploid cells. Importantly, since recombination occurs at least once between every pair of homologs, each of the four daughter cells is not only different from each other, but also contains a unique combination of genes that may not be found in either parent. Meiosis reliably executes Mendel's laws of segregation and independent assortment to perpetuate the diversity that natural selection and genetic drift act on, allowing organisms to evolve and either adapt or perish. However, there are exceptions to these rules.

Deviations to Mendel's laws fall under a broad category known as genomic conflict, indicating the quest for self preservation that a genomic element displays without regard for host fitness. Examples

of genomic conflict encompass everything from transposable elements to auxiliary (B) chromosomes to autosomal segregation distorters [1]. Transposable elements are short regions of DNA (a few hundred to a few thousand base pairs in length) that encode proteins to move and/or copy themselves independent of replication [2]. They were first discovered by Barbara McClintock in maize, and have since been identified in all eukaryotes [3,4]. B chromosomes are entire superfluous chromosomes that do not contribute to the necessary genes in their host organisms. They have unknown origins but have been identified in many eukaryotic species [5]. B chromosomes increase in number generally through non-disjunction during meiosis [5,6]. Autosomal segregation distorters are large gene complexes that typically cheat Mendel's rules in their favor, i.e. they are transmitted at rates higher than the expected 50%. However, segregation distorters can also refer to regions showing reduced transmission. Segregation distortion systems have been found in multiple independent lineages in all three major eukaryotic taxonomic groups (plants, animals and fungi) [1]. Although all autosomal segregation distorters use different, independently evolved mechanisms to alter Mendelian genetics, they share key structural similarities. In brief, most autosomal segregation distorters involve multiple loci that are contained in a large, genetically isolated haplotype.

The structural commonalities of autosomal segregation distorters can be seen in examples that show reduced transmission as well as examples that show increased transmission. One example of reduced sexual transmission is the aposporous apomixis haplotype (ASGR) that confers apospory in *Pennisetum squamulatum* and the closely related species *Cenchrus ciliaris* [7,8]. Apospory is a type of asexual reproduction that allows embryo development from an unreduced egg. Since apospory bypasses meiosis all together, ASGR produces the ideal system to ensure its own transmission. When this system fails (in the rare sexual female) this haplotype exhibits severely reduced transmission, as low as 12% in some sexual F1 hybrids of *Pennisetum glaucum* x *Pennisetum squamulatum*. ASGR also shows slightly reduced transmission through pollen (42%) [9]. It is unclear mechanistically why this region shows reduced transmission, but it likely has to do with post-meiotic gametogenesis inhibition and not an actual

alteration of meiotic processes [9,10]. The ASGR region occupies a large region (approximately 50 Mb) found on the arm of its carrier chromosome [8,11], which exhibits reduced recombination [12] and is speculated to have originated due to gross chromosomal rearrangements (translocation and inversion) [13]. It is likely these rearrangements that cause the reduced recombination, thus ensuring the functional gene complex is inherited as a single unit. However, the lack of recombination could easily allow for the accumulation and propagation of deleterious fitness alleles that contribute to the observed transmission reduction.

Preferential autosomal distortion systems (systems that show increased transmission) also rely on large isolated haplotypes. Three classic examples include the *t*-haplotype in *Mus musculus*, spore killer (Sk) in *Neurospora crassa*, and Segregation Distorter (SD) in *Drosophila melanogaster* [1,14,15]. While they are frequently referred to as meiotic drive systems due to their preferential transmission these three examples act on the products of meiosis rather than the process itself [1]. Specifically, they achieve preferential transmission by inducing dysfunction in male gametes that do not contain them [16-18]. To this end, each of these systems contains several trans-acting factors including a killing locus, a responder locus, and a locus (or allele of the responder) that is insensitive to the killing effects [1]. The major components of *t*, Sk, and SD are located in large haplotypes in or near pericentromeric heterochromatin (areas of low recombination) [19-22]. The lack of recombination within these haplotypes ensures that the functional gene complexes will be transmitted together so that meiocytes with the killing locus will survive and all other meiocytes (sensitive meiocytes) will die. As with AGSR, the downfall of such tight linkage over a large region is that recombination can no longer act to eliminate deleterious alleles. Furthermore, in the cases of *t*, Sk, and SD not only will deleterious alleles cease to be eliminated, but they will be preferentially transmitted.

Deleterious alleles have not been identified in all driving haplotypes in part due to lack of molecular characterization of many systems, however *t* and SD are known to contain deleterious alleles [23,24]. The measured observed outcome of both characterized and unidentified deleterious alleles in

these and other drive systems include lethality, reductions in fertility, and male sterility [1,14,25]. The negative effects of such deleterious alleles likely contribute to the observation that most drive systems do not become fixed in their host populations. In fact, the frequency of many driving haplotypes is so low (averages around 5% in the case of *t* and SD) that active suppressors and/ or competitors of autosomal drive systems likely co-exist in natural populations. There is good evidence of such competition between different SDs in African drosophila populations [22].

In addition to post-meiotic drivers like SD, Sk, and t, examples of true meiotic preferential segregation also exist in nature. These systems achieve their victory by using the natural asymmetry of female meiosis to their advantage. Specifically, they are able to strategically place themselves in the meiocyte that will inherently develop into the functional egg. Once such driving locus, D, has been recently discovered in *Mimulus guttatus* [26]. Importantly, in the symmetrical meiosis of males (where a true meiotic drive system would have no advantage) D shows Mendelian segregation [27]. In hybrids of *Mimulus guttatus* and *M. nasutus*, D is transmitted to 98% of the progeny through the female; however, within *M. guttatus* populations D only drives up to 58% [27]. These observations as well as FISH data from *M. guttatus* inbred lines point to centromere divergence between *M. guttatus* and *M. nasutus* as the cause for the strong drive of D in these hybrids [27]. Several reasons have been speculated to contribute to the weaker drive observed within *M. guttatus* compared to the hybrid, including, genomic differences between the hybrid parents aggravating chromosome competition, and/ or the evolution of a drive suppressor within *M. guttatus* [27]. Notably, this drive system adheres to the characteristic of being contained within a large, genetically isolated haplotype (in this case the centromere). Moreover, homozygous D shows a reduction in pollen viability indicative of deleterious alleles trapped in the driving haplotype [27].

True meiotic drive has been hypothesized as the evolutionary force controlling all centromere evolution. Centromeres, the primary constriction of chromosome, are responsible for recruiting the kinetochore, a large protein complex that bridges the interaction of the chromosome and the spindle

microtubules. The interaction of kinetochore proteins with centromeric DNA is highly conserved across eukaryotes, independent of DNA sequence, and believed to be controlled by a competition between epigenetic and genetic forces [28-30]. Nearly all eukaryotic centromeric DNA is made of tandem repeats and plant centromeres also contain retrotransposons [31], but only one centromere is known to contain any genes (rice centromere 8) [32]. Centromeric DNA does not undergo recombination and the sequence of the repeats is not conserved between even species in the same family [33]. It is thus interesting that the repetitive nature of centromeric DNA is conserved, and that the key DNA binding kinetochore proteins have homologs across eukaryotes [28,33]. The paradoxical nature of centromeric DNA and kinetochore proteins has led to the proposition that meiotic drive governs centromere evolution [28,34-36].

Tandem Repeats and Knobs

The term tandem repeat can refer to any DNA sequence of two or more bases that is repeated, in tandem, at least twice. There are different categories of tandem repeats that have been found across all eukaryotic taxa. Microsatellites are tandem repeats where each repeated unit is less than ten nucleotides in length and repeat units from ten to about 100 nucleotides are typically called minisatellites [37]. Tandem repeats can have a repeat unit greater than 100 nucleotides, but these do not have a universal name [38]. Often tandem repeats, particularly repeats of larger unit size, are referred to by their location in the genome or their function, for example, centromere repeats. Some types of tandem repeats, such as microsatellites, do not fulfill any particular role in organism function, but can be used as a tool to examine relatedness of individuals within populations [39]. Other tandem repeats hold very important roles in organisms, for example telomeres protect chromosomes from losing valuable genetic information each round of cell division [40]. Still other repeats, such as centromere repeats, while useful, are dispensable. Tandem repeats on occasion can have very negative effects on organism health as in Huntington's disease in human [41].

When tandem repeats are dispensable (i.e. have nothing actively perpetuating their existence) random chance governs their evolution and gives them an equal likelihood of expanding or contracting in

number. There are two main ways in which tandem repeat number can change, strand slippage and unequal crossing over [2,38]. Strand slippage during DNA replication generally involves short intervals of DNA so may act more as a mechanism to generate tandem repeats than a method to drastically alter their number. Tandem repeats can also be subject to unequal crossing over during meiosis and mitosis which potentially can cause dramatic changes in copy number. *Zea* species contain a unique set of tandem repeats that form large, cytologically visible, heterochromatic structures called knobs.

Knobs serve no known function, but are extremely abundant [42]. Knobs can be composed of either one or both of two different tandem repeats: a 180 bp repeat called knob 180, and a 350 bp repeat called TR-1 [43,44]. Perhaps the most famous knobs are located on the variants of chromosome 10, termed abnormal chromosome 10, but cytologically visible knobs have been found on all ten chromosomes of all *Zea* species, and knobs composed of just the 180 bp repeat have been observed in the sister taxa to *Zea*, *Tripsacum* [42,45]. Thus, it seems the 180 bp repeat evolved earlier in evolutionary time than the TR-1 repeat. There has been some speculation that TR-1 evolved from the 180 bp repeat, however they are so far diverged today that they only share two short regions of homology, a 31 bp region and a 12 bp region (with less than 65% homology) [43].

Knob position and size are non-random and highly polymorphic. Knobs are found in gene rich regions of chromosome arms, though all 34 potential knob loci [46] are not simultaneously occupied, and knob positions and sizes vary greatly among lines and individuals of open pollinated landraces and teosintes [42]. The accumulated mass of knob repeats can account for up to nearly 17% of the total DNA sequence in maize [47]. Additionally, knob size polymorphism accounts for 23% of the genome size variation seen among examined individuals [47]. Knobs contain no known coding information and typically are inert structures believed to have a deleterious effect on genomes if any, due to their large size, which could slow replication. Thus, it does not make sense that they have accumulated to such a high degree. Buckler *et al*. [46] showed that the position and abundance of knobs in *Zea* correlates with the theory that they are under the control of the abnormal chromosome 10 (Ab10) meiotic drive system.

Also, since knobs are found in gene rich regions, Ab10 is presumed to have had a major impact on the frequency of alleles linked to knobs.

Maize Abnormal Chromosome 10

Abnormal chromosome 10 (Ab10) has been implicated as the primary cause of knob evolution in *Zea* because of its trans-acting meiotic drive abilities. The Ab10 drive system is arguably the most famous example of a true meiotic drive system. Ab10 is able to use the inherent asymmetry of female meiosis to place itself and other knobbed chromatids in the meiocyte that will develop into the egg [48]. The mechanism of meiotic drive in maize was proposed in 1952 by Marcus Rhoades [49] and is diagrammed in figure 2.1b. At the onset of meiosis a recombination event occurs between the Ab10 haplotype (or knob locus) and the centromere. This creates a heteromorphic dyad in which each pair of homologs consists of one Ab10 (or knobbed chromatid) and one N10 (or unknobbed sister). Once chromosomes are aligned on the metaphase plate all knobs begin to quickly and dramatically travel along spindle microtubules to the poles. This movement is termed neocentromere activity and allows knobbed chromatids to set up a distinctive outward orientation when they reach the spindle poles. Moreover, each product of meiosis I will carry one knobbed chromatid. The outward orientation and neocentromere activity are maintained through meiosis II, placing the knobbed chromatids in the top and bottom cells of the linear tetrad at the end of female meiosis. The bottom cell naturally develops into the functional egg, so one of the knobbed chromatids will be preferentially transmitted. Though neocentromeres can be seen during male meiosis, preferential transmission through males is not possible in this system since all four male meiocytes develop into pollen grains.

Theoretically knobbed chromatids can reach close to 100% meiotic drive by this mechanism, but measured meiotic drive levels usually average at 70% transmission [48,50]. The degree of preferential transmission is limited by recombination, the efficiency of neocentromere formation, and other postulated factors [50]. Recombination between structural heterozygotes (e.g. knobbed and unknobbed homologs) to create a heteromorphic dyad is absolutely essential for drive to occur [48]. If a heteromorphic dyad

does not form the knobbed homologs will both travel to the same pole in meiosis I. They will maintain a strong outward orientation, but this orientation has an equal likelihood of being apical or basal. Thus, without the formation of a heteromorphic dyad, the knobbed chromatids will finish in the top most cells of the tetrad 50% of the time and the bottom most cells 50% of the time, and Mendelian inheritance will be restored. Though it is obligatory to have one recombination event per pair of homologs, Ab10 is known to increase recombination between structural heterozygotes [48,51]. This recombination effect works not only on Ab10 and N10 homologs but on other maize chromosomes that structurally differ.

Knob movement is termed neocentromere activity since the centromere (primary constriction) is typically the only part of a chromosome known to interact with the spindle microtubules. However there are key differences between centromere and maize neocentomere function. The poleward movement of centromeres is dictated by the kinetochore, whereas neocentromere activity is mediated by unique, non-kinetochore proteins [52]. Neocentromere movement of knobs not only utilizes distinctive proteins, but also behaves very differently from the kinetochore-mediated movement observed at centromeres. Kinetochores bond the plus ends of spindle microtubules to centromeric DNA, and then microtubule flux (polymerization and depolymerization) generates chromosome movement. Alternatively, neocentromeres actively move themselves laterally along the sides of microtubules, and their movement is independent of microtubule flux [53,54].

The Ab10 drive system, like other segregation distorters, is held within a large (55 cM), genetically isolated haplotype [55]. This haplotype is located on the end of chromosome 10 and is what distinguishes the Ab10 chromosome from the canonical maize chromosome 10 (N10). Importantly, all of the functions required for meiotic drive have been roughly mapped to the Ab10 haplotype [50]. The Ab10 haplotype consists of four main sections, the TR-1 region, the shared euchromatic region, the large 180 bp knob, and the distal euchromatic tip (Figure 2.1a) [50]. The two euchromatic portions of the Ab10 haplotype contain sections of sequence also found on N10 as well as sections of unknown, presumably unique sequence [55,56]. Four known morphological markers located distal to the *colored1* (*R1*) locus on

N10, have been mapped to the shared euchromatic portion of Ab10. These loci are *white seedling2* (*W2*), *opaque endosperm7* (*O7*), *luteus13* (*L13*) and *striate leaves2* (*Sr2*) but they have undergone a major inversion within the Ab10 haplotype, placing them in the following order: *L13*, *O7*, *W2*, *Sr2* [56]. A few molecular markers from N10L of the B73 reference genome have also been crudely mapped to the TR-1 region and the shared euchromatic region on Ab10 [55]. The entire Ab10 haplotype lies distal to the *R1* locus. It is tightly linked to *R1*, undergoing less than 2% recombination with the corresponding region on N10 distal to *R1*. [57].

There are two known structural variants of Ab10, Ab10-I and Ab10-II, both of which are able to preferentially transmit themselves over an N10 homolog at comparable rates [51]. However, both haplotypes do not show the same neocentromere activity. Ab10-I confers neocentromere activity to knobs composed of both the TR-1 repeat and the knob 180 repeat, but Ab10-II is only able to activate knobs with the 180bp repeat as neocentromeres [55]. This observation indicates that the two knob repeats may be activated by different proteins, and thus have independent activity. Accordingly, the neocentromere activies of TR-1 and knob 180 have been mapped to different locations within the Ab10-I haplotype [50,53]. Moreover, TR-1 neocentromeres are able to move even faster than knob 180 neocentromeres [53]. In spite of their differences, Ab10-I and Ab10-II appear to be more similar to each other than either is to N10. Ab10-I and Ab10-II share the same inversion of the *L13*, *O7*, *W2*, and *Sr2* loci, but otherwise differ [58] (Figure 2.1a). Ab10-I contains three small TR-1 knobs in its most proximal domain, and one large knob composed of the knob 180 repeat followed by a euchromatic distal tip. Ab10-II contains one very small TR-1 knob, two 180 bp knobs, and no distal tip. Ab10-I has been found in domesticated maize, but Ab10-II has only been observed in teosinte (the wild progenitor of maize) [51,59].

Similar to other autosomal segregation distorters Ab10 appears to be maintained at low levels in wild maize populations. McClintock *et al*. [59] surveyed approximately 1300 landrace populations and 54 teosinte populations noting Ab10 presence among other things. They found that 14% of the maize

landrace populations they surveyed contained Ab10-I, and none contained Ab10-II. Though the sample size of teosinte populations was smaller, Ab10 appears to be more prevalent in teosinte than maize. Ab10-I was found in 9% of the populations and Ab10-II was found in 26% of the 54 teosinte populations. Possible explanations for the low frequency of Ab10 include a reduced male transmission of Ab10 [46]. In a homozygous state Ab10 appears to cause reduced vigor and seed set in the homozygous condition, but the underlying mechanisms causing poor performance as a homozygote are unknown. No specific deleterious alleles or suppressor elements have been characterized though it is likely some exist.

Purpose of Study

There are no sequence data or molecular markers from the Ab10 haplotype, and the sequence of the key genes involved in neocentromere activity and meiotic drive remain unknown. Though extensive, the last survey of Ab10 abundance was performed in 1981, before the advent of modern cytological resources such as fluorescent *in situ* hybridization (FISH), and before teosintes were split into subgroups (parviglumis, mexicana and huehuetenangensis) [60]. We now know that the subspecies, parviglumis, was the primary genetic contributor to maize [61] and that Ab10 has been found in parviglumis. This proves that Ab10 is more ancient than the domestication of maize and it is speculated to be very ancient, perhaps dating back to the divergence of the *Zea* species.

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CHAPTER II:

RAPID DIVERSIFICATION OF THE MAIZE ABNORMAL 10 MEIOTIC DRIVE COMPLEX[1]

[1] Kanizay, L. B., Shan, X., Hufford, M., Peterson, D.G., Ross-Ibarra, J. and Dawe, R. K. To be submitted to PLOS Genetics.

Abstract

Maize Abnormal chromosome 10 (Ab10) is a classic example of meiotic drive whereby genes exploit the mechanics of chromosome segregation to increase their transmission advantage. When Ab10 is present, normally quiescent heterochromatic knobs become neocentromeres that are preferentially transmitted in female meiosis. As most maize lines contain multiple knobs on different chromosomes, Ab10 is thought to have had a major impact on maize genome structure and allele frequency. There have been no molecular studies of Ab10. To begin a study of the molecular landscape of Ab10, we constructed a BAC library and sequenced eleven BACs that lie within the Ab10-I haplotype. From the resulting ~1.1 Mb of new Ab10 sequence, we developed three Ab10-specific PCR markers and scored for the chromosome in a sample of 355 individuals from natural teosinte populations and cultivated maize landraces. The data indicate that Ab10 occurs throughout most if not all populations of *Zea mays*, including early domesticated forms. The results further revealed a reservoir of previously unsuspected Ab10 haplotype diversity, including at least one new, cytologically distinct form called Ab10-III. The fact that multiple forms of Ab10 coexist within populations suggests that they are actively competing with each other, rapidly spawning new forms of the haplotype that subsequently influence the frequency of knobs and their linked loci throughout the genome.

Introduction

Eukaryotic sexual reproduction requires the elaborate process of meiosis, in which the diploid state is reduced to unique haploid gametes. The defining step of meiosis is the first division (meiosis I), where homologous chromosomes pair, recombine, and separate. Meiosis II follows a mitosis-like pattern to produce haploid cells that contain the parents' genomes in randomized arrangements. It is a naturally diversifying process that is fair in the sense that all alleles are represented equally. However the meiotic daughter cells can begin to take different paths as they mature into gametes. In males, all four meiotic products develop into sperm cells that may compete at the level of fertilization. In females, the potential for competition starts immediately following meiosis when only one of the four daughters of meiosis is

chosen to become an egg. The selectivity of female gametogenesis opens the possibility that genes and chromosomes might compete during meiosis to gain access to the reproductive cell, disrupting the fairness of Mendelian segregation itself [1].

Major deviations from Mendel's rules have been termed meiotic drive, signifying preferential transmission (higher than the expected 50%) of certain genomic regions to progeny. Although deviations in meiosis are implied by the term, the most heavily studied examples of meiotic drive affect post-meiotic events. Three well characterized examples are the *t*-haplotype in *Mus musculus*, Segregation Distorter (SD) in *Drosophila melanogaster*, and Spore Killer (Sk) in *Neurospora* [2]. In these examples meiotic drive is controlled by multiple loci that encode transacting factors, including a driving locus and a responder. The loci involved are tightly linked in a haplotype enforced by genomic rearrangements and/or proximity to pericentromeric heterochromatin [3,4]. The genes involved in the *t*, SD, and Sk systems are different, but in all cases they disrupt Mendelian transmission by causing sperm sterility or aborted spores [5,6].

Maize abnormal chromosome 10 (Ab10) causes meiotic drive of itself and knobbed chromosomes through the female to ensure that 60-80% of progeny contain it [7]. Much like *t*, SD, and Sk, the Ab10 meiotic drive system is held within a large, genetically isolated haplotype containing megabases of DNA [8] (Figure 2.1a). Within the haplotype there are at least three genes that encode trans-acting driving factors, and these critical genes are unique to the Ab10 haplotype [7]. Ab10 directly manipulates the process of meiosis to increase the likelihood that it is transmitted to the basal cell that becomes the egg. The driven loci are large heterochromatic repeat arrays called knobs. Knobs are composed of the knob 180 repeat, the TR-1 repeat, or a combination of the two repeats (Figure 2.1a). Notably, knobs are not unique to Ab10, but are frequently found on other chromosomes as well. Any single knob can contain thousands of repeats, and when multiple knobs are present, they can increase genome size by as much as 23% [9-11].

The mechanism of Ab10-mediated meiotic drive in maize was proposed in 1952 by Marcus Rhoades [12] (Figure 2.1b). It begins with an obligatory recombination event between the centromere and a knob locus, or in the case of Ab10, between the centromere and the Ab10 haplotype, creating heteromorphic dyads. Once the chromosomes are aligned on the metaphase plate, knobs move rapidly poleward along microtubules. This activity is termed neocentromere activity because of its superficial similarity to centromeres, though the mechanisms of motility are very different. Neocentromere activity is mediated by non-kinetochore proteins [13] that enable the knob-containing chromosome arms to travel faster than centromeres, and to stretch along the microtubules towards the spindle poles. This fast and directed movement allows neocentromeres to create a distinctive outward orientation that is maintained through meiosis II so that knobbed chromatids are segregated to the upper and lower cells of the linear tetrad. The bottom cell naturally develops into the functional egg in females. Thus, one of the knob-containing chromatids will be passed on regardless of Mendelian rules. In theory knobbed chromosomes can reach 83% [15] (or higher) transmission by this mechanism although measured meiotic drive levels typically range from 65-80% [7,14]. The degree of preferential transmission is limited by several factors including, recombination, the efficiency of neocentromere formation, and other postulated factors that all must perform optimally and in conjunction [7]. The Ab10 drive system has been implicated as the primary cause of knob evolution in *Zea*, and is presumed to have had a major impact on the frequency of alleles linked to knobs [15].

Though knobs have become extremely prevalent in maize, cytological surveys suggest that Ab10 is found in only 14% of sampled maize landrace populations [16]. It is not unusual for meiotic drive systems to be maintained at low levels in nature either due to inherent fitness defects or selection for host-encoded suppressors that reduce meiotic drive [2,17]. Possible explanations for the low frequency of Ab10 include a reduced male transmission of Ab10 [15] and the fact that Ab10 causes reduced vigor and seed set in the homozygous condition. In addition, it is possible that both the frequency and diversity of Ab10 were reduced as maize was domesticated into landraces, and then further reduced as maize races

were converted to inbreds. No modern inbreds appear to carry Ab10, and there is only one known form of Ab10 in landraces, which is the Ab10-I type that was originally described by Rhoades [18]. In the principle ancestors of maize, *Zea parviglumis* and *Zea mexicana* [19], there are at least two forms – the Ab10-I type and a cytologically different form known as Ab10-II [16,20,21]. Other minor variations in Ab10 would probably not have been noticed in the original karyotype surveys, which did not take advantage of fluorescent *in situ* hybridization (FISH).

Ab10 is well understood at a functional genetic level; however, from a molecular genetics standpoint, it remains largely uncharacterized [8] and the molecular relationship of Ab10-I and Ab10-II is unclear. Currently, there are no sequence data from Ab10 and no molecular markers for the haplotype, making additional surveys labor intensive, even with modern cytological techniques. Here we describe the sequence of multiple BAC clones from Ab10, and use Ab10-specific PCR markers to conduct a survey of the chromosome in natural teosinte populations and domesticated landraces. The data revealed that Ab10 is more prevalent and diverse than originally expected, with many more than two haplotypes observed. Multiple molecular variants may exist within single teosinte populations. A cytological sampling of four landraces revealed a novel structural variant (Ab10-III) which clearly differs from both Ab10-I and Ab10-II. These data suggest that Ab10 chromosomes are actively competing with each other in natural populations, driving rapid rates of change both within the haplotype as well as at the 34 other knob sites that indirectly respond to Ab10.

Materials and Methods

BAC library creation and gridding onto high-density filters

Tissue from a plant homozygous for the Ab10-I haplotype was used to create a BAC library. The BAC library was created in the pIndigoBAC-5 vector as described previously [22] using HindIII restriction enzyme option and the "Y" method for nuclei extraction, with minor modifications [23]. Clones were gridded and fixed onto 5 nylon membranes as in [24].

Overgo probe design and library hybridization

Single copy cDNA sequences from chromosome 10L of the B73 reference genome, located between the marker *rsp11* and the end of the chromosome were identified via BLAST at PlantGDB.org. These sequences were submitted to an overgo prediction program used in Dan Peterson's lab at Mississippi State University. Twenty-eight single copy overgos were chosen to screen the BAC library.

Overgo probes were diluted to a working concentration of 20 µM. Half a microliter of each probe was randomly labeled with ^{32}P-dCTP and ^{32}P-dATP, pooled, and hybridized to membranes at 55 °C, as in [24]. Ninety-six positive clones were handpicked and re-grown in a 96 well plate then spotted onto six new nylon membranes. The sub-library membranes were reprobed with overlapping pools of probes by row and column to identify which colony corresponded to which probe(s). Nine colonies corresponding to different probes than each other, as well as two colonies corresponding to the same probes were chosen for sequencing (for a total of 11 colonies). The BACs are referred to by the probe number(s) that hybridized to them.

BAC preparation and sequenicng

The eleven BACs were purified using the Qiagen Large-Construct Kit. The quality of the purified plasmids and size of insert was verified by digesting with NotI and running samples on a CHEF gel system [23]. Intact BACs were submitted to the Georgia Genomics Facility for 454 Titanium FLX sequencing. From our sequence data we found no chloroplast genes and less than 1% of assembled sequence was derived from *E.coli*.

RNA isolation and cDNA preparation

Immature tassels were dissected from Ab10 and N10 sibling plants. Anthers from each tassel were removed from florets and placed in PBS. Male meiocytes were extruded from the anthers, placed on a slide, stained with DAPI and visually staged from pre-meiotic through mature pollen. Anthers of the correct size to capture the range of stages of meiosis were then collected in 1.5 ml eppendorf tubes and

frozen in liquid nitrogen. RNA was extracted from the anthers using the Qiaquick RNA extraction kit. cDNA was synthesized using the Evrogen Mint kit, then normalized with the Evrogen Trimmer kit. The Ab10 and N10 normalized cDNA libraries were submitted to Emory University for 100 bp paired-end Illumina sequencing. cDNA Illumina reads were assembled by the core facility at Emory.

BAC sequence assembly and analysis

The 454 reads were assembled with both MIRA and Newbler [25,26]. These assemblies and remaining raw reads were then further assembled using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA). The two BACs that correspond to the same probes (11-12), were assembled together and subsequently treated as one BAC. Final assemblies were run through RepeatMasker [27] to eliminate TEs. The masked contigs were then BLASTed to the non redundant nucleotide and protein databases at NCBI, all with an e-value cut off of 10^{-5}, and *E. coli* hits were eliminated. Genes were identified using a combination of BLAST and the gene prediction programs, SNAP [28], FGenesH [29] and Augustus [30]. Expression of identified genes was confirmed by cDNA analysis. BAC contigs were uploaded to DNA Subway (http://dnasubway.iplantcollaborative.org/) for display purposes with Gbrowse [31,32].

Mapping of BACs to the Ab10 haplotype

Nine repeat junction primers (RJ) were identified using RJPrimers: v1.0 [33-35]. Six intron size polymorphism primers were identified by BLASTing the set of 35 complete genes identified in the Ab10 BACs and comparing them to their homologs on the B73 N10 chromosome [36]. Introns that differed in size by at least 50 bp in this comparison were tested as markers. PCR was used to map the BACs within the Ab10 haplotype using the 15 primers in a series of DNAs extracted from Ab10-I and Ab10-II deficiency lines. Reaction conditions were as follows: per reaction we used 1x Sigma PCR buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.25 µM primers, 1-2 unit Sigma Taq polymerase, 10-30 ng DNA. Reactions were denatured at 94 °C 5 minutes, followed by 40 cycles of 94 °C 30 seconds, 55 °C-59.5 °C 20 seconds, 72 °C 30-60 seconds, followed by a final extension at 72°C for 5 minutes. PCR reactions were run on 2% agarose gels.

Teosinte and landrace screen

DNA from a collection of 355 individuals from 20 teosinte populations, 114 maize landraces and one *Tripsacum* individual were obtained from Jeff Ross-Ibarra's lab at the University of California, Davis. Three dominant repeat junction PCR makers were tested in a set of 53 maize inbreds [37] and found to be unique to Ab10. These three markers (A1ISPB1, D6AllRJJM6, and G8RJJM3) were then used to screen the set of 355 DNA samples. PCRs and gel analysis were carried out as for mapping.

Slide preparation, probe design, and fluorescence in situ *hybridization (FISH) of four landraces*

Seedlings were germinated and slides prepared for FISH in mitotic chromosomes as previously published [38]. For TR-1 and CentC, consensus sequences were examined by eye and regions from 20-23 nt in length that showed general conservation were chosen for probes. Four DNA oligos (20-21 nt long) for TR-1 and four DNA oligos (20-23 nt long) for CentC were ordered from Integrated DNA Technologies (IDT) (http://www.idtdna.com/) as 5' end labeled oligos. The TR-1 oligos were 5' Cy3 labeled and the CentC oligos were 5' Cy5 labeled. Ten FITC labeled DNA oligo probes for knob180 were previously designed [39].

All oligos were resuspended in 2x SSC (saline sodium citrate) buffer to 100 µM. The oligos for each repeat were then mixed together in equal molar amounts and diluted to a final mixture concentration of 10 µM. The diluted probe mixtures were used directly for fluorescence *in situ* hybridization (FISH) of prepared slides. Each slide contained: 0.5 µl TR-1 probe mix (10 µM), 0.5 µl CentC probe mix (10 µM), 0.2 µl knob180 probe mix, 5 µl salmon sperm DNA (140 ng/µl), and 3.8 µl 2x SSC in 1x TE (Tris EDTA) buffer. This mixture was dropped onto the slide and covered with a glass coverslip. The samples (slides) were then denatured 5-10 minutes in a boiling water bath and allowed to hybridize at room temperature in a humid chamber for 1-3 hours. Following hybridization the slides were dipped in SSC at room temperature twice to remove coverslips then air dried. Ten microliters of Vectashield with DAPI (Vector Laboratories, Burlingame, CA, USA) was added to each slide and the slides were covered with

fresh coverslips. Images were collected using Zeiss Axio Imager and processed using Slidebook 5.0 (Intelligent Imaging Innovations, Denver, CO, USA).

DNA extractions and PCR of four landraces used for FISH

Four landraces carrying Ab10 were obtained from the National Plant Germplasm System (http://www.ars-grin.gov/npgs). The 6-15 seed that were germinated for FISH were planted after their primary root tip was used for FISH. Approximately 100 µg of fresh leaf tissue was harvested from 3-5 week old seedlings and ground in liquid nitrogen. The plants continued to grow so that we could examine male meiocytes during pachytene and better understand the composition of the new Ab10. Each sample was lysed with 550 µl 2x CTAB solution, 100 µl 5M Sodium Chloride (NaCl), 13.5 µl β-mercaptoethanol (BME), and 6.5 µl 100 µg/µl RNaseA at 65 °C for 3hrs. DNA was extracted with an equal volume of Phenol:Chloroform:Isoamyl alcohol (25:24:1) saturated with Tris EDTA buffer (TE) pH 8.0. The samples were inverted 20 times to mix, and centrifuged in a bench top centrifuge at 14,000 rpm for 5 minutes. The aqueous layer was removed and extracted a second time with an equal volume of Chloroform: Isoamyl alcohol (24:1). Tubes were mixed and centrifuged as before, and the aqueous layer was removed to a new tube. DNA was precipitated with isopropanol, washed with 70% ethanol, and pellets were air dried and resuspended in 50 µl of TE pH 8.0.

PCRs were carried out on the extracted DNA as for the initial screen of the 355 DNA samples.

Pachytene FISH of the new Ab10 variant, Ab10-III

Immature tassels from plants known to contain Ab10-III based on root tip spread FISH results were staged as for cDNA extractions. Anthers contain meiotic cells were fixed in 4% paraformaldehyde and coverslips of meiocytes were prepared [40].

Results

Identification of 11 BACs from within the Ab10 haplotype

Obtaining molecular markers from Ab10 requires access to the sequences within the meiotic drive haplotype, which is megabases in size and shows extensive molecular homology to N10 [8]. Prior data suggest that much of the N10 chromosome distal to the *R1* gene is embedded within Ab10; however the sequences are scattered, rearranged, and mixed with other unknown sequence and transposable elements (Figure 2.1a, 2.2) [8,21]. We reasoned that if we sequenced long sections of the N10-Ab10 shared region we could identify sequences and/ or polymorphisms unique to Ab10. To this end, a bacterial artificial chromosome (BAC) library from a plant homozygous for Ab10-I was created in the pIndigoBAC-5 vector. The resulting library contained 90,960 clones with an average insert of 112kb, providing roughly 97% (4x) genome coverage. The library was hybridized with short probes (overgos) homologous to 28 single-copy sequences distributed across the shared region (Figure 2.2). Of these 28, four did not hybridize and 24 identified more than one BAC (between two and eleven colonies). Several BACs hybridized to more than one probe, and have compound names to reflect this (e.g. BAC22-23) (Table 2.1).

The shared region of Ab10 is largely syntenous with N10

Eleven BACs that were estimated to be clustered around the presumed edges of the meiotic drive haplotype were chosen for sequencing by 454 technology (Table 2.1, Figure 2.2). The resulting reads were assembled then analyzed for gene and repeat content (Table 2.2, 2.3). Two of the eleven BACs correspond to the same probe and were assembled together and treated as one BAC for analysis (reducing the total to ten). As a control, we also analyzed the published sequence of thirteen BACs from the corresponding regions in the B73 reference genome (results not shown). The repeat content of the Ab10 BACs ranged from 44-80% per BAC with an average of 67% repeats. The corresponding B73 BACs ranged from 49-79% repeats with an average of 63%, which is not statistically less than the Ab10 repeat content. In addition, gene prediction software revealed the presence of 43 genes within the Ab10 BAC.

To determine if the predicted genes were expressed in Ab10 lines, we created a cDNA library from anthers undergoing meiosis in an Ab10 plant, and sequenced it using 100bp paired-end Illumina technology. We compared the assembled cDNA contigs with the predicted genes using BLAST, and confirmed that 35 of the 43 are transcribed in this tissue. As expected, 33 of the genes showed high homology to genes on the long arm of chromosome 10 from the B73 inbred. The other two genes showed highest homology to genes on chromosomes four (DW814383.1) and nine (BT066625.1). The Ab10 gene displaying the best homology with chromosome four also showed high homology with a B73 gene on 10L, indicating that this gene has been duplicated within the maize genome. The gene that had the best hit to B73 chromosome nine showed no good homology with any B73 genes from 10L.

The homology between Ab10 and N10 lies proximal to the 180 bp knobs

We sought to identify at least one PCR marker for each BAC so as to map them within the haplotype (Figure 2.2). Although multiple small SSR regions were found on the BACs, none of these proved to be reliable markers. As an alternative we tested 30 primer pairs that amplify introns for size polymorphism between Ab10 and N10. Six of the intron amplicons proved useful to the extent that they are polymorphic between Ab10 and either the B73 or W23 N10 chromosomes (B73 and W23 are lab tester strains). However, these primers were less reliable in other genetic backgrounds. In addition, we assayed intergenic spaces for unique transposon insertion sites that can be used as PCR product markers [33-35]. From this approach we identified nine useful markers, including three Ab10-specific dominant markers, which proved useful in any inbred genetic background. One polymorphic PCR marker was identified for each BAC, although BAC10 and BAC22-23 could not be mapped on both Ab10-I and Ab10-II. In the case of BAC10 this was due to a lack of polymorphism between Ab10-I and either B73 or W23, whereas the BAC22-23 marker is unique to Ab10-I and thus is not amplified in Ab10-II (Table 2.3).

One defining feature of Ab10 is that it does not recombine with N10 [18]. This property makes it impossible to carry out standard genetic mapping. Deletion mapping is a viable alternative since large

terminal deficiencies of Ab10 can be tolerated, although the most severe deficiencies are homozygous unviable due to the fact that Ab10 shares required genes with N10 [40]. Previous studies have used deficiencies of Ab10-I and Ab10-II to map shared genes that have mutant phenotypes: *white seedling2* (*W2*), *opaque endosperm7* (*O7*), *luteus13* (*L13*), and *striate leaves2* (*Sr2*) [21,42]. Ab10-I deficiencies have also been used to map RFLP markers [8] (Figure 2.2). We used deficiencies of Ab10-I and Ab10-II with breakpoints distributed along the haplotype to map the position of the BACs, which in turn allowed us to compare the overall structure of Ab10-I, Ab10-II, and N10 (Figure 2.2).

Our mapping data indicate that Ab10-I and Ab10-II are similar in overall structure. BAC2-3 mapped upstream of the most proximal breakpoints of both the Ab10-I and Ab10-II haplotypes, suggesting that it lies proximal to the functional meiotic drive haplotype. All other mappable BACs are located between the Df(I) and Df(F) breakpoints of Ab10-I. On the Ab10-II chromosome, the BACs mapped according to known rearrangements [42]. Four BACs that correspond to distal regions of N10 map to the proximal side of the Ab10-II haplotype (between Df(Q) and Df(M)), while the remaining four BACs map distal to Ab10-II Df(M) (Figure 2.2). These data confirm that a major inversion of the *W2*, *O7* and *L13* loci occurred after Ab10 diverged from N10, but before Ab10-I and Ab10-II diverged from each other. Moreover, the data strongly support the prior assertion that all homology between N10 and Ab10 is limited to the region proximal to the major 180 bp knobs [7,8,41], and suggest that the distal tip region is not derived from N10. The one area of disagreement between our data and prior results [8] is that we would have expected the most distal BAC (28) to map proximal to Ab10-I Df(I), which was not the case. This result suggests that the sequences at proximal edge of the Ab10-I haplotype (which equates to the distal end of N10) have sustained multiple rearrangements.

Ab10 is more prevalent in teosinte than maize

Ab10 has not been cytologically observed in any of at least 100 maize inbred lines [43], and it is likely that it is absent in all others since in our hands homozygous Ab10 plants are not vigorous. Hence, in order to test the specificity of our three Ab10 specific dominant markers we performed PCR on a core

set of 53 inbreds that represents about 73% of the diversity in maize [37,44]. We found that the markers are absent in all 53 of these inbreds. We decided to use these three markers to assess the frequency of Ab10 in more natural settings: open pollinated landrace populations and wild teosinte (predecessor of maize) populations. Previous cytological surveys of natural landrace populations throughout North and South America indicate Ab10 is segregating in 14% of the surveyed populations [16]. Multiple teosinte populations from Mexico were also surveyed, showing that Ab10 is segregating in 35% of these populations [16]. At the level of individuals Ab10 is present at very rare, about 1% of total surveyed maize individuals and 6% of total teosinte individuals were found to contain an Ab10 chromosome. Within populations that segregate Ab10 the maximum frequency of the chromosome was 2/3 (66%), though most populations maintained Ab10 at less than 50% [16].

To assess the frequency of Ab10 in natural populations of Z. *parviglumis* and Z. *mexicana*, we collected DNA samples from 12 individuals from ten populations of each native teosinte. We also examined single individuals from 114 landraces accessions and inbred lines, as well as a single *Tripsacum* (sister genus to *Zea*) individual. The three dominant Ab10-specific markers were used to score the full panel of 355 DNA samples. The combined data suggest that Ab10 is found in an average of 19% of the total individuals (landraces and teosintes). Ab10 is also widely distributed among the lines sampled, being found in 24 individuals of *Zea mays ssp. parviglumis* accessions, 20 from *Zea mays ssp. mexicana*, and 22 from maize landraces (Table 2.4). Within the teosinte populations, at least one Ab10 marker was observed in 85% (17) of the populations. These data suggest a higher frequency of Ab10 in teosinte populations than previously thought (85% compared to 35%), although we sampled different populations than those surveyed previously [16].

We were interested to find that many Ab10 chromosomes lacked one or two of the Ab10-I markers, though some of the patterns that did not match Ab10-I did match our pattern for Ab10-II. All 68 of the Ab10 chromosomes contained either a single marker or a combination of two different markers (Table 2.4). No individual contained all three markers at once, indicating that the original Ab10-I

accession from Marcus Rhoades was not resampled. There are at least two likely explanations for this observation: 1) there are multiple Ab10 variants and they can recombine with each other, and 2) some N10 chromosomes in natural populations contain one or more of the markers. In support of the first possibility, we found five *Z. mexicana* and five *Z. parviglumis* that seem to be segregating more than one haplotype variant of Ab10, based on different patterns of positive marker results (Table 2.4). To test the possibility that we may be amplifying N10, we expanded our analysis of four landraces that tested positive for one marker. Multiple seeds (6-15) from each line were germinated and scored by both PCR and fluorescence in situ hybridization (FISH) on mitotic root tip chromosome spreads (Table 2.5). The results support the assertion that one marker is sufficient to identify Ab10: All four lines were confirmed to be segregating Ab10, and each individual that was positive for a PCR marker was also found to contain at least one copy of the Ab10 chromosome by FISH. Likewise, individuals that lacked the Ab10 chromosome scored negative for all three PCR markers. In total, 16/46 individuals contained one variant of Ab10.

Discovery of a new cytological variant, Ab10-III, by population screening

During the course of our cytological confirmations (Figure 2.4), we observed that two landraces contained what appeared to be Ab10-I, and unexpectedly, the other two landraces showed a new cytological variation of the Ab10 haplotype that we term Ab10-III. Ab10-III contains a second TR-1-rich domain appended to the end of the main knob, which on Ab10-I is composed entirely of the 180 bp repeat (Figure 2.3). This staining pattern has not been previously described, though it is quite distinct from Ab10-I and Ab10-II. Using classical techniques [16] Ab10-III would probably have been confused with Ab10-I since both variants contain three small knobs (what we now know are the TR1 knobs), followed by what would appear as one large knob without the ability to distinguish TR-1 and knob180 (Figure 2.1a). The fact that multiple haplotypes were observed in our population screen (Table 2.4), and that at least a portion of the molecular variation is correlated with cytological variation indicates that the Ab10 haplotype is highly variable in natural populations of maize and teosinte.

Discussion

The fascinating phenotypes of Ab10 have been known for nearly 70 years, but the molecular characterization of this haplotype has been slow relative to the rapid advances in our understanding of the rest of the maize genome. Since Ab10 confers no known benefit to maize, and it is absent from modern inbreds, it has been left out of all comprehensive mapping, sequencing, and genotyping efforts to date. Here we have taken the first steps to characterize Ab10 molecularly, beginning by creating a BAC library from a line that includes the Ab10-I haplotype. We used probes from regions shared between Ab10 and N10 to identify eleven BAC clones that formed the basis of the study. Sequencing and mapping of the BACs revealed that most of the homology between N10 and Ab10 lies within a cytologically small section of Ab10 stretching between the deficiency breakpoints Df(I) and Df(F) (Figure 2.1, 2.3). This central region does not contain any known meiotic drive functions, but is genetically trapped by multiple inversions and the large knobs that flank it, making it an inseparable, if non-functional component of the Ab10 haplotype.

From an accumulated span of 1.1 Mb of contiged Ab10 sequence, we identified 35 expressed genes and extensive transposon-rich intergenic spaces. The intergenic regions proved to contain three reliable Ab10-specific markers that we ultimately used to score 355 *Zea mays* accessions for Ab10. These data revealed that Ab10 is present in an estimated 18% of teosinte (*Zea mays ssp. mexicana* and *ssp. parviglumis*) individuals, and that at least 85% of these natural teosinte populations are segregating the haplotype (Table 2.4). The actual distribution of Ab10 may be higher than 85% since population estimates are limited by sampling, which in our case was 12 individuals in each of 20 populations. Earlier cytological surveys of teosintes found Ab10 to be less abundant. It was identified in an average of only 6 % of total individuals and only 35% of teosinte populations contained these individuals [16]. These past surveys relied on fewer individuals per population (six), so are also likely to be an underestimate of Ab10s prevalence. While our data indicate that Ab10 is far more prevalent than previously thought, the populations we surveyed are not the same populations that were previously examined. Additionally we

examined twice as many individuals per population on average giving us a slightly higher power of detection.

In the 1970s McClintock *et al*. [16] surveyed a total of 1246 landrace populations across North and South America and found that Ab10-I was present in 14% of the populations, and only an average of 1% of individuals in these populations contained Ab10-I. Our data with landraces suggest the actual abundance maybe substantially higher than 14%, though we were only able to examine one individual per landrace. Our landraces populations ranged from Arizona through Mexico and Guatemala, and along the northwest coast of South America to the northern part of Argentina. The previously surveyed populations span a much larger area across the United States (US), Central, and South America compared to our populations. In several of those US populations outside of Arizona, as well as most of the South American populations from Brasil, Paraguay and Argentina there were no Ab10 chromosomes [16] (we did not sample from these regions of South America). In addition to low sampling within populations, the difference in Ab10 abundance between our survey and previous results might be explained by sampling from different locations. Nevertheless the discrepancy of Ab10 frequency in our data compared to the previous surveys led us to question if our markers were picking up false positives, for example N10 chromosomes.

Expanded studies with a larger set of randomly chosen landraces will help us address remaining concerns that our assay is subject to false positive identification. Although, our first round of tests (Table 2.5) suggests that one marker is sufficient to identify Ab10, and that our molecular assay provides an accurate assessment of Ab10 frequencies in teosintes and maize landraces. One major conclusion that can be drawn from both our data and the previous cytological data is that Ab10 appears to be present in a higher percentage of teosinte populations than landrace populations. By examining a larger set of landraces we might also be able to discover if Ab10 is more molecularly diverse in teosinte populations compared to landraces. Further comparisons of Ab10 in teosinte and maize will provide insights as to how, or if, the domestication process affected Ab10.

There is little doubt that Ab10 evolved from an early form of modern N10. Within the region between Df(I) and Df(F), we found 43 full length genes, 35 which were expressed in developing anthers. All but one of these genes showed primary homology to modern N10, with the single exception having apparently been derived from a modern day chromosome 9. This probably reflects the fact that maize is an ancient paleopolyploid with extensive remaining redundancy that can be lost without phenotypic consequence. Comparisons between normal, homologous maize chromosomes have revealed similar inconsistencies in gene content, known as position-absence variation or PAV [45,46]. It is possible that Ab10 evolved either before or soon after the ancient tetraploidy event, and since it has been genetically isolated for much of this period, such variation was anticipated. The fact that we based our study on the premise of homology with N10 could have limited the discovery of larger discrepancies.

Ancient polyploidy and genome rearrangement may also explain the origin of the large region of Ab10 distal to Df(F). This entire region, which we have shown is almost entirely non-syntenous with N10L may represent a remnant of the ancient whole genome duplication that was lost in B73 and other N10s. The origin of the distal tip in particular has been in question since 1942 [18]. The current hypothesis on its origin is that it was introgressed from a wide cross with a different grass species [8,47]. In light of our data as well as the growing body of literature on the abundance of PAV in maize it seems more likely that the distal tip of the haplotype was translocated and/or duplicated during the last whole genome duplication event and consists of an amalgamation of other portions of the genome. It is particularly intriguing that the distal tip of Ab10 contains the gene conferring knob 180 neocentromere activity, which is the single most important meiotic drive function (Chapter 4).

Our data suggest that there are many more Ab10 haplotypes than previously suspected: at the least, there are three major cytological forms and multiple molecular variants. The structural homology among different Ab10 haplotypes (Figure 2.2) suggest that recombination is contributing to the high level of molecular and cytological variation we observed. Ab10 is inherently competitive and the observation that teosinte populations are segregating more than one Ab10 variant leads to the idea that Ab10 variants

compete with not only N10, but with each other. It is known that larger knobs drive over smaller knobs when paired together [48], and the knobs on Ab10 seem much larger than all other knobs. Further evidence for Ab10 haplotype competition comes from the fact that the cytological variants of Ab10 contain different amounts of the two knob repeats. Previous work has shown these repeats to have independent neocentromere activities, with TR-1 being able to move poleward faster than the knob180 repeat [49] (Chapter 3). The fact that the new Ab10 variant described here (Ab10-III) differs primarily with respect to the abundance of TR1 suggests that TR1 is part an Ab10-versus-Ab10 genetic arms race [50,51]. Arms-race like competition is thought to drive rapid sequence change, and may help explain the molecular diversity we have observed in Ab10.

Knobs have become extremely abundant in all *Zea* due to the strong and continuous selection Ab10 places on them. Cytologically visible knobs have been documented at 34 loci on all ten chromosomes [15] and are highly polymorphic among lines in *Zea* and *Tripsacum* [43,52,53]. Recent cytological surveys reveal that maize accessions also differ widely in the proportion of knobs with 180 bp and TR-1 repeats [43]. Since Ab10 causes all knobs to display meiotic drive [54] and is statistically correlated with their overall abundance in nature [15], these results indicate that much of the genome is under the influence of Ab10 [8,15]. Issues relating to the size, repeat content and relative strength of the Ab10 chromosomes have direct relevance to overall genome size gradients [9,11] and the frequencies of the many thousands of alleles in linkage knobs.

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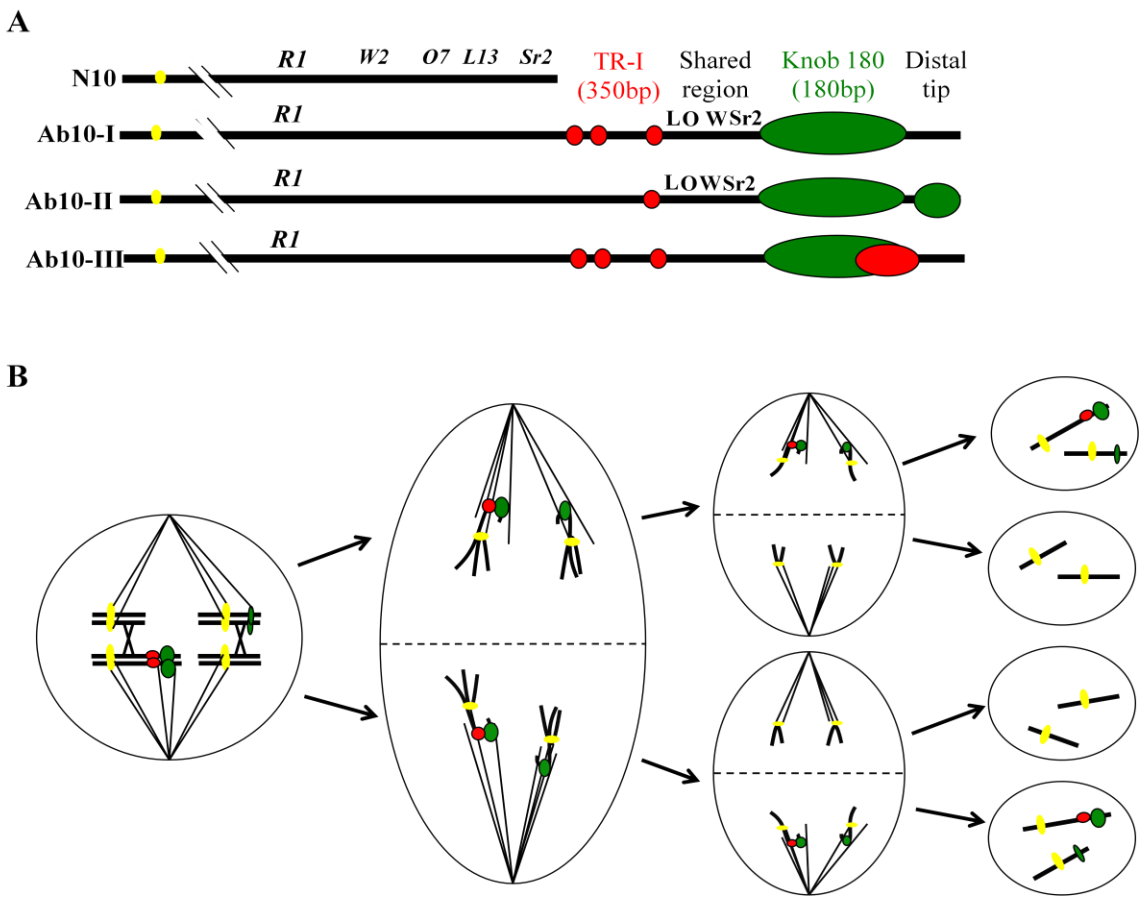

Figure 2.1: Abnormal chromosome 10 promotes meiotic drive.

A) The four variations of maize chromosome 10: normal 10 (N10), abnormal 10 type 1 (Ab10-I), abnormal 10 type 2 (Ab10-II), and abnormal 10 type 3 (Ab10-III). The chromosomes differ in length and knob content, with N10 possessing no knobs and the Ab10s containing different amounts of the TR-1 (red) and knob180 (green) repeats. The extended region on the Ab10s is responsible for meiotic drive and has been split into four main domains (the TR-1 region, shared region, 180 bp knob, and distal tip). B) Meiotic Drive model, based off Rhoades, 1952. Drive begins with a recombination event between the centromere (yellow) and knobs (red and green). In anaphase I, neocentromeres move laterally along the spindle poles ahead of the centromere, creating a strong outward orientation. Neocentromere activity and the outward orientation are maintained through anaphase II. These events place knobbed chromatids in the top and bottom most cells of the linear tetrad. The bottom cell naturally develops into the egg.

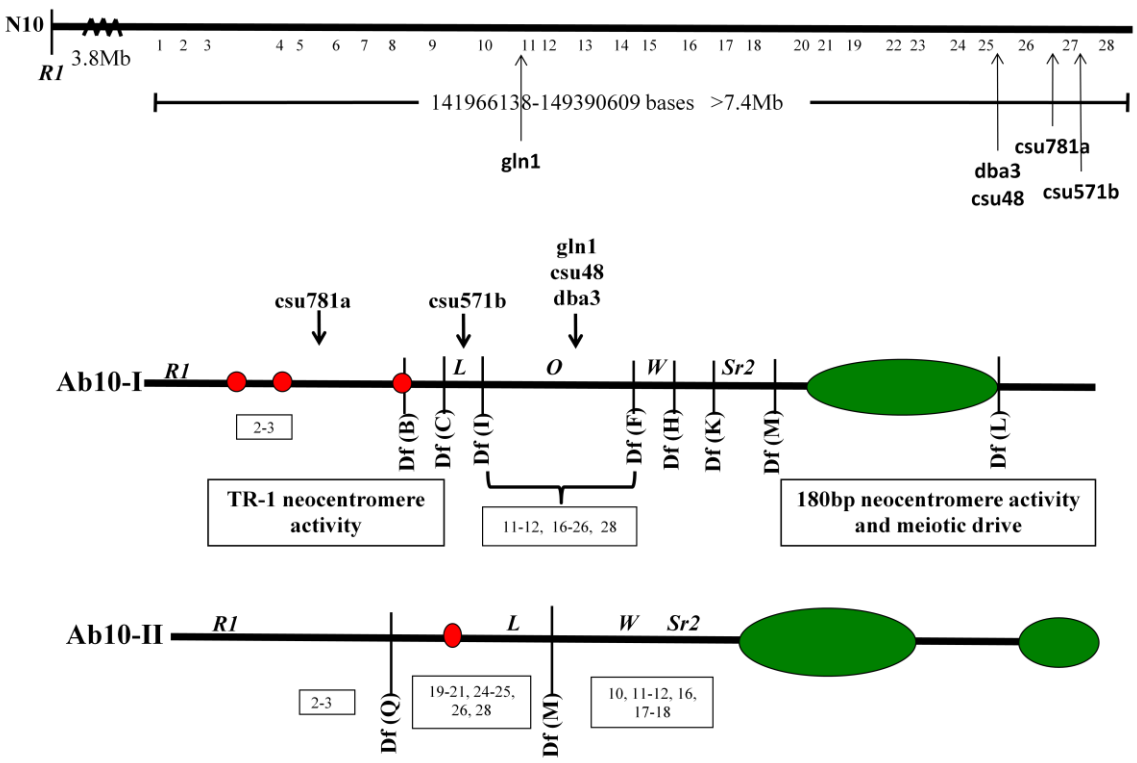

Figure 2.2: Positions of probes on N10 and corresponding BACs on Ab10-I and Ab10-II.

Relative probe positions on N10L are shown at the top. The positions of known markers are indicated by arrows. The location of the corresponding Ab10 BACs is indicated below blown up diagrams of the Ab10 haplotypes in boxes. The BACs were mapped on the Ab10s using deficiencies of the haplotypes (denoted by vertical lines). BAC2-3 mapped farthest upstream on both haplotypes. All other BACs mapped between Df(I) and Df(F) on Ab10-I. We were unable to map BAC10 on Ab10-I. On Ab10-II four BACs mapped between Df(Q) and Df(M), and four mapped distal to Df(M). We were unable to map BAC22-23 on Ab10-II. The position of the TR-1 and knob 180 neocentromere activities as well as the position of the N10 markers are shown in the Ab10-I haplotype. These positions are based on previous work [7,8].

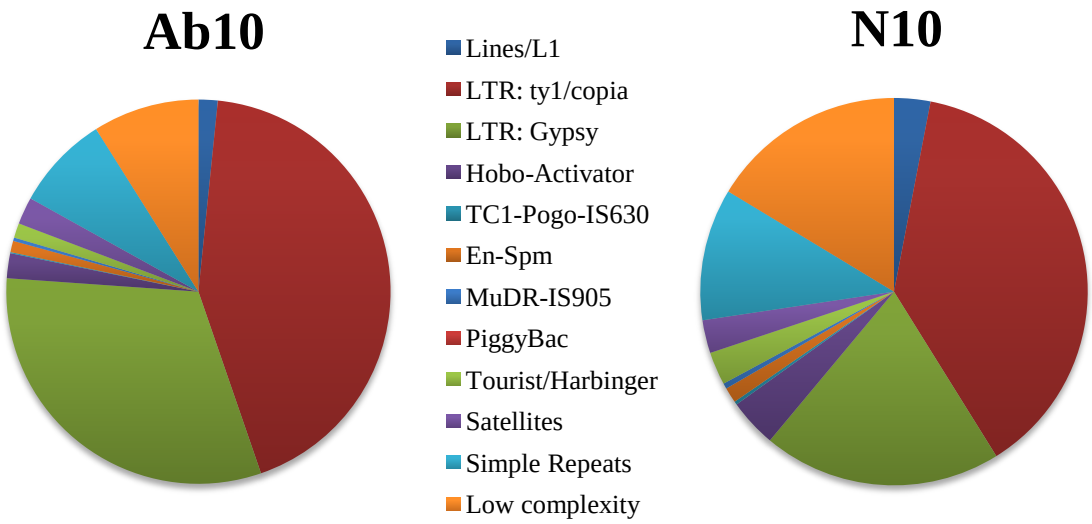

Figure 2.3: Repeat content of Ab10 BACs and the corresponding region on N10 of B73.

Ab10 BAC sequences were run through RepeatMasker. Corresponding N10 BAC sequences were downloaded from MaizeGDB and run through RepeatMasker. The amount of repeats on Ab10 is not significantly different from N10.

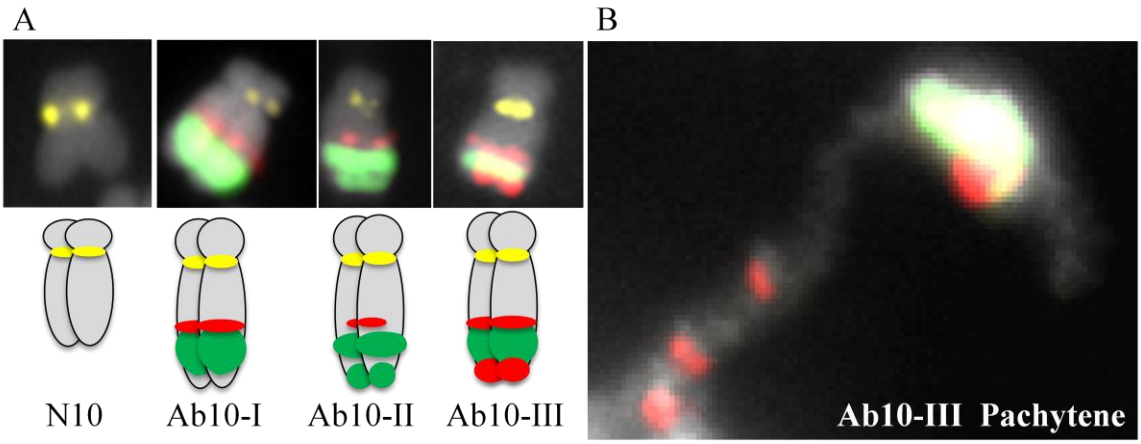

Figure 2.4: Karyotypes of maize chromosome 10.

A) From left to right FISH images from root tip spreads show N10, Ab10-I, Ab10-II, Ab10-III. Fluorescence in situ hybridization images are paired with cartoon depictions of the chromosomes. B) Pachytene image from a male meiocyte showing the Ab10-III haplotype. There are three small TR-I knobs, followed by a euchromatic region, a large 180 bp knob, another TR-1 knob, and a distal euchromatic tip. In all images chromosomes are stained with DAPI (grey) and hybridized with probes to centc (yellow), TR-1 (red) and knob180 (green).

Probe	cDNA genbank number	probe location in B73 Refgen_v2	distance between probes (bp)	BAC ID	probe sequence
1	BT034135.1	141966138 - 141966173		0	TTACACAACTTGCAAACGCATGGCTGGGCATTGCTG
2	EU958727.1	142092723 - 142092757	126550	2-3	CTGCCAAGGGGATTGCTATACCTCTTAGATCGTCAC
3	AY110965.1	142188234 - 142188269	95477	2-3	CCCGCCTATTTGTGGATGGTGTTTCTCGTGCCATTT
4	DQ244249.1	143442452 - 143442487	1254183	N/A	CTACCATGGAGAGTTTCGGCTTCAAGCAAATGGCAG
5	EU969545.1	143501006 - 143501041	58519	N/A	CTCACTTCTGAAGAATGACAGCCGTGGCCCTGGCGC
6	EU945786.1	143852056 - 143852021	351015	0	TCCATCGCAAGACGGGGAAAAGTGAGCACATTCTTG
7	EU941341.1	144142759 - 144142794	290738	N/A	GTGCTACAGGTCCATGCACCTTTTGCTAGATTGTCC
8	AY110814.2	144611585 - 144611620	468791	N/A	CAGGCATGAATGATCGAGAGATCCTAACAGGGAGTG
9	AY109294.1	145122333 - 145122368	510713	N/A	GCTGTTTTGGCTGAGCGTTTCATTGAAGCAGGTAGC
10	EU966139.1	145894946 - 145894981	772578	10	CGATTCCGAGGATGACTCGGAAGAAGATGATGACGA
11	BT041728.1	146469868 - 146469896	574887	11-12	AATCAAGGACGATTTCGAAACCCGTGGAGGATCCCT
12	EU953450.1	146473106 - 146473141	3210	11-12	TTTCTTGCCCCGCTATCGCTTCTGATGAGCTTGACA
13	EU971129.1	146690236 - 146690271	217095	N/A	AGTGTGTGTGCATTATCAGGCAGATGGATGGACTCG
14	EU965644.1	147254651 - 147254686	564380	0	CTGAGTAGACTTGTGTTTTCGCTTCCCCTTCCCCAT
15	EU964514.1	147361765 - 147361800	107079	0	GTAGCAGTAGCAGCCGTTTTCCTCTCTTCTTGCAGT
16	EU975708.1	147705944 - 147705979	344144	16	TTTAGCAACGTGTGCATTCCTGCCGTGCTTTGCACA
17	EU956118.1	147855441 - 147855476	149462	17-18	AGTAGTGTACTGGGATAGGGGTACTGCTACAGCTCA

18	EU963375.1	147885728 - 147885763	30252	17-18	AAAAATCCATAACGCAATCGCAAGGCGCTGCCTCCA
20	EE681409.1	148085796 - 148085831	200033	19-21	AAGGGTAGAACTTCTTGGTGCTCTGCAGGGAGTCAT
21	EE681409.1	148086813 - 148086848	982	19-21	CAAGGTGGCCGGCGTCTTGGTCGACGTCTTCATCAA
19	EU967632.1	148106614 - 148106649	19766	19-21	TTTCATGGGCTGACCCGGTTATGTGGCCTTGTTAGT
22	EU975794.1	148328503 - 148328538	221854	22-23	CCGTGGTTCGGCCTAATCCTCCTCTCTTTACTTTGT
23	EU975794.1	148328805 - 148328840	267	22-23	TTGGCAACTTGTTGCACGTACTCAGACCCTCTCTCT
24	BT042549.1	148503188 - 148503223	174348	24-25	ATAACGACGCTGGTGGATATGCCACTCAACAGCTTC
25	EU952272.1	148515337 - 148515302	12114	24-25	CATTAGCTGTACTCTAAGCTGGTACCGCACTTGGTC
26	BT037801.1	148638301 - 148638336	122999	26	CTATGTCACGGTGACTAAGCAGCCCCTTCTGTTATG
27	EU962249.1	148996297 - 148996332	357961	N/A	ATGAATAGGCCAAGTCCATACGTTTACCGTGTGGCG
28	BT039443.1	149390574 - 149390609	394242	28	GTGTACGCGCAGATTATGACGCTGTTCAACAAGCAG

Table 2.1: BAC probe information and location on B73 N10 .

N/A indicates that an Ab10 BAC corresponding to that probe was not sequenced.

BAC	BAC size (kb)	sequence coverage (x)	total # of contigs	# of conitgs >50kb	# of contigs 30kb – 50 kb	# of contigs 5kb – 30kb	sequence from LTR retro elements	sequence from other repeats	sequence from genes	# of genes with cdna support	# of full length genes	predicted gene #	gene fragment #
2-3	150	35.3	4	1	0	3	39 %	5.0%	14%	10	5	2	11
10	50	37.3	2	1	0	2	52%	1.4%	3.8%	3	3	0	1
11-12	120	9.2	26	2	0	10	65%	5.2%	7.1%	7	5	3	7
16	75	14.9	33	0	1	9	74%	0.6%	5.0%	9	6	1	8
17-18	115	41.1	7	0	1	6	43%	3.2%	5.0%	3	2	1	4
19-21	70	12.2	33	0	0	10	72%	0.6%	9.8%	8	6	1	5
22-23	90	8.6	82	0	0	13	68%	2.2%	2.7%	2	3	1	1
24-25	130	25.1	3	1	1	3	80%	1.3%	10%	10	7	1	3
26	50	31.9	4	1	1	3	51%	3.7%	15%	4	5	1	8
28	N/A	N/A	4	1	1	4	66%	0.3%	4.4%%	4	1	0	4

Table 2.2: BAC sequence information

N/A indicates the BAC size was not measured so sequence coverage could not be calculated for BAC28. BAC size was estimated from NotI digests run on a CHEF gel system. There were few reads that could not be assembled into contigs

Marker name	BAC	marker type	polymorphism	N10 size (bp)	Ab10-I size (bp)	Ab10-II size (bp)	Forward primer	Reverse primer
A1 34In7	22-23	intron	codominant	350, 500	400, 500	400, 500	AACGTGGAAGCTTGGGAACT	GGACCACTGTACTTGCGAAGA
A3 87In2	16	intron	codominant	N/A	1100	1100	TGTCACAAAGGCAAAGCGTA	GCTGGAGACGCTCATACCTC
C1 32In7	19-21	intron	codominant	350	300	300	GGGAAGCCTTTGTTGGACTT	TCTTCTTGTATGCATCCTCACC
C4 35In3	2-3	intron	codominant	500	750	750	GTGCCTTCACTTCCTCCTTG	GCTTCTATAGGCTGGCGTTG
D2 25In1	24-25	intron	codominant	N/A	400	N/A	CCAGCTTATGCCAAGCATTC	ATCCTCCCAGTTCGCTGATA
D2 25aIn9	24-25	intron	codominant	225	400	400	GGATCATGATTTGCAAGACG	TCTCTGATAGCCGCTTCCTC
C6 RJM5	17-18	repeat junction	dominant	N/A	700	700	CCTCTAGGCGACTATCAGCTTG	TGCAGACTATACTGCACAGACTC
C6 RJM12	17-18	repeat junction	dominant	N/A	400	400	TTCGGAAAAAAAATCACGTT	GGTGGAGCACAAGGCTTCTA
C2 RJJM2	26	repeat junction	dominant	N/A	150	150	TACAGCTCCGGTCACCTGTC	AAAGACTGTTGTTTCCCCTCC
C2 RJJM5	26	repeat junction	dominant	N/A	750	750	AGCCTCACTCTACCGCCTC	AACGAAACACATCTTAAAGCTATCC
C2 RJJM6	26	repeat junction	dominant	N/A	700	700	AACAGTGGAGGGAGGGGA	AACGAAACACATCTTAAAGCTATCC
H10 RJJM1	10	repeat junction	dominant	N/A	250	250	CGTAGTGTGAGGAGGACCTATCC	CTTTGACCACCCAGATGGA
A1 ISPB	22-23	repeat junction	dominant	N/A	550	N/A	GAGAACCGTTTGTCGAGCTC	GCTTCTGTAGCTCCGAATGG
D6A11 RJJM6	11-12	repeat junction	dominant	N/A	700	N/A	GCTTGTTTGGTTAATAAAGCGC	GTCTGCCCGACCATAACACT
G8 RJJM3	28	repeat junction	dominant	N/A	350	350	TTGAGTGCTGATGGGGCA	AGGGCATAATGATCACCCTG

Table 2.3: Molecular marker information.

N/A indicates no band (no PCR product) was produced in that sample.

MEXICANA				PARVIGLUMIS				MAIZE LANDRACES			
Sample ID	D6 A11	G8	A1	Sample ID	D6 A11	G8	A1	Sample ID	D6 A11	G8	A1
RIMME0021	**+**	**+**		**RIMPA0086**		**+**	**+**	RIMMA0710.1	+		
RIMME0021 (C1)1.1	+	+		RIMPA0086.2		+		RIMMA0385.1	+		
RIMME0021 (C2)1.1		+		RIMPA0086.3		+		RIMMA0621.1	+		
RIMME0026	**+**	**+**		RIMPA0086.7			+	RIMMA0661.1	+		
RIMME0026 (C6)1.1	+			RIMPA0086.9		+		RIMMA0433.1	+	+	
RIMME0026 (C7)1.1	+			RIMPA0086.12		+		RIMMA0729.1	+	+	
RIMME0026 (C10)1.1	+			**RIMPA0087**	**+**			RIMMA0701.1	+	+	
RIMME0026 (C14)1.1		+		RIMPA0087.5	+			RIMMA0436.1	+		
RIMME0028	**+**			RIMPA0087.10	+			RIMMA0700.1	+		
RIMME0028.3	+			**RIMPA0096**			**+**	RIMMA0721.1	+		
RIMME0028.4	+			RIMPA0096.7			+	RIMMA0392.1	+		
RIMME0028.6	+			**RIMPA0135**		**+**		RIMMA0396.1		+	
RIMME0029	**+**			RIMPA0135.1		+		RIMMA0687.1		+	
RIMME0029.10	+			RIMPA0135.4		+		RIMMA0731.1		+	
RIMME0029.11	+			RIMPA0135.8		+		RIMMA0391.1 B		+	
RIMME0030	**+**	**+**		**RIMPA0142**			**+**	RIMMA0680.1		+	
RIMME0030.4		+		RIMPA0142.12			+	RIMMA0404.1		+	
RIMME0030.13	+			**RIMPA0155**		**+**	**+**	RIMMA0614.1		+	
RIMME0032		**+**	**+**	RIMPA0155(C23)			+	RIMMA0709.1		+	
RIMME0032.10			+	RIMPA0155(C28)		+		RIMMA0397.1		+	
RIMME0032.12		+		**RIMPA0156**	**+**		**+**	RIMMA0398.1			+
RIMME0032.13		+		RIMPA0156(C15)	+			RIMMA0439.1			+
RIMME0033	**+**		**+**	RIMPA0156(C43)			+				
RIMME0033.5	+			**RIMPA0157**	**+**	**+**	**+**				
RIMME0033.10	+			RIMPA0157(C3)			+				
RIMME0033.11	+		+	RIMPA0157(C22)			+				
RIMME0035	**+**			RIMPA0157(C45)	+						
RIMME0035.6	+			RIMPA0157(C48)		+					
				RIMPA0158	**+**	**+**					
				RIMPA0158(C3)	+						
				RIMPA0158(C6)		+					
				RIMPA0158(C29)	+						
				RIMPA0158(C44)	+						

Table 2.4: Marker pattern for positive teosinte and landrace individuals.

Summary of teosinte populations is shown in bold before the individual breakdown within the population.

Landrace individual	Sample ID	Marker positive	FISH type
PI444296-1	RIMMA391	no	N10
PI444296-2	RIMMA391	G8RJJM3	Ab10-III
PI444296-3	RIMMA391	no	N10
PI444296-4	RIMMA391	G8RJJM3	Ab10-III
PI444296-5	RIMMA391	no	N10
PI444296-6	RIMMA391	G8RJJM3	Ab10-III
PI444296-7	RIMMA391	no	N10
PI444296-8	RIMMA391	G8RJJM3	Ab10-III
PI444296-9	RIMMA391	no	N10
PI444296-10	RIMMA391	no	N10
PI444296-11	RIMMA391	no	N10
PI444296-12	RIMMA391	no	N10
PI444296-13	RIMMA391	G8RJJM3	Ab10-III
PI444834-1	RIMMA396	no	N10
PI444834-2	RIMMA396	no	N10
PI444834-3	RIMMA396	no	N10
PI444834-4	RIMMA396	no	N10
PI444834-5	RIMMA396	no	N10
PI444834-6	RIMMA396	no	N10
PI444834-7	RIMMA396	no	N10
PI444834-8	RIMMA396	no	N10
PI444834-9	RIMMA396	no	N10
PI444834-10	RIMMA396	no	N10
PI444834-11	RIMMA396	no	N10
PI444834-12	RIMMA396	G8RJJM3	Ab10-I
PI490825-1	RIMMA433	no	N10
PI490825-2	RIMMA433	G8RJJM3, D6A11RJJM6	Ab10-III
PI490825-3	RIMMA433	no	N10
PI490825-4	RIMMA433	G8RJJM3, D6A11RJJM6	Ab10-III
PI490825-5	RIMMA433	G8RJJM3, D6A11RJJM6	Ab10-III
PI490825-6	RIMMA433	no	N10
PI490825-7	RIMMA433	no	N10
PI490825-8	RIMMA433	G8RJJM3, D6A11RJJM6	Ab10-III
PI490825-9	RIMMA433	G8RJJM3, D6A11RJJM6	Ab10-III
PI490825-10	RIMMA433	G8RJJM3, D6A11RJJM6	Ab10-III
PI490825-11	RIMMA433	no	N10
PI490825-12	RIMMA433	no	N10
PI490825-13	RIMMA433	no	N10
PI490825-14	RIMMA433	No	N10

PI490825-15	RIMMA433	no	N10
PI628445-1	RIMMA614	G8RJJM3	Ab10-I
PI628445-2	RIMMA614	G8RJJM3	Ab10-I
PI628445-3	RIMMA614	G8RJJM3	Ab10-I
PI628445-4	RIMMA614	G8RJJM3	Ab10-I
PI628445-5	RIMMA614	no	N10
PI628445-6	RIMMA614	no	N10

Table 2.5: Landrace FISH screen and PCR marker check

The populations are separated by a double line.

CHAPTER III:

A FOURTH VARIANT OF MAIZE CHROMOSOME 10 (AB10-L2) PROVIDES EVIDENCE FOR THE EVOLUTION OF TR-1 AS A SUPPRESSOR OF KNOB 180 MEIOTIC DRIVE[2]

[2] Kanizay, L., Ghaffari, R., Albert, P., Birchler, J., and Dawe, R.K. To be submitted to Genetics.

Abstract

Examples of meiotic drive, the non-Mendelian segregation of a specific genomic region, have been identified in several eukaryotic species. All known meiotic drive systems contain multiple interacting alleles within large and genetically isolated haplotypes. The lack of recombination within drive haplotypes allows for the perpetuation of the system, but also can lead to an accumulation of deleterious alleles that can reduce the population abundance of a drive haplotype. Unlinked suppressors of meiotic drive may also evolve to limit the spread of meiotic drive systems. *Zea mays* contains the well characterized abnormal chromosome 10 (Ab10) driving haplotype. When Ab10 type 1 (Ab10-I) is present, all knobs are activated as neocentromeres and preferentially transmitted. Knobs are large heterochromatic tandem repeat arrays composed of the TR-1 repeat and/ or knob 180 repeat. They are the agents by which Ab10 drive works, and thus have become wide spread in all *Zea* species. Although knobs are abundant in most maize lines, Ab10 is only found in 18% of individuals in wild populations, raising questions about what properties are limiting the further spread of Ab10. Here we describe a variant of chromosome 10, Ab10-L2, which contains two large TR-1 knobs on its long arm, and no knob 180. Ab10-L2 produces strong TR-1 neocentromeres, but contrary to the other forms of Ab10 that contain both knob repeats, Ab10-L2 does not confer strong meiotic drive. Rather, when paired with driving Ab10 haplotypes, Ab10-L2 reduces their ability to drive. Moreover, Ab10-L2 leads to an overall increase in the abundance of TR-1 knobs and a decrease in 180 bp knobs. These data indicate that TR-1 evolved on Ab10-L2, independently of Ab10-I and has since been selected for to down regulate meiotic drive.

Introduction

When eukaryotic cell division occurs chromosome movement is mediated by the kinetochore proteins that bridge the interaction between the centromere DNA (primary constriction) and the spindle microtubules [1]. During chromosome segregation the arms of chromosomes remain inert structures. However, in Zea mays, maize, the abnormal chromosome 10 (Ab10) meiotic drive system confers

motility to chromosome arms via large heterochromatic tandem repeat arrays, termed knobs. Knobs are composed of the 350 bp TR-1 repeat and/or the 180 bp knob180 repeat [2,3]. Knob motility has been termed neocentromere activity and is required for meiotic drive to occur [4]. In the case of maize, meiotic drive refers to the preferential transmission of a knobbed chromosome over an unknobbed chromosome or of a large knob over a smaller knob [5-7]. Knobs contain no coding information, so neocentromere activity and the subsequent meiotic drive of knobbed chromosomes only occurs when Ab10 is present.

Structurally, Ab10 is mostly similar to the canonical chromosome 10 (N10), but contains a large, supernumerary haplotype (the driving haplotype) on the end of its long arm [8] (Figure 3.1a). This haplotype encodes all the genes necessary for neocentromere activity and meiotic drive [8,9]. Additionally, the Ab10 haplotype does not recombine with N10 [10,11]. There are two structural variants of the Ab10 haplotype that have been shown to confer drive, Ab10-I and Ab10-II. They differ in knob repeat content, with Ab10-I containing three small TR-1 knobs followed by a large 180 bp knob, and Ab10-II containing one TR-1 knob and two large knob 180 knobs (Figure 3.1a). Not only do TR-1 and knob 180 occupy different domains within Ab10, the two repeats appear to have independent neocentromere activity, with TR-1 being the stronger [12]. When either variant is present, the trans-acting factors encoded within them activate knobs on other chromosomes as neocentromeres [12]. When they are activated as neocentromeres, knobs dramatically move poleward along the meiotic spindle ahead of the centromere [13]. The outcome of neocentromere activity is that knobbed chromatids end up in the top and bottom most cells of a naturally linear tetrad, the bottom of which becomes the egg in maize [4] (Figure 3.1b). Thus, Ab10 alters chromosome movement during meiosis which leads to the non-Mendelian, preferential transmission of itself and other knobbed chromosomes.

Since knobs are superfluous structures composed of tandem repeats they should evolve as other tandem repeats evolve: through unequal recombination and replication strand slippage, both of which produce an equal chance for knobs to expand or contract in size [14]. However, drive acts directly on knob repeats creating a clear bias toward the expansion of both knob repeats in all *Zea* species [15].

Since both Ab10-I and Ab10-II transmit knobbed chromosomes over unknobbed chromosomes, increased knob size is continuously under selection in maize. Knobs have been documented on all chromosome arms at approximately 34 different loci and account for up to 23% of the maize genome [15,16]. In general the knob 180 repeat is more abundant than TR-1 [2,17,18]. Knob loci are highly polymorphic in repeat number (size) and type of repeat [18]. Some loci contain mixed knobs (both repeats) while others contain only one of the two repeats [12,19]. Interestingly, in mixed knobs, like in Ab10, TR-1 and knob 180 appear to be contained in different domains [12]. This observation fits with the idea that the neocentromere activity of the two repeats is independently regulated. However, both repeats are believed to be driven in the presence of Ab10-I, so it is not clear why the 180 bp repeat is much more abundant than TR-1.

Meiotic drive systems are believed to be very common, and have been identified in nearly all heavily studied model organisms as well as some less well characterized organisms [20-22]. The classic drive systems (*t*-haplotype in *Mus musculus*, segregation distorter (SD) in *Drosophila melanogaster*, and spore killer (*Sk*) in *Neurospora*) achieve preferential transmission with a completely different strategy than Ab10 – by killing male gametes (or spores) that do not contain them [23]. But, like Ab10, these systems rely on large, genetically isolated haplotypes. Specifically, these systems encode killer loci and responder (protective) loci that are found in haplotypes near centromeres (in areas of low recombination) [24-27]. The lack of recombination ensures the killer and responder loci will be transmitted together so that meiocytes without the responder (sensitive meiocytes) will die while meiocytes that contain the killer and responder will live [28-30]. However, the lack of recombination also allows deleterious alleles to accrue, and these will also be preferentially transmitted [20].

Deleterious alleles likely contribute to the observed population dynamics of drive systems. Most known drive systems, including Ab10, are present in less than 20% of individuals on average (Chapter 2) [20,23,31]. The *t*-haplotype contains recessive alleles that cause lethality and male sterility, and SD is hypothesized to cause reduced male fertility [20]. The severe effects of these alleles have contributed to

very low abundance of these two systems in natural populations [20,23]. In contrast, *Sk* and Ab10 do not contain lethal deleterious alleles and both are maintained in a wide range of levels within host populations [31,32]. *Sk* ranges from 0% to 100% in some populations [32], but Ab10 ranges from 0%-42% (Chapter 2) [31]. While homozygous Ab10 plants show reduced fitness and male fertility, they are not sterile and Ab10 can be maintained in a homozygous state (personal observations).

Suppressors are a second explanation for the low frequency of drive systems in populations. The SD system in *Drosophila* is the most extensively studied drive system and several modifiers of SD have been identified, both enhancers and suppressors [33-36]. The cumulative decades of work on SD have concluded that suppressors exist, suppressors that are unlinked to the driving haplotype can reach fixation, and new suppressors can arise through recombination, competition of drive loci, and *de novo* mutation [20,33,37]. The idea of drive suppressors fits within the larger context of modifier genes. Altenberg and Feldman [38] in particular discuss the relationship between modifier genes and transmission, concluding in part that modifier genes can arise and subsist if they act to restore random chance in transmission [38]. This is precisely what drive suppressors do. Since Ab10 does not contain any known lethal alleles, is highly variable, and is maintained at low levels, like SD, it is highly possible that suppressors of Ab10 mediated drive exist.

Here we describe the characterization of chromosome 10 from the CI66 inbred line (Ab10-L2) that contains a previously examined knob, K10L2. We compare the structure, neocentromere activity and meiotic drive of Ab10-L2 with the other known Ab10 variants. Our data show that Ab10-L2 contains two large TR-1 knobs, but no 180 bp knobs. Ab10-L2 confers consistent and strong neocentromere activity to the TR-1 repeat, but cannot preferentially transmit itself at rates above 51%. Previous work showed that knobs are prevalent and polymorphic because Ab10 selects for them [15]. Since drive does not seem to be a strong selective agent with respect to TR-1, we propose that Ab10-L2 selects TR-1as a drive suppressor. Our crossing data and examination of knob content in various landrace populations

support this hypothesis and indicate that the opposing selection placed on TR-1 and knob 180 influence the knob polymorphism throughout the genome.

Materials and Methods

Cytological characterization of mitotic root tip and pachytene chromosomes

Root tip spreads were made from three day old primary roots from the CI66 inbred line and 1-10 individuals from each of 31 maize landraces as in Chapter 2. Tassels were staged under a dissecting microscope and anthers of the correct stages (pachytene through anaphase II) were fixed in 4% paraformaldehyde, and processed for FISH as described in Chapter 2.

Crossing for drive tests and recombination

We created heterozygous F1 progeny of all possible combinations of Ab10-I, Ab10-II, Ab10-L2 and N10 chromosomes by crossing homozygous parents (Table 3.2) in the University of Georgia Plant Biology Greenhouse facilities. F1 heterozygous plants were test crossed to N10/N10 plants carrying either of two recessive allele of the *R1* gene, (*R1-st* or *r1)*. Two seasons of test crosses were carried out for Ab10-I/N10, Ab10-II/N10, and Ab10-L2/N10. The first season was summer 2010 on the University of Georgia farm land. The test crosses for the second season, winter 2010, were performed in Hawaii. The competition test crosses (Ab10-I/Ab10-II, Ab10-I/Ab10-L2, Ab10-II/Ab10-L2) as well as two more combinations of Ab10-L2/N10 were carried out for one season, winter 2010 in Hawaii. The additional Ab10-L2/N10 testcrosses were performed with different *R1* alleles on N10 to control for any transmission associated with a specific *R1* allele. At the end of each season all kernels from each cross were counted for *R1* allele pigmentation (yellow, purple, or purple patterning) and chi-squared was calculated to test for deviation from Mendelian transmission.

Recombination testing

In order to test for recombination between haplotypes, 65-106 seed from individual testcrosses were planted, and DNA was extracted using CTAB as in Chapter 2. Polymerase chain reaction (PCR)

was performed using one of three dominant markers depending on the particular cross. One marker marks both Ab10-I and Ab10-II, one marks only Ab10-I, and one marks Ab10-L2. The markers are listed in Table 2.3 in Chapter 2. Recombination between these markers and the *R1* locus was measured. *R1* is tightly linked to the Ab10 haplotype and produces anthocyanin in kernels that is easily scored prior to planting.

Results

The maize inbred CI66 contains an unusually large TR-1 rich knob on chromosome 10

The inbred lines of maize do not typically contain knobs on the tenth chromosome [18]. As a part of a large scale karyotyping project [18] we observed one inbred, CI66, that contained a variant of chromosome 10 with a very large TR-1-rich knob on 10L. No extended knob 180 repeat arrays can be detected on this knob, at least as judged by our standard FISH assay (Figure 2a). We further examined the substructure of the knob at the pachytene substage of meiosis I, where the spatial resolution is much higher. These images revealed that there are actually two TR-1 knobs of different sizes followed by a distal portion of chromatin (Figure 3.2d). The overall structure of the knob at pachytene appears to be very similar to a previously described knob called K10L2 [31] (Figure 3.2c).

The knob on CI66 chromosome 10 is likely K10L2

In order to test the hypothesis that the TR-1 knob from chromosome 10 in CI66 is equivalent to K10L2, we used FISH to karyotype 31 distinct maize landraces. We believed these landraces might contain the K10L2 chromosome based on their name and/ or location compared to previous cytological surveys [31]. We compared these karyotypes with the karyotype of CI66 (Table 3.1, Figure 3.2b). We identified the K10L2 knob in 12 of the 31 landraces (39%). Within individual populations this knob ranged in frequency from 12%-100% (Table 3.1). We were able to grow individuals from four of the 12 landraces we identified as carrying a TR-1 knob on 10L to look at pachytene-staged meiocytes. We observed the same structure (two TR-1 knobs) in these landraces as we saw on chromosome 10 from CI66 (Figure 2e). Though we were not able to examine all 12 landraces in pachytene we are confident

that the knob on chromosome 10 of CI66 is the same (or similar to) the K10L2 knob. We have renamed this knob Ab10-L2.

Ab10-L2 produces TR-1 neocentromeres but no knob 180 neocentromeres

Neocentromere activity is a hallmark of other knobbed variants of chromosome 10 (Ab10-I and Ab10-II) so we were curious if Ab10-L2 produced neocentromeres. We looked at male meiocytes undergoing anaphase from CI66, Ab10-I, Ab10-II and a deficiency of Ab10-I that only retains the TR-1 knobs, Df(B) (Figure 3.1a) [39]. We also examined four landraces containing K10L2. Ab10-I produced very strong TR-1 and knob 180 neocentromeres (Figure 3.3a), whereas Ab10-II produced only knob 180 neocentromeres (Figure 3.3b). These results are consistent with previous data [8,12]. Df(B) produced weak TR-1 neocentromeres and no knob 180 neocentromeres (Figure 3.3d). Surprisingly, CI66 individuals showed extremely strong, dramatic neocentromere activity of all TR-1 knobs, but not of the knob180 repeat (Figure 3.3c). This result suggests that Ab10-L2 may be related to Df(B), and have other properties similar to Df(B). We were able to obtain good anaphase staged anthers from one of the four K10L2 landraces, and it also showed TR-1 neocentromere activity (weaker than that seen in CI66, but similar to the TR-1 activity in Df(B)) and no knob180 activity (Figure 3.3e).

Ab10-L2 confers very weak meiotic drive, if any

The observation that Ab10-L2 confers very strong TR-1 neocentromere activity but no knob 180 neocentromeres led us to question if this chromosome also confers preferential transmission of knobs (like Ab10-I) or if it has only neocentromere activity and no drive (like Df(B) [39]). To this end we created heterozygous Ab10-I/N10, Ab10-II/N10, and Ab10-L2/N10 seed. In all cases the Ab10 variant was linked to a recessive allele *r1* (that produces yellow seed), and the N10 chromosome was linked to *R1-nj*, which produces purple capped seeds. These heterozygotes were test crossed to N10/N10 plants carrying the *R1-st* allele that produces purple spotted seeds. The use of different *R1* alleles allowed test crosses to be visually screened for non-Mendelian inheritance of the Ab10 chromosomes. When either Ab10-I or Ab10-II was paired with N10, the Ab10 chromosome averaged upwards of 70% transmission

(Table 3.2). However, when Ab10-L2 competed against N10 the average drive of Ab10-L2 was only 51%. Over 10,000 kernels were counted and this was shown to be statistically different from the Mendelian expectation of 50%, though this is clearly weaker than they typical drive exhibited by Ab10-I and Ab10-II. This indicates Ab10-L2 maybe more like an N10 chromosome than an Ab10 chromosome from a behavioral standpoint.

Ab10-L2 pairs more often with N10 than with other Ab10 chromosomes

The fact that Ab10-L2 structurally looks like K10L2 and does not have strong drive, but shows very strong neocentromere activity made us further question the origin of this chromosome. Since the Ab10 haplotype is largely superfluous it is possible that Ab10-L2 occurred from either Ab10-I or Ab10-II losing some distal portion of chromatin including the 180 bp knob, producing something reminiscent of Df(B). Ab10-I seems a better candidate for this because it contains more TR-1 than Ab10-II, and it confers TR-1 neocentromeres, but Ab10-II does not (Figure 3a, b). In order to examine the relationship between Ab10-L2, Ab10-I, Ab10-II and N10 we examined pairing in heterozygous plants during pachytene. In addition we used dominant Ab10 molecular markers to measure recombination frequency in the progeny of our meiotic drive test crosses.

First, pairing of Ab10-L2 with N10 was observed. We did not examine the pairing of Ab10-I and Ab10-II with N10 because this was previously done [40,41], showing that the Ab10 haplotypes do not pair with N10, and are more similar to each other than to N10 [42]. Unlike Ab10-I and Ab10-II, Ab10-L2 appeared to pair with N10 (Table 3.3, Figure 3.4b). Next we examined the pairing of Ab10-L2 with Ab10-II, Ab10-I and the Ab10-I_Df(B). We used Df(B) to rule out possible mis-pairing of Ab10-I and Ab10-L2 due to structural interference of the 180 bp knob on the distal portion of Ab10-I. Based on misalignment of the TR-1 knobs, we were able to conclude that Ab10-L2 displayed very inconsistent paring or no pairing with Ab10-I and Ab10-I_Df(B) (Figure 3.4c, d). It proved too difficult to visually tell whether Ab10-II and Ab10-L2 paired. Since Ab10-II only has one TR-1 knob and the two Ab10-L2 knobs are very close together, we observed a large globular TR-1 structure in these heterozygotes and

were unable to tell if the euchromatic portions were pairing or not (data not shown). We were however able to score Ab10-I/Ab10-II heterozygotes for pairing, finding that they paired most of the time (Table 3.3, Figure 3.4a).

Ab10-L2 recombines more often with N10 than with other Ab10 chromosomes

The recombination data confirm our cytological observations. We measured recombination between the *R1* locus (a visual marker) and various molecular Ab10 markers in progeny from our drive test crosses (Table 3.4). We chose to use three different markers (D6AllRJJM6, G8RJJM3, and C2RJJM2). These markers are transposon junction markers that were developed from Ab10-I BAC sequence data and are dominant to the Ab10 haplotypes (none amplify in N10) (Chapter 2). All three markers have been previously mapped to the central euchromatin of Ab10-I between Df(I) and Df(F) (Chapter 2). D6A11RJJM6 was previously shown to be only present in Ab10-I. G8RJJM3 was previously shown to be present in both Ab10-I and Ab10-II, and mapped between Df(Q) and Df(M) of Ab10-II (Chapter 2). The final marker, C2RJJM2, was present in Ab10-I, Ab10-II and Ab10-L2. It mapped between Df(Q) and Df(M) on Ab10-II (Chapter 2). We were unable to map it to a precise location on Ab10-L2 because we have no sequence information or deficiency breakpoints from this chromosome.

Though the BACs containing these markers are clustered together on Ab10-I and Ab10-II, their homologous sequences spread along the distal portion of N10L (Chapter 2). D6A11 is approximately 27 cM distal to *R1* on N10 and C2 and G8 are about 34 cM distal to *R1* on B73. Our recombination data confirm previous observations that Ab10-I recombines less than 2% with N10 [10] (Table 3.4). Further we show that Ab10-I and Ab10-II show less than 2% recombination with Ab10-L2 (Table 3.4). We measured about 15% recombination between Ab10-I and Ab10-II, and 11% recombination between Ab10-L2 and N10. These data support the pairing data and the conclusion that Ab10-I and Ab10-II are most similar to each other and Ab10-L2 is most similar to N10. Together these data suggest that Ab10-L2 did not originate as a deficiency of Ab10-I or Ab10-II, but as a novel form of chromosome 10.

Meiotic drive competition exists between the different forms of Ab10

Since Ab10-L2 seems to be more closely related to N10 than either Ab10-I or Ab10-II, and because it did not have strong preferential transmission itself compared to N10, we wondered if this chromosome would affect the preferential transmission of the other Ab10 chromosomes. In order to investigate this we created individual plants that were heterozygous for all the combinations of chromosome 10, ensuring that in each heterozygous progeny the different forms of chromosome 10 were linked to different *R1* alleles to enable visual screening (Table 3.2). We used Ab10/N10 heterozygous plants as meiotic drive controls because drive strength can vary from season to season. Test crosses of the heterozygous individuals to N10/N10 plants linked to the recessive *r1* allele were performed. In spite of its strong neocentromere activity, we expected Ab10-L2 to have no effect on the preferential transmission of Ab10-I or Ab10-II since it cannot preferentially transmit itself. We also anticipated that in an Ab10-I/Ab10-II test cross, Mendelian segregation would be nearly restored since the drive of these chromosomes is roughly equal when paired with N10.

The results contradicted our expectations on all accounts. In Ab10-L2/Ab10-II testcrosses, the average drive of Ab10-II was reduced by 15% compared with Ab10-II/N10 testcrosses, from 77% transmission when paired with N10 to 62% transmission when paired with Ab10-L2 (Table 2). Moreover, in Ab10-I/Ab10-L2 testcrosses, the drive of Ab10-I was severely reduced, to an average of only 54% transmission (Table 3.2). In our Ab10-I/Ab10-II testcrosses Mendelian segregation was not restored, rather Ab10-I was transmitted on average 63% of the time. We were astonished that Ab10-L2 suppressed the drive of Ab10-I to such a degree, but that Ab10-II did not have nearly as strong an effect on Ab10-I.

TR-1 accumulates in Ab10-L2 populations and knob 180 decreases in abundance

The unexpected results from our drive competition crosses point not only to competition between the various Ab10 haplotypes, but suggest that Ab10-L2 may have a parasitic or suppressive effect on the other haplotypes. Moreover, though Ab10-L2 has very weak drive when paired with an N10

chromosome, it contains a very large TR-1 knob. Since knobs theoretically only increase in size in the presence of a selective agent, we believe the TR-1 is under selection as either a parasite of the 180 bp repeat or as a drive suppressor. To test the suppressor theory we examined the knob content of our 31 landraces, counting the number of knobs containing only knob 180, only TR-1, or both repeats and averaging them (Table 3.5). If TR-1 is only being selected for by drive, in cases where Ab10-I is present, we would not expect to see any difference in TR-1 knob content in Ab10-L2 populations compared to N10 populations. However, if TR-1 is also being selected for as a suppressor we would expect to see an increase in TR-1 knobs in Ab10-L2 populations compared to populations with only N10. Using a T-test, we found that in populations that are segregating for the Ab10-L2 chromosome not only was the average number of TR-1 only knobs significantly higher, but also the average number of knob 180 knobs was significantly lower compared to populations containing only N10 (Table 3.5). Accordingly, the number of mixed (TR-1 and knob 180) knobs was not different in N10 vs. Ab10-L2 populations (Table 3.5).

Discussion

To date, no regulators of meiotic drive have been identified in maize, but several lines of evidence support the theory that a drive suppressor of sorts should exist [33,37,38]. Previous population studies have shown that Ab10 is likely segregating in all teosinte (wild progenitor of maize) populations, but it never reaches above 50% within any single population, and is maintained at an average of 18% (Chapter 2). If Ab10 were uncontested and contained no detrimental alleles that reduced its viability, it would have gone to fixation throughout the *Zea*. Although we believe that Ab10 has accumulated (probably multiple) mildly detrimental alleles over time, none cause lethality or sterility and Ab10 strains can be easily maintained as pure breeding strains. Though any detrimental alleles that confer reduced fitness could decrease the ability of Ab10 homozygous plants to compete in the wild, these adverse fitness affects alone are likely not strong enough to consistently hold Ab10 at such a low frequency within populations. In other meiotic drive systems that do not reach fixation, for example, the *t*-haplotype in *Mus* lethal alleles have been found within the driving haplotypes [20]. In the case of segregation distorter (SD)

in *Drosophila*, drive modifiers exist [33]. Alternatively, in the spore killer (Sk) system of *Neurospora*, like in maize, no deleterious alleles or suppressors have been identified, and Sk has reached fixation in some populations. Here we show that Ab10 is not always uncontested, and maize harbors at least one trans-acting chromosome that helps to regulate the strong drive of Ab10-I and Ab10-II, thus helping to keep the frequency of Ab10 at relatively low levels.

Our initial cytological karyotyping of the CI66 inbred as well further analysis of multiple landraces revealed that many chromosomes contain a large TR-1-rich knob on the end of N10. The knob was initially described as K10L2 [31] but here we renamed it Ab10-L2 to accommodate the fact that the knob exhibits neocentromere activity, which has so far only been described as occurring when Ab10-I or Ab10-II are present. We found that Ab10-L2 confers strong TR-1 neocentromeres but does not activate knob 180 neocentromeres, definitively confirming that the two knob repeats have evolved independent neocentromere activities, *i.e.* they are mobilized by different proteins [12]. In spite of the neocentromere activity of Ab10-L2, our pachytene pairing analysis and recombination results strongly indicate that Ab10-L2 is more closely related to N10 than to other known Ab10 haplotypes. In support of this, we found that although the TR-1 neocentromere activity of Ab10-L2 is strong, Ab10-L2 itself does not confer strong meiotic drive, if any drive. Rather, it appears to decrease the drive strength of the Ab10-I and Ab10-II haplotypes.

The observation that Ab10-L2 does not show strong drive, but that the Ab10-L2 knob is very large led us to question whether Ab10-L2 has an effect on the abundance of TR-1 knobs throughout the maize genome. The Ab10-I and Ab10-II drive system encodes trans-acting factors that allow for the preferential transmission of all chromosomes that are heterozygous for a knob [5]. Ab10 not only drives a knobbed chromosome over an unknobbed chromosome, if both homologs have a knob Ab10 will drive the larger of the two [7]. For example, chromosome 9 can have a cytologically small, medium or large knob on its short arm. Studies showed that Ab10 will cause preferential transmission of the large knob over the medium knob and the medium over the small knob [7]. Though Ab10 itself has not reached fixation, both TR-1 and knob180, the tandem repeats that form knobs, have become extremely prevalent

in teosinte, maize and all *Zea* [18]. Several of these knobs have nearly reached fixation in maize [18]. The extremely large knobs in maize presumably create a drag on replication during the cell cycle due to their size and heterochromatic nature. It is in the best interest of the genome to rid itself of knobs. Hence, without being selected for increased size by drive, knobs, like other tandem repeats, would expand and contract in equal proportion [43], and we know that Ab10 relies on knobs for neocentromere activity and drive. However, the results here clearly show that the different variants of Ab10 exhibit different drive and neocentromere strengths. Since both Ab10-I and Ab10-II exhibit knob 180 neocentromeres and strong meiotic drive [8], it makes sense that the knob 180 repeat is so abundant. However, the abundance of TR-1 is less clear.

There are two possible mechanisms by which Ab10-L2 might function to down regulate drive and perpetuate TR-1 (Figure 3.5). First, Ab10-L2 may play a direct suppressive role on the neocentromere activity and strong drive exhibited when the 180bp knob is present as in Ab10-I and Ab10-II. For example, Ab10-L2 may contain genes that inactivate either the 180 bp knob neocentromeres or other drive components. Alternately, the TR-1 repeat may be able to parasitize the drive machinery contained within the Ab10-I and Ab10-II haplotypes. In this scenario the large size of the Ab10-L2 knobs and their strong neocenromere activity would compete during anaphase. It is known that TR-1 moves poleward faster than knob 180 [12], and that larger knobs drive over smaller knobs [7], thus Ab10-L2 would be an excellent competitor of both Ab10-I and Ab10-II which contain less TR-1 repeat. Both scenarios would lead to the restoration of Mendelian transmission, while allowing both knob repeats to be maintained throughout maize populations.

It is known that TR-1 arose later in evolutionary history than knob 180 [19], and though TR-1 is contained on Ab10-I, Ab10-II, and Ab10-L2, only Ab10-I confers TR-1 neocentromere activity and strong drive. Ab10-II confers strong drive but does not activate TR-1 knobs as neocentromeres, so can only drive TR-1 if it is present at the same locus as ("attached" to) knob 180, and we know Ab10-L2 does not confer strong drive. In fact, is unclear whether the TR-1 repeat alone is ever subject to meiotic drive. Even though Ab10-I activates TR-1 neocentromeres, it has never been directly tested whether only 180 bp

knobs are driving in the presence of Ab10 I, or all knobs. In order to test if Ab10-I can drive TR-1 independently of knob180, the transmission of a TR-1 only knob needs to be examined in an Ab10-I test cross. It is possible that Ab10-I does not actually drive TR-1 independently of knob 180 since the three TR-1 knobs located within the haplotype are very small, much smaller than the Ab10-L2 knobs. Moreover, Ab10-I_Df(B), that only retains the TR-1 region, shows TR-1 neocentromeres, but shows reduced transmission (~45%) [9]. The added advantage of the very strong neocentromere activity TR-1 exhibits may provide Ab10-I with the observed driving advantage over Ab10-II, which only produces knob 180 neocentromeres (Figure 3.3, Table 3.2). For this reason, Ab10-I may assert some selection on TR-1.

Although Ab10-L2 does not drive it seems to have a positive effect on TR-1 abundance. We found that landrace populations segregating Ab10-L2 contain more TR-1 knobs than populations with only N10. Even if Ab10-I confers meiotic drive to TR-1, this alone would not explain why TR-1 abundance is different in Ab10-L2 and N10 populations, because Ab10-I is not a factor in these populations. The second observation from the 31 landrace populations strongly supports the idea that Ab10-L2 selects TR-1 to suppress the 180 bp knob repeat (that is always associated with strong drive). We found that the Ab10-L2 populations examined here contain significantly less knob 180 knobs than populations with only N10. If Ab10-L2 and the TR-1 repeat were simply acting a parasite on the strong drive that Ab10-I confers we would not expect knob 180 to be selected against. If this were a simple case of parasitism, Ab10-L2 populations should not show any significant difference in knob 180 repeat compared to N10 populations because this repeat is not being used in either group. When Ab10-I is not present, and knobs are not being selected for, they should have an equal chance of decreasing in size in both N10 and Ab10-L2 populations [15,43]. These results provide preliminary evidence that Ab10-L2 is acting as a suppressor of drive and thus the TR-1 repeat is under selection as a suppressor.

In further support of our suppression theory we went back and re-analyzed surveys of Ab10-I, Ab10-II, and Ab10-L2 (which was referred to as K10L2) distribution. Kato found that in teosinte populations Ab10-L2 is more common than both Ab10-I and Ab10-II, and that 68% (13/19 populations)

of the populations that contain Ab10-I or Ab10-II also contain Ab10-L2 [31]. If Ab10-L2 is acting as a suppressor it makes sense that it would occur with the haplotypes it suppresses most of the time.

Since Ab10-L2 is most closely related to N10 it is possible that TR-1 has been selected for by genes on N10 that want to survive in Ab10 populations. How else would these genes stand a chance when paired with a driving Ab10 haplotype? We know that knobs are found in gene dense regions of chromosome arms and that they suppress recombination in their vicinity (unpublished, dawe lab). For this reason Ab10 is potentially preferentially transmitting not only genetically inert knob repeats, but multiple deleterious alleles across the genome. Logically, selection might favor the perpetuation of an element that acts to even the playing field, such as Ab10-L2. Our combined lines of evidence regarding the relation of Ab10-L2 to N10, the abundance and large size of TR-1 knobs in Ab10-L2, as well as the adverse affect it has on meiotic drive of the other Ab10 haplotypes, strongly support Ab10-L2 as the first drive suppressor identified in *Zea mays*.

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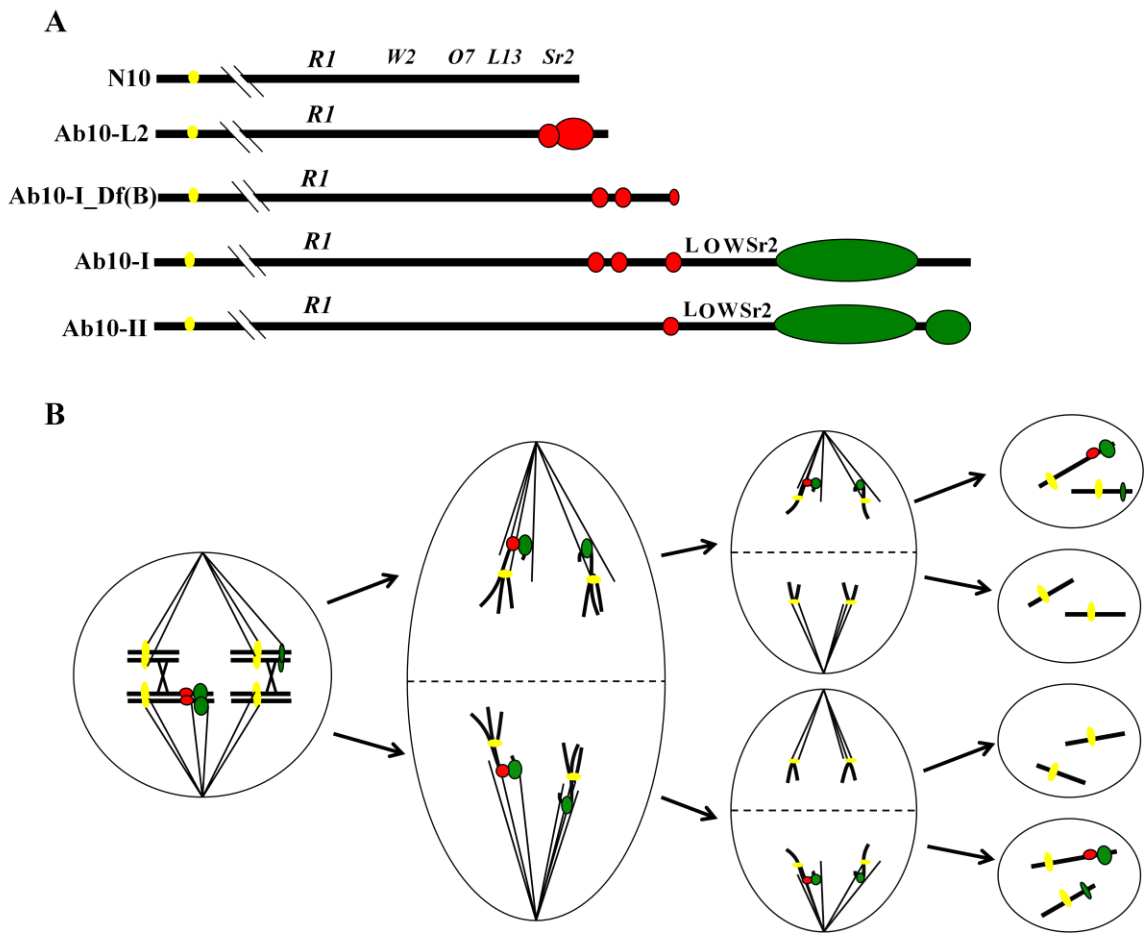

Figure 3.1: Variants of maize chromosome 10 and meiotic drive model.

Five variations of maize chromosome 10 (A): normal 10 (N10), chromosome 10 with K10L2 knob (Ab10-L2), abnormal 10 type 1 deficiency B (Ab10-I_Df(B)), abnormal 10 type 1 (Ab10-I), and abnormal 10 type 2 (Ab10-II). N10 is shortest and has no knobs and the Ab10s containing different amounts of the TR-1 (red) and knob180 (green) repeats. The extended region on Ab10-I and Ab10-II is responsible for meiotic drive and has been split into four main domains (the TR-1 region, shared region, 180 bp knob, and distal tip). Meiotic Drive model, based off of Rhoades, 1952 (B). Drive begins with recombination between the centromere (yellow) and knobs (red and green). In anaphase I, knobs move laterally along the spindle poles ahead of the centromere, creating a strong outward orientation, that is maintained through anaphase II. These events place knobbed chromatids in the top and bottom most cells of the linear tetrad. The bottom cell naturally develops into the egg.

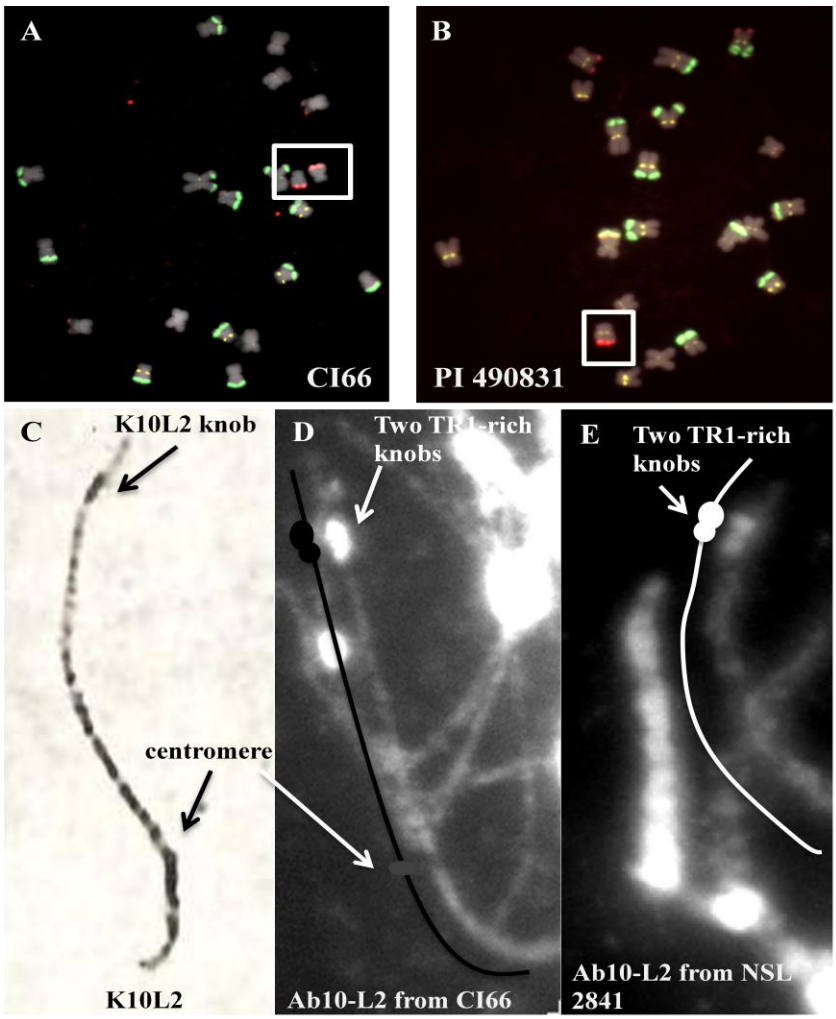

Figure 3.2: Fluorescence in situ hybridization (FISH) of root tip spreads and pachytene meiocytes

Root tip FISH karyotypes of inbred CI66 (A) and landrace PI490831 (B) show the K10L2 TR-1 knob (white boxes) in a homozygous and heterozygous state respectively. Eleven other landraces showed similar staining for K10L2. In pachytene, it is clear that the TR-1 knobs on chromosome 10 from CI66 (D) and landraces (E) resemble the K10L2 knob previously identified [31] (C). In images A and B TR-1 is red, knob 180 is green, centc (centromere) is yellow and DAPI is grey. Images D and E are showing only DAPI DNA staining (grey), but these slides were hybridized with the same probes used in A and B to verify that Ab10-L2 only contains TR-1. Image C was provided through personal communication with Dr. Kato.

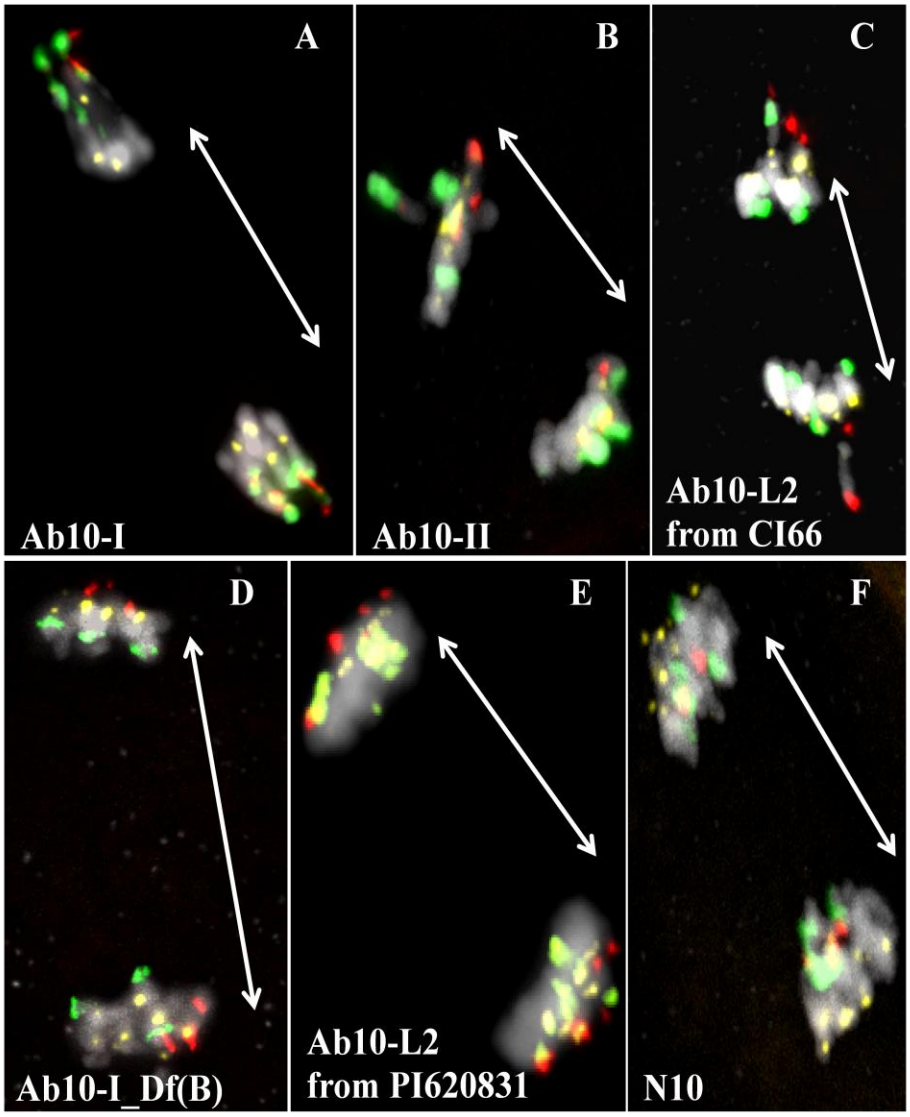

Figure 3.3: Fluorescence in situ hybridization (FISH) of anaphase meiocytes

Ab10-I shows strong neocentromere activity of both repeats (A), whereas Ab10-II only shows knob 180 neocentromeres (B). CI66 shows strong TR-1 neocentromeres, but knob 180 is not activated (C). Both Ab10-I_Df(B) (D) and the K10L2 containing landrace PI620831 (E) show weak activity of TR-1 and no 180 bp activity. In N10 (negative control) the centromere clearly leads the way to the spindle poles (F). In all images neocentromere activity is scored as either knob 180 (green), TR-1 (red) or both repeats moving poleward ahead of the centromere (yellow). Arrows indicate the direction of chromosome movement.

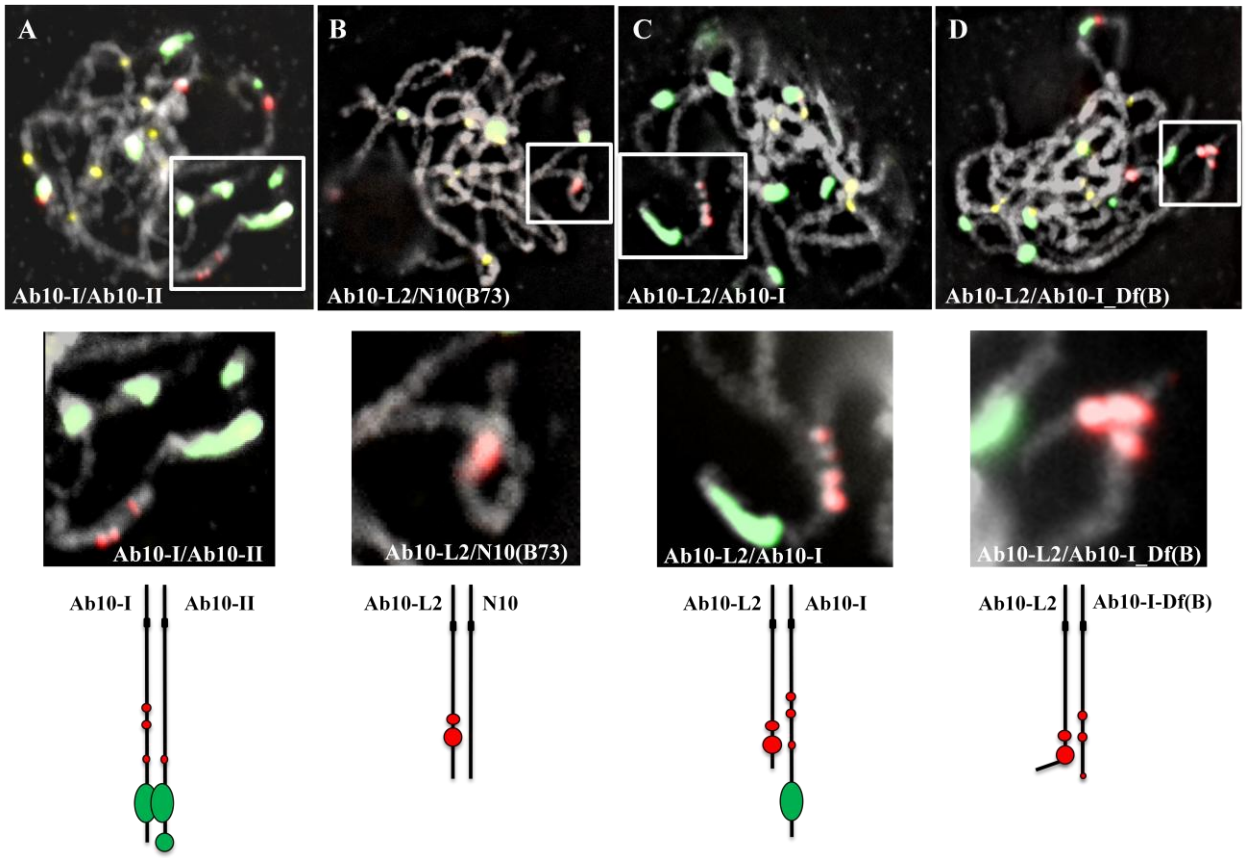

Figure 3.4: Fluorescence *in situ* hybridization (FISH) showing pairing of chromosome 10 variants in maize.

Pairing was examined in four heterozygous plants. Ab10-I and Ab10-II paired as depicted in (A) some of the time, however sometimes the two chromosomes did not pair (TR-1 and knob 180 knobs resembled a blob or were all separate). Ab10-L2 paired with N10 in all cells (B). Ab10-L2 showed inconsistent pairing with Ab10-I. The TR-1 knobs never consistently aligned. One example is shown in (C). Ab10-L2 and Df(B) also showed inconsistent pairing (D). In all images DNA is grey, TR-1 is red, knob 180 is green and the centromere is yellow. The ends of chromosome 10 are boxed and blown up. Cartoon images are drawn below each blow up to illustrate pairing.

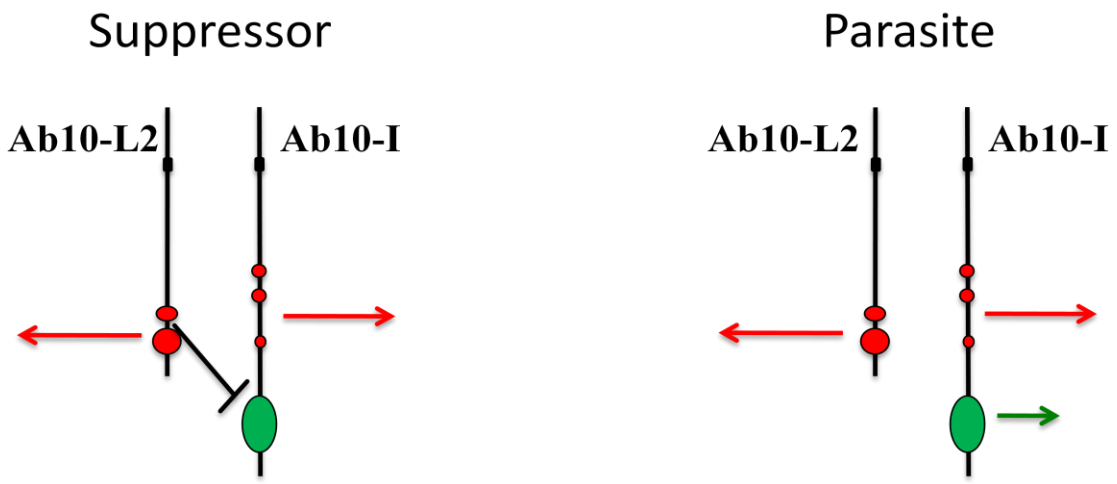

Figure 3.5: Possible mechanisms of Ab10-L2 to decrease the driving ability of other Ab10 haplotypes.

In the suppressor scenario Ab10-L2 is hypothesized to encode some sort of regulatory factors that act to inactivate or down regulate either the neocentromere activity of the knob 180 repeat or other genes that are required for meiotic drive. In the parasite scenario Ab10-L2 would have no adverse effects on other Ab10 haplotypes. Rather it would utilize the trans-acting drive factors contained within Ab10-I and Ab10-II to give itself a competitive advantage. Knob180 is depicted in green and TR-1 is red.

Race ID, location and individual	TR-1 knobs	Mixed knobs	180bp knobs	Ab10-I	Ab10-L2	Ab10-III	B-chromosomes
AMES 10264, Louisiana, USA							
1	0	6	6				
2	0	6	6				
3	0	6	6				
4	0	6	8				
5	0	6	8				
6	0	6	8				
7	0	6	8				
AMES 19980, Oaxaca, Mexico							
1	1	13	13			1	1
2	2	10	13				
3	2	10	14				1
4	1	13	13				
5	2	11	12			1	2
6	0	13	15			1	3
7	0	14	11			1	
8	1	13	12				
Ames 22673, Texas, USA							
1	0	8	14				
2	0	8	13				
3	0	9	13				
4	0	8	12				
5	0	8	14				
6	0	8	14				
AMES 21972, Illinois, USA							
1	2	6	10		1		
2	2	8	10		1		
3	2	7	10		1		
4	2	8	12		1		
PI 420244, Sonora, Mexico							
1	0	9	9				
2	2	8	10		1		3
3	0	13	8				2
4	0	13	9				1
5	1	9	10				
PI 451694, Iowa, USA							

1	1	6	7				
2	0	6	10				
3	1	7	5				
4	1	6	9				
5	2	5	10				
PI 483314, South Carolina, USA							
1	1	4	7				
2	0	8	7				
3	1	5	12				
4	1	6	7				
5	0	8	8				
6	1	4	8				
7	1	4	11				
PI 490921, Nayarit, Mexico							
1	0	6	11				1
2	1	8	13				1
3	0	6	10				2
4	0	8	13				
5	0	7	8				1
6	0	7	2				1
7	0	8	2				
8	0	10	8				2
9	1	7	12				2
PI 503574, Sinaloa, Mexcio							
1	1	10	11		1		1
2	0	11	12				
3	0	11	12				
4	2	7	11				
5	0	6	10				2
6	0	9	11				
7	2	6	11		1		
PI 539859, Mississippi, USA							
1	2	6	8				
2	2	6	12				
3	2	6	12				
4	2	6	12				
5	2	6	11				
6	2	5	11				
7	2	6	10				

8	2	6	9				
9	2	6	12				
PI 595530, Federal District, Mexico							
1	0	6	12				
2	0	5	14				
3	0	6	13				
4	0	4	11				
5	0	6	11				
6	0	6	12				
PI 614819, Mississippi, USA							
1	2	6	7		2		
2	2	5	6		2		
3	2	6	6		2		
4	2	4	6		2		
5	2	6	8		2		
6	2	5	8		2		
7	2	6	6		2		
8	2	6	8		2		
9	2	6	8		2		
10	2	6	6		2		
PI 620775, Chiapas, Mexico							
1	1	9	10		1		
2	0	14	11				
3	0	10	11				
PI 620831, Mexico, Mexico *							
1	1	6	8		1		
2	1	3	9				
3	1	7	5				
4	0	6	6				
5	0	6	8				
6	1	5	8				1
7	0	4	8				
8	0	6	4				1
PI 620859, Sonora, Mexico *							
1	2	11	9		1		2
2	0	12	10			1	1
3	1	7	11				
4	0	10	6				3

5	0	9	11				
6	1	11	8				
PI 628470, Oaxaca, Mexico							
1	1	11	16				2
2	1	12	12				2
3	0	13	13				2
4	1	13	12				
5	0	15	14				1
6	0	15	14				1
PI 628473, Oaxaca, Mexico							
1	0	14	7				
2	0	15	11				
3	0	15	12				
4	1	13	10				
5	0	12	8				
PI 645891, Michoacan, Mexico							
1	1	2	3				1
2	1	6	4				3
3	0	2	10				3
4	0	2	9				
5	0	2	7				1
6	0	2	8				1
PI 645866, Mexico, Mexico							
1	0	4	11				
2	0	5	10				
3	0	6	8				
4	0	11	4				
5	0	4	10				
6	1	5	9				
7	0	5	9				
PI 645933, Oaxaca, Mexico							
1	0	6	12				
2	2	7	11				
3	0	6	10				
4	0	8	11				
5	9	11	11				
NSL 18, Cundinamarca, Colombia							

1	3	2	7		1		
2	1	3	12				
3	1	3	10				
4	2	7	7			1	
5	0	6	10				
NSL2824, Mexico							
1	1	2	4		1		
2	2	2	4				
3	0	2	6				
4	2	4	4				
5	0	3	4				
6	1	4	3				
NSL 2827, Mexico *							
1	1	8	13	1	1		4
2	0	8	11				5
3	0	6	13				
4	1	7	12		1		5
5	1	8	11		1		5
6	1	8	10		1		5
7	0	7	15				5
8	2	9	10		2		4
9	1	9	11		1		
NSL2828, Mexico							
1	1	12	10		1		
2	0	13	12				
3	0	16	10				
4	0	15	12			1	
5	1	14	13				
6	0	14	10			1	
7	1	10	11				
NSL2833, Mexico							
1	0	11	12				
2	1	9	9				
3	1	7	11				
4	2	9	9				
5	1	9	8				
6	1	9	9				
7	1	7	7				2
NSL 2836, Mexico							
1	1	9	9				
2	1	9	8				

3	0	10	10				
4	0	12	7				
5	0	10	10				
6	2	9	10				
7	0	10	11				
NS 2837, Mexico							
1	0	3	8				
NSL 2840, Mexico							
1	0	11	13				6
2	0	13	12				2
3	0	12	9				
4	0	13	14				1
5	0	10	16				
6	0	10	10				
NSL 2841, Mexico*							
1	2	10	5		1		
2	1	10	6		1		
4	0	11	6				
5	0	9	4				
6	1	12	4		1		
7	2	8	10		1		
8	1	11	7		1		
NSL 2843, Mexico, Mexico							
1	0	5	8				
2	3	5	10				
3	0	4	10				1
4	2	4	9				
5	1	3	11				1
NSL2844, Mexico, Mexico							
1	1	6	8				
2	1	13	8	2			
3	0	9	7				
4	0	4	12				
5	0	7	12				

Table 3.1: Chromosome content of individuals from 31 landraces

Race ID and location are bolded. Ab10-II is not listed because it was never observed.

female genotype	male tester genotype	driving haplotype	average drive season 1 (Georgia)	average drive season 2 (Hawaii)
*R-nj*_N10/*r*_Ab10-I	*R-st*_N10/*R-st*_N10	*r*_Ab10-I	71%**	79%**
*R-nj*_N10/*r*_Ab10-II	*R-st*_N10/*R-st*_N10	*r*_Ab10-II	70%**	77%**
*R-nj*_N10/*r*_Ab10-L2	*R-st*_N10/*R-st*_N10	*r*_Ab10-L2	51%*[$]	50%
*R-st*_N10/*r*_Ab10-L2	*r*_N10/*r*_N10	*r*_Ab10-L2	N/A	52%*
*R*_N10/*r*_Ab10-L2	*r*_N10/*r*_N10	*r*_Ab10-L2	N/A	51%
*R*_Ab10-I/*r*_Ab10-II	*r*_N10/*r*_N10	*R*_Ab10-I	N/A	63%**
*R*_Ab10-I/*r*_Ab10-L2	*r*_N10/*r*_N10	*R*_Ab10-I	N/A	54%*
*R*_Ab10-II/*r*_Ab10-L2	*r*_N10/*r*_N10	*R*_Ab10-II	N/A	62%**

Table 3.2: Test crosses for meiotic drive.

* indicates significant deviation from Mendelian segregation, measured by chi squared test ($p < 0.05$).

** indicates significant deviation from Mendelian segregation, measured by chi squared test ($p < 0.01$).

[$] > 10,000 seed were counted for this cross.

Heterozygous line	# paired cells/ total cells observed
Ab10-I/Ab10-II	6/10
Ab10-I/Ab10-L2	1/10
Df(B)/Ab10-L2	2/10
Ab10-L2/N10	10/10

Table 3.3: Pairing counts for Ab10 heterozygotes during pachytene.

Ten pachytene cells where chromosome 10 was clearly visible were examined for pairing from each of four heterozygous lines. Pairing was counted based on alignment of TR-1 knobs and euchromatic regions.

female parent test cross	# recombinants/ total individuals	% recombination
Ab10-I/N10	1/100	1.00%
Ab10-I/Ab10-L2	1/94	1.06%
Ab10-II/Ab10-L2	1/71	1.41%
Ab10-L2/N10	12/106	11.32%
Ab10-I/Ab10-II	10/65	15.38%

Table 3.4: Recombination data from progeny of heterozygous test crosses.

The female heterozygotes were test crossed to N10/N10 males and progeny from a single ear of each test cross were used to measure recombination between *R1* and three molecular markers.

knob type	average # in Ab10-L2 populations	average # in N10 populations	unpaired t-test p-value
TR-1	0.97	0.65	0.0293
knob 180	8.77	10.18	0.0029
mixed	7.87	7.81	0.9045

Table 3.5: Knob count statistics from Ab10-L2 compared to N10 landraces.

The average number of TR-1 knobs is significantly higher and the average number of knob 180 knobs is significantly lower in populations with Ab10-L2. Mixed knobs show no significant difference.

CHAPTER IV

UNCOVERING AB10 SPECIFIC GENES

Abstract

The maize abnormal chromosome 10 (Ab10) possesses the unique ability to activate knobs (long tandem repeat arrays) as neocentromeres during meiosis. In turn, this neocentromere activity promotes the non-Mendelian, preferential transmission (meiotic drive) of the knobbed chromatid over its unknobbed sister. The genes underlying these processes remain unknown, but the functions have been definitively mapped to the extra chromatin on Ab10, termed the Ab10 haplotype. The haplotype is very large (tens of megabases) and does not recombine with the typical chromosome 10 (N10), hence traditional cloning methods are not feasible to isolate the unique genes found on Ab10. Moreover no sequence from the functional portions of the haplotype exists. As a first step toward neocentromere and meiotic drive gene discovery cDNA libraries from two Ab10-I lines, an Ab10-II line, an Ab10-L2 line, as well as three different wild type (WT) N10 lines were prepared, sequenced, and analyzed. Initial analysis of cDNA has shown that functional genes on Ab10 are elusive. They are not highly expressed, and/or are not as novel as presumed. Additionally, three Mutator (Mu) transposon insertion mutants of meiotic drive (*Smd1*, *smd3* and Df(L)) were cytologically characterized and prepared for Illumina sequencing. The mutant analysis shows that neocentromere activity of the 180 bp repeat is absolutely required for meiotic drive to occur. One of the mutants, Df(L), was a deficiency line that allowed a finer mapping of the drive locus to the distal tip of the haplotype. Once the insertion mutants are sequenced, candidate genes will be analyzed and their expression verified using the cDNA libraries.

Introduction

Zea mays, maize, contains several structural and molecular variants of its tenth chromosome (Chapter 2 and 3). The canonical chromosome ten is normal ten (N10) and the other variants are referred

to as abnormal chromosome 10s (Ab10). The Ab10s are all characterized as having supernumerary chromatin on the end of their long arms, termed the Ab10 haplotypes. The haplotypes vary in size, but are generally large, estimated to be up to 55 cM [1]. Ab10 haplotypes include both euchromatic sections with genes as well as different amounts of heterochromatic knobs (Chapter 2 and 3). The knobs are composed of long arrays of tandem repeats and can contain exclusively the knob 180 bp repeat or the TR-1 350 bp repeat, or they can hold a mixture of both repeats [2,3]. Notably, knobs are also found on the arms of all nine other *Zea* chromosomes [4]. The gene content of the Ab10 haplotypes largely includes genes that are also found on the long arm of N10, distal to the *R1* locus (Chapter 2), but this supernumerary chromatin also must contain novel trans-acting genes that are unique to the Ab10 haplotypes [5,6].

Previous mutant screens of Ab10-I have yielded three meiotic drive mutants (*Smd1*, *smd3* and Df(L)) that have been mapped to the Ab10 haplotype [5,7]. According to cytological characterization in pachytene, *Smd1* and *smd3* are cytologically intact mutants whereas Df(L) lacks the distal euchromatic tip of the Ab10-I haplotype [5,7]. *Smd1* was shown to have reduced levels of meiotic drive (~55%) in test crosses, and accordingly showed inconsistent neocentromere activity [7]. Df(L) and *smd3* both exhibit a total lack of meiotic drive, and in fact show reduced transmission (both show near 45% transmission), however both appear to have unaffected neocentromere activity [5]. Complementation tests were carried out that indicate that *smd3* and Df(L) complement each other, but *Smd1* was found to be a dominant mutant so could not be used in the complementation tests [5].

The novel genes underlying these mutations as well as any other genes involved in drive are unknown, as are the proteins they encode. Nevertheless, a strong body of evidence points to their existence on the Ab10 haplotype. Namely, Ab10 confers unique functions to knobs that lead to the preferential transmission (meiotic drive) of knobbed chromatids during female meiosis. These events only occur when Ab10 is present. Meiotic drive of knobbed chromatids requires several factors: 1) a heterozygous state (i.e. one homolog has a knob and the other does not), 2) a recombination event

between heterozygous homologs to create a heteromorphic dyad, 3) neocentromere activity of knobs, and 4) other presumed factors regarding orientation of sister chromatids. All of these functions have been postulated according to Rhoades' model for meiotic drive [8]. Importantly, all necessary functions have been roughly mapped to the Ab10 haplotype, proving that it contains the loci that control these activities [5].

It is known that neocentromere movement is quite different from centromere movement. Neocentromere movement is an active, lateral movement of knobs along microtubules and does not depend on microtubule flux (polymerization and depolymerization) like centromeres do [3]. Moreover, neocentromere movement is not mediated by any of the known plant kinetochore proteins (that bridge the centromere to the microtubules and mediate chromosome movement) [9]. For these reasons, the genes that mediate neocentromere activity of knobs are presumed to be novel, Ab10 specific genes.

Though neocentromere and other Ab10 functional proteins are unknown there are several known proteins that contain functional domains that would meet the requirements for Ab10's unique behavior. Kinesins are the only known plant motor proteins that can move laterally along microtubules and as such are good candidate neocentromere proteins. DNA binding proteins, such as transcription factors, may be involved in many steps of meiotic drive, for instance, as a bridge to connect chromosomal knob DNA to microtubules, or as expression regulators of drive genes. SUN domain proteins are meiosis-specific proteins that are involved in telomere binding to the nuclear envelope and subsequent homologous pairing and recombination. It is possible that a protein with this type of functional domain is involved in recombination of structural heterozygotes (such as knobbed and unknobbed homologs) as well as maintaining an outward orientation of knobbed chromatids during telophase. The functional Ab10 genes may be completely unique, but they may also be splice variants of pre-existing proteins, or even chimeric fusions of different functional domains from pre-existing proteins. Currently, the preferred explanation of major drive genes is that they are completely novel proteins [1].

In order to uncover the functional genes contained within the Ab10 haplotype, we took two approaches. First, we created, sequenced, and analyzed cDNA libraries from anthers of several wild type (N10) and Ab10 containing plants undergoing meiosis. Second, we characterized two point mutations of meiotic drive (*Smd1* and *smd3*) as well as one deficiency line (Df(L)) (Figure 4.4) that lacks meiotic drive. These three mutants had been previously characterized genetically, but their cytological phenotypes have not been thoroughly examined [5,7]. The meiotic drive mutants will be sequenced with an Illumina sequencing method developed to identify Mu insertion mutants [10]. The candidate sequences will be compared to the cDNA data in order to verify expression in Ab10.

Materials and Methods

Seed stocks used for cDNA libraries

Four to six individuals from each of seven different genotypes (four different Ab10 lines and three different N10 lines) were grown to be the source of RNA for our cDNA libraries. All of these lines are stocks that have been maintained in the Dawe lab: Ab10-I, Ab10-II, Ab10-L2, T1-Ab10-I, *Rnj*, *ameiotic1*, and wild type sibs of the *ameiotic1* mutant individuals. The Ab10-I and *Rnj* individuals are also sibling progeny, from an Ab10-I/*Rnj* self cross.

Extraction of RNA from meiotic anthers and preparation of cDNA

Immature tassels were dissected from individuals of all seven lines, and processed to create normalized cDNA libraries as in Chapter 2. The normalized cDNA libraries were submitted to the University of Georgia genomics core facility for 454 sequencing. All seven cDNA libraries were barcoded and sequenced on one and a half plates. The Ab10-I and *Rnj* sibling libraries were also submitted to Emory University's sequencing facility for Illumina sequencing as described in Chapter 2.

Assembly and analysis of cDNA sequence

The 454 reads were assembled using MIRA two independent times, and the two sets of assemblies were compared for accuracy. The 454 data were run through a analysis pipeline, NGMagic

[11], to determine the abundance of reads in each assembly and the best BLASTx hit to the EMBL Uniprot data base (http://www.ebi.ac.uk/uniprot/).

The Illumina reads were assembled at Emory University's core sequencing facility. The Illumina contigs from Ab10 were mapped back to the wild type *Rnj* contigs using CLC genomics workbench. The contigs from Ab10 that did not map back, meaning they were unique to Ab10, were then mapped to maize repeats from TIGR (http://maize.jcvi.org/repeat_db.shtml) and the B73 filtered gene set available at (http://ftp.maizesequence.org/current/filtered-set/). Seventy-four contigs were chosen to map to the Ab10 haplotype using the deficiency breakpoints used for mapping BACs in Chapter 1. The 74 contigs were chosen based on size (length). Primers were designed to each of the 74 contigs using Primer3Plus [12] and contigs were mapped by scoring for polymorphism with B73 or W23 inbreds as in Chapter 1. See Figure 4.2 for a diagram of Illumina cDNA analysis. As a separate line of analysis specific meiosis genes and genes we believed might be involved in drive from B73 were BLASTed to the Illumina Ab10 cDNA.

Preparation of mutant meiocytes and fluorescent in situ hybridization (FISH)

Four individuals from each mutant line (*Smd1*, *smd3* and Df(L)) as well as N10, Ab10-I, Ab10-II and Ab10-L2 control lines were grown in the University of Georgia Plant Biology greenhouses during the 2010-2011 winter season (October – January). Tassels were collected, anthers were fixed, coverslips were prepared, and fluorescence *in situ* hybridization (FISH) was performed as in Chapter 2.

Cytological phenotype scoring

The meiocyte FISH slides were scored for neocentromere activity during either anaphase I or anaphase II. The number of anaphase cells showing neocentromere activity out of 25 total anaphase cells per slide was recorded. At least two individuals from each mutant and control were counted. The type of neocentromere activity was noted for each line (TR-1, knob 180, or both).

Verification of homozygous mutant individuals and preparation of DNA for Mu insertion sequencing

We plan to sequence the genomes of our two point mutants, *Smd1* and *smd3*, with Illumina. The sequences will be assembled and analyzed with a pipeline created in Alice Barkan's lab at the University of Oregon to identify the causative Mu insertion in each mutant. The pipeline requires comparison of sequence from four homozygous individuals from two lineages of each mutant to enable detection of causative Mu elements over background Mu elements that are present across the maize genome. In order to verify homozygosity of our individuals for sequencing, FISH was performed on *Smd1* and *smd3* individual root tip spreads as in Chapter 2. Two homozygous individuals from each of four independent mutant lines (two *Smd1* lines and two *smd3* lines) were grown for DNA extractions, for a total of 8 samples. DNA was extracted using urea as previously described [13]. A total of 5-10ug DNA per individual was sent to Alice Barkan's lab at the University of Oregon for the Illumina sequencing and Mu insertion analysis.

Results

Comparative cDNA analysis alone is unlikely to identify the genes required for meiotic drive

The genes that confer neocentromere activity and other functions required for meiotic drive are unknown. Genomic sequence and cDNA sequence from Ab10 have only recently been generated (Chapter 2). Here we created seven cDNA libraries. The strength and consistency of neocentromere activity and meiotic drive varies between Ab10 lines and between seasons for the same line (*i.e.* it depends on environment). For this reason we were unsure how abundant any transcripts associated with the unique functions of Ab10 would be during the time we extracted RNA from our tissue, and so chose to normalize our cDNA libraries before sequencing them. First, we sequenced all seven libraries using 454 technology on 1.5 plates, yielding a total of approximately one million reads (Table 4.1).

The first analysis we performed on the 454 data measured how many transcripts composed each cDNA contig. Though we normalized our libraries we ran our sequence data through a pipeline, NGMagic, to determine if the Ab10 libraries contained any obviously over-represented contigs that were

not present in the N10 samples. We were unable to identify good candidates by this method using the 454 data set. Since the 454 analysis was limited by low sequence coverage, as modeled by Ken Jones at UGA Genomics Facility (Figure 4.1), we proceeded to carry out a similar analysis using Illumina technology, which provides shorter reads but far higher sequence depth.

We sequenced our Ab10-I and sibling *Rnj* (N10) individuals with 100bp paired-end Illumina sequencing. These reads were assembled at Emory University. The analysis of the Illumina reads is diagrammed in Figure 4.2. First, the Ab10 contigs were filtered to determine which were unique to the Ab10 transcriptome. To begin the filtering process, the 67,725 Ab10 contigs were mapped back to the 46,498 *Rnj* control contigs. This step identified 49,478 contigs as unique to Ab10. The set of 49,478 contigs was then mapped to the TIGR maize repeat dataset the B73 filtered gene set, leaving 21,150 contigs that were unique to Ab10. From here, we filtered out all contigs less than 1,000 bp. At this point there were 74 contigs left. In order to determine if any of these contigs corresponded to known genes, we used BLASTx to compare them to NCBI's nr protein database. Most of them produced hits to maize proteins with e-values above $10e^{-5}$ (Figure 4.2).

In order to determine if these 74 contigs were good candidates for genes involved in meiotic drive we attempted to map them to the Ab10 haplotype. To this end we designed primers from each contig and tested them for polymorphism in our mapping lines, which are heterozygous for Ab10 and either the B73 or W23 N10 chromosomes (Chapter 2). Unfortunately, none of the PCR products showed polymorphism between Ab10 and N10. The lack of polymorphism in these genes indicates either that the chosen genes do not map to Ab10, or that there is little divergence between the Ab10 and N10 alleles. These sequences were not pursued further.

Neocentromere and meiotic drive genes are not easily identifiable

We also manually screened the cDNA library for sequences that matched our initial expectation that a neocentromere gene will have the characteristics of a DNA binding or chromosome motility

protein. Specifically we BLASTed maize kinesins and SUN domain proteins, to the 49,478 "unique" Ab10 contigs (that were not observed in the *Rnj* control). We observed several high likelihood hits for each candidate. However, these genes are all also present in wild type lines such as B73. The only way to definitively prove that we are finding potential neocentromere genes would be to map them to the Ab10 haplotype. This proved impossible to do without further sequencing because we could not design primers that would not amplify the WT genes. These sequences remain good candidates but deeper analysis is necessary to determine if the Ab10 transcriptome contains alternate transcripts or chimeric proteins involving the SUN or kinesin functional domains. Genomic DNA and/ or longer cDNA contigs would enable this analysis, though these data are not available at this time.

All known meiotic drive mutants are deficient in knob 180 neocentromere activity

As a second approach to identify the genes required for meiotic drive, we scored the cytological phenotype of three meiotic drive mutants of Ab10-I (*Smd1*, *smd3*, Df(L)) identified in prior mutant screens using *Robertson's mutator* (Mu) [5,7]. Mu is an endogenous maize DNA transposon that has been widely used to conduct large-scale mutant screens [14]. Mu is highly active and abundant in maize and preferentially inserts into unlinked, low copy regions (genes) [15]. Though these mutants have been previously characterized for neocentromere activity, there exists doubt as to the accuracy of the methods used. Additionally, cytology has greatly improved in recent years. In order to better characterize the cytological mutant phenotype of these mutants twenty-five anaphase cells from two individuals of each mutant and wild type line were scored for neocentromere activity (Table 4.2). We found that *Smd1*, *smd3* and Df(L) show the same general cytological phenotype: All three mutants lack neocentromere activity at all knob 180 loci, but retain TR-1 neocentromeres. In the case of *Smd1* TR-1 neocentromere activity was extremely variable, ranging from dramatic to weak (Figure 4.3e, f). This observation supports the previous characterization of *Smd1* neocentromere activity [7], but clarifies that only TR-1 contributes to neocentromere formation. The TR-1 activity in *smd3* and Df(L) was weaker but more consistent than *Smd1* (Figure 4.3c, d). The data presented here on Df(L) and smd3 provide a novel characterization of

these mutants. Although all three mutants retain TR-1 neocentromere activity, it is not as consistent as the TR-1 activity observed in the Ab10-L2 control (Table 4.2, Figure 4.3d).

Genomic DNA from the point mutants, *Smd1* and *smd3* has been submitted to Alice Barkan's lab from Illumina sequencing. Four individuals from two lineages of each mutant (for a total of eight individuals) were verified to be homozygous with fluorescent *in situ* hybridization (FISH) (Figure 4.5). These plants were grown up and DNA was extracted from young leaves as previously described [13]. The sequence from these samples will be analyzed as published to identify any potential causative *Mu* insertions [10].

Discussion and Future Plans

Meiotic drive in maize has been studied since the 1940s and the maize genome sequence has been available since 2008. Knob heterozygosity, recombination, neocentromere formation, and orientation of sister chromatids all contribute to meiotic drive. The genes underlying meiotic drive have been mapped within a large, superfluous haplotype known as the Ab10 haplotype [1,5]. Unfortunately, the sequenced B73 reference genome does not contain this haplotype and so the drive genes remain unknown. Here the first transcriptome data from an Ab10 genome were presented.

Although meiotic drive genes were presumed to be unique and likely easy to identify based on the prior knowledge of neocentromere and drive functions [3,9], the analysis of Ab10 specific transcripts yielded no obvious meiotic drive candidate genes. In part this was due to lack of completely novel BLAST hits, and in part to an inability to map transcripts to the Ab10 haplotype. Since the cDNA data we analyzed was from an entire transcriptome, not only the Ab10 haplotype, we could not be sure if any interesting BLAST hits were located within the Ab10 haplotype without mapping them. Mapping failed primarily because of a lack of detectable polymorphism between the candidate genes and the homologous or closely related sequences in the normal (non-Ab10) lines. The lack of size polymorphism in the 74 candidates we examined indicates that these genes are likely not involved in meiotic drive. However, the

lack of polymorphism in these genes may also indicate that (if they are involved in meiotic drive) they are used as splice variants of the N10 genes. If drive genes exist as splice variants or chimeric proteins they would be difficult to detect. It would be necessary to amplify the alternately spliced regions or the fusion point of a chimeric gene in order to detect a difference between the Ab10 and wild type genes.

The initial analysis of our cDNA produced no obvious candidates for meiotic drive genes therefore we turned to a second method to identify candidates. We cytologically characterized three mutants of meiotic drive that were identified in mutant screens using active *Mu* transposons. Previous work showed that one of these mutants, *Smd1*, has reduced drive and the other two, *smd3* and Df(L), have no drive. When examined in anaphase these mutants all retained TR-1 neocentromere activity, but none exhibited knob 180 neocentromeres. Since these mutants all lack meiotic drive, our results indicate that neocentromere activity of the 180 bp repeat is necessary for meiotic drive to occur. Moreover, these results support findings from Chapter 3 that TR-1 neocentromere activity is insufficient for meiotic drive to occur. If TR-1 could confer strong drive we would not expect all three mutants to retain TR-1 neocentromere activity.

Characterization of the Df(L) mutant refined previous mapping of drive functions within the Ab10 haplotype. Previously the Ab10 haplotype had been split into four functional regions, with knob 180 neocentromere activity mapping to a large region (distal to Df(K)) [5]. The data on neocentromere activity presented here show that knob180 activity maps distal to Df(L). This signifies that the elusive distal tip function is knob 180 neocentromere activity. These results combined with the characterization of Df(B) in Chapter 3, show that there are three prominent functions conferred by the Ab10 haplotype (TR-1 neocentromeres, knob 180 neocentromers, and meiotic drive). Moreover, these functions map to two regions on the Ab10 haplotype, proximal to Df(B) and distal to Df(L) (Figure 4.4). Thus, all necessary components of the drive system lie in the distal tip, and the sequence of this region remains completely unknown.

DNA was extracted from the two point mutants, *Smd1* and *smd3* and submitted to the Barkan Lab for Illumina sequencing and *Mu* insertion analysis. The Barkan Lab has developed an informatics pipeline to identify *Mu* insertions that may be the cause of a particular mutant phenotype [10]. In brief they analyze *Mu* insertions in four individuals (two individuals from two lineages) representing a single, original mutation event. The pipeline examines *Mu* insertions in common between the four individuals, but not contained in the B73 reference genome [10]. The Barkan lab will sequence our point mutants and provide us with a list of possible sequences based on their pipeline. We will then map candidate genes to the Ab10 haplotpe and compare these candidates to our Ab10 cDNA data to verify their expression. Ultimately well characterized candidates that map to the correct location on the Ab10 haplotype (distal to Df(L)) will be fused with YFP. Transgenic plants will be examined for YFP localization to knobs during meiosis.

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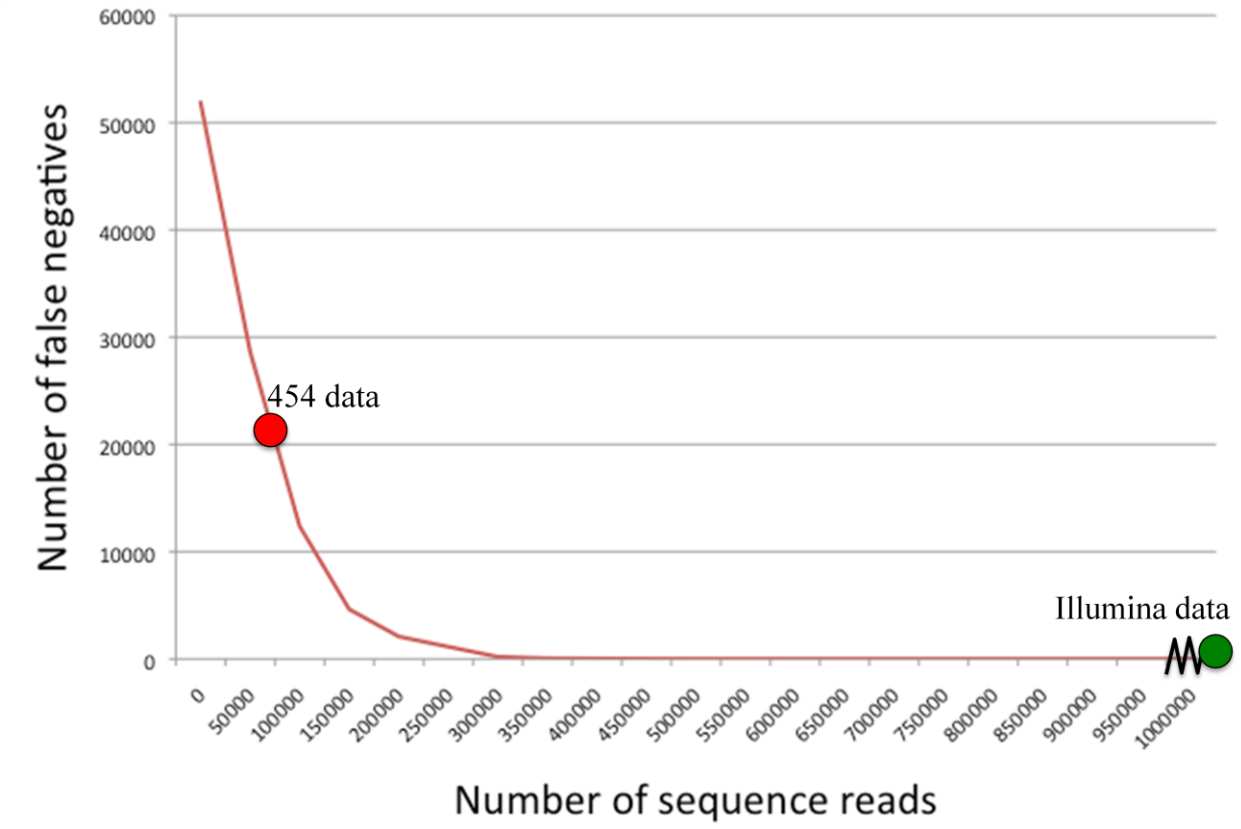

Figure 4.1: Estimation of reads needed to ensure there are no false negatives

The graph was generated by Ken Jones, at the UGA Genomics facility. This graph represents an estimate of the number of 454 reads needed per individual, to ensure no false negatives (i.e. if we did not find a gene it is because it doesn't exist, not because our sequence coverage was not high enough). A transcript number of 51,000 was used when making the graph. Our total 454 reads are represented by a red circle, indicating that even if all of our 454 reads had come from one individual we still risk missing 20,000 genes. Our Illumina sequence (green circle) yielded over 40 million reads per sample which is more than enough to ensure all transcripts are represented in our sequence data.

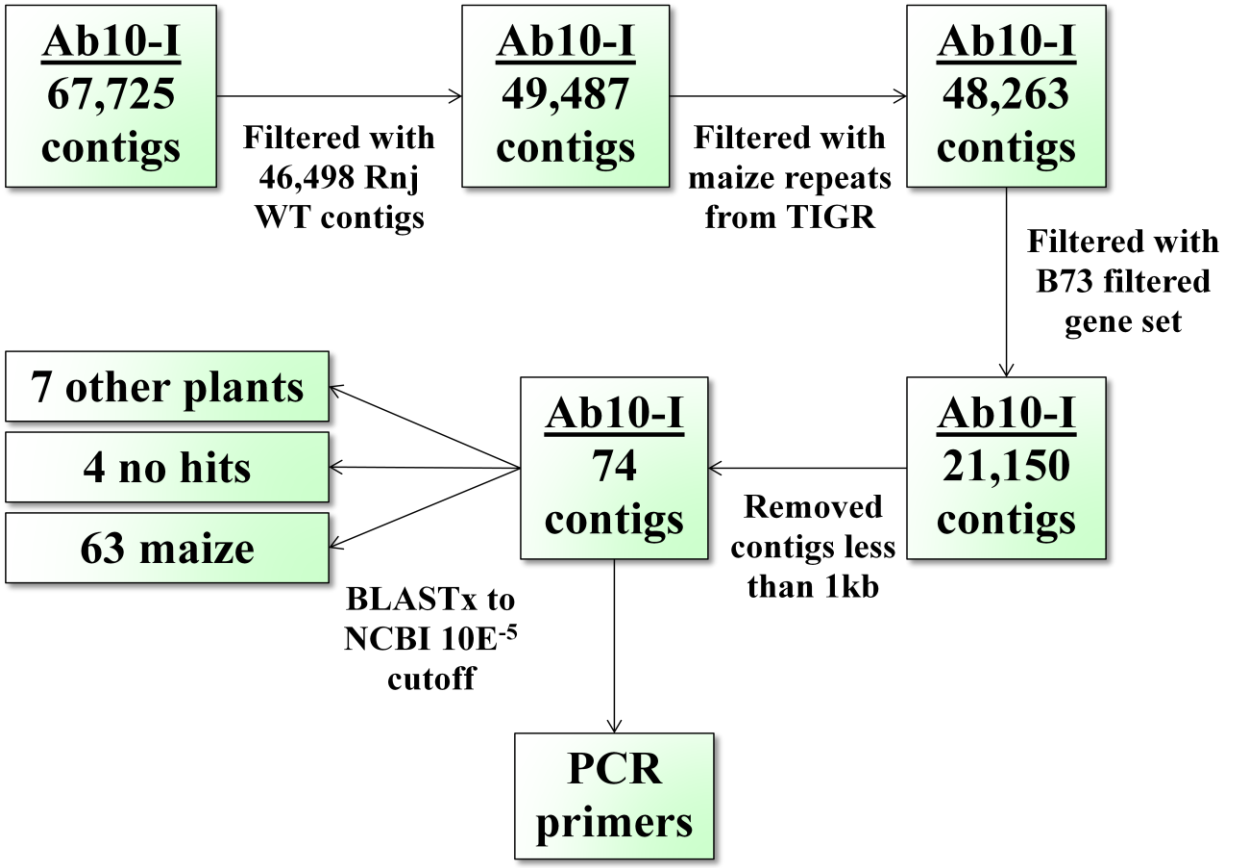

Figure 4.2: Flow chart of Illumina cDNA analysis.

The sequencing core facility at Emory assembled our Ab10-I and Rnj (wild type) reads. From there, we used CLC genomics workbench to map Rnj, maize repeat and B73 transcripts back to our Ab10-I contigs in a stepwise fashion. We then filtered out all contigs less than 1,000 bp. Primer 3 plus was used to design primers to the 74 remaining contigs. The number of Ab10-I contigs remaining after each filtering step is shown in a green box.

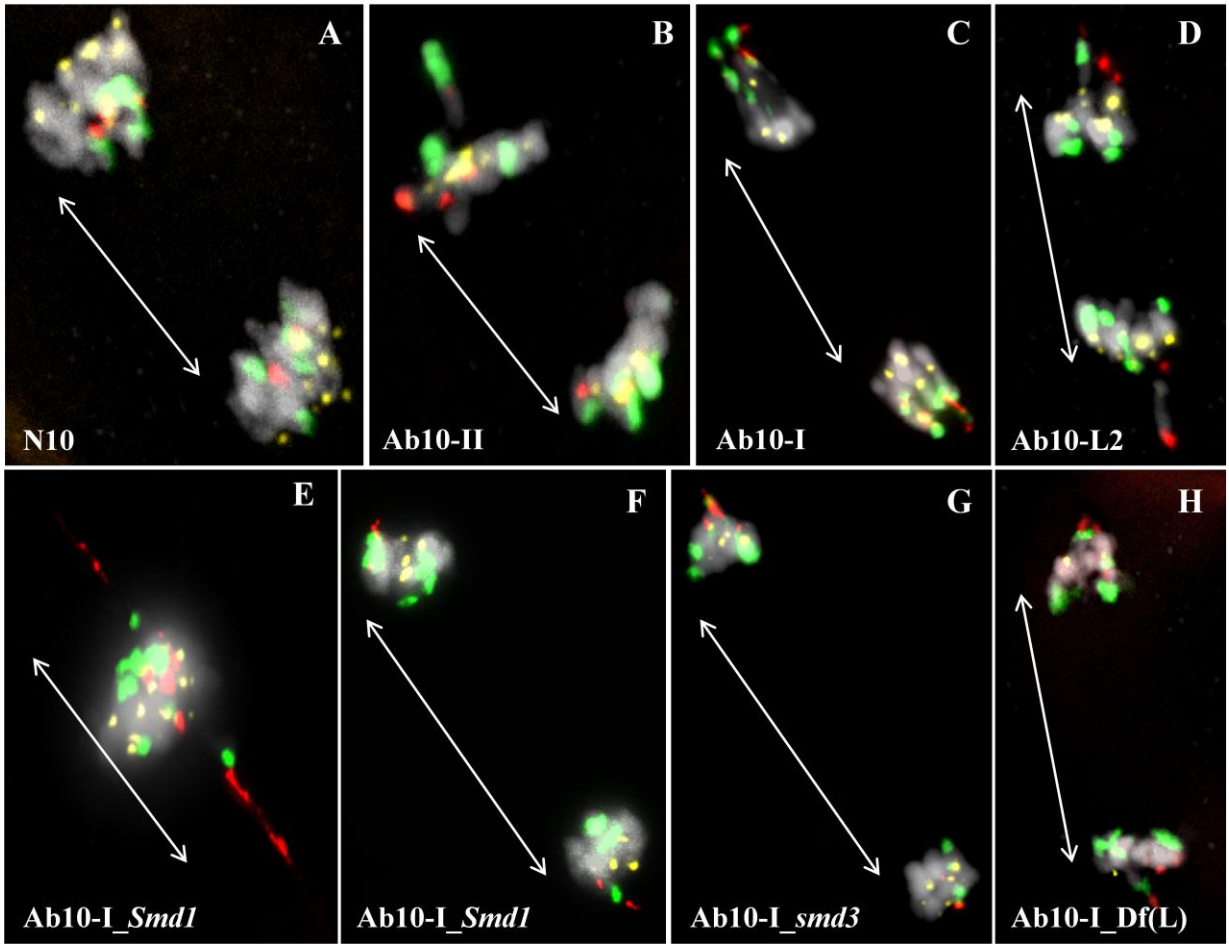

Figure 4.3: Neocentromere activity of control Ab10s and mutant lines depicted by fluorescence *in situ* hybridization (FISH).

Example images of control and mutant anaphase cells depicting varying levels of neocentromere activity. N10 (A) shows no neocentromeres, Ab10-II (B) only shows knob 180 neocentromeres, Ab10-I (C) shows both TR-1 and knob 180 neocentromeres and Ab10-L2 (D) shows only TR-1 neocentromeres. The mutants all show only TR-1 neocentromeres (E-H). *Smd1* showed very strong TR-1 neocentromeres that began at late metaphase/ early anaphase (E), as well as weaker neocentromeres (F). Df(L) and smd3 both showed consistently weak TR-1 neocentromeres (G, H). In all images the centromere repeat, centc, is yellow, TR-1 is red, and knob 180 is green. DNA was stained with DAPI and is grey here. Arrows indicate direction of movement.

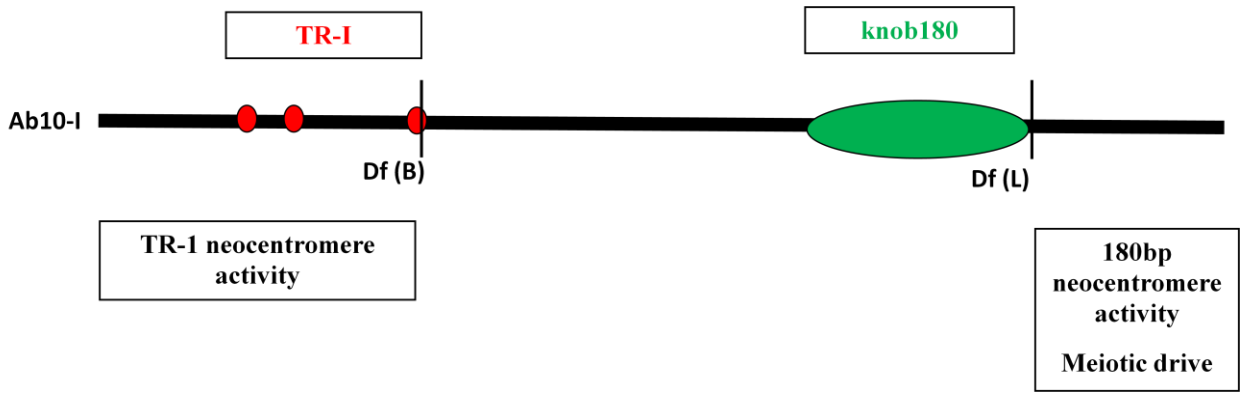

Figure 4.4: New mapping of key Ab10-I functions

Zoomed in cartoon of the Ab10-I haplotype showing the regions necessary for TR-1 neocentromeres, 180 bp neocentromeres and meiotic drive. TR-1 neocentromere activity maps proximal to Df(B) (Chapter 3). Both knob 180 neocentromeres and key meiotic drive genes are now mapped distal to Df(L), in the euchromatic distal tip of the haplotype.

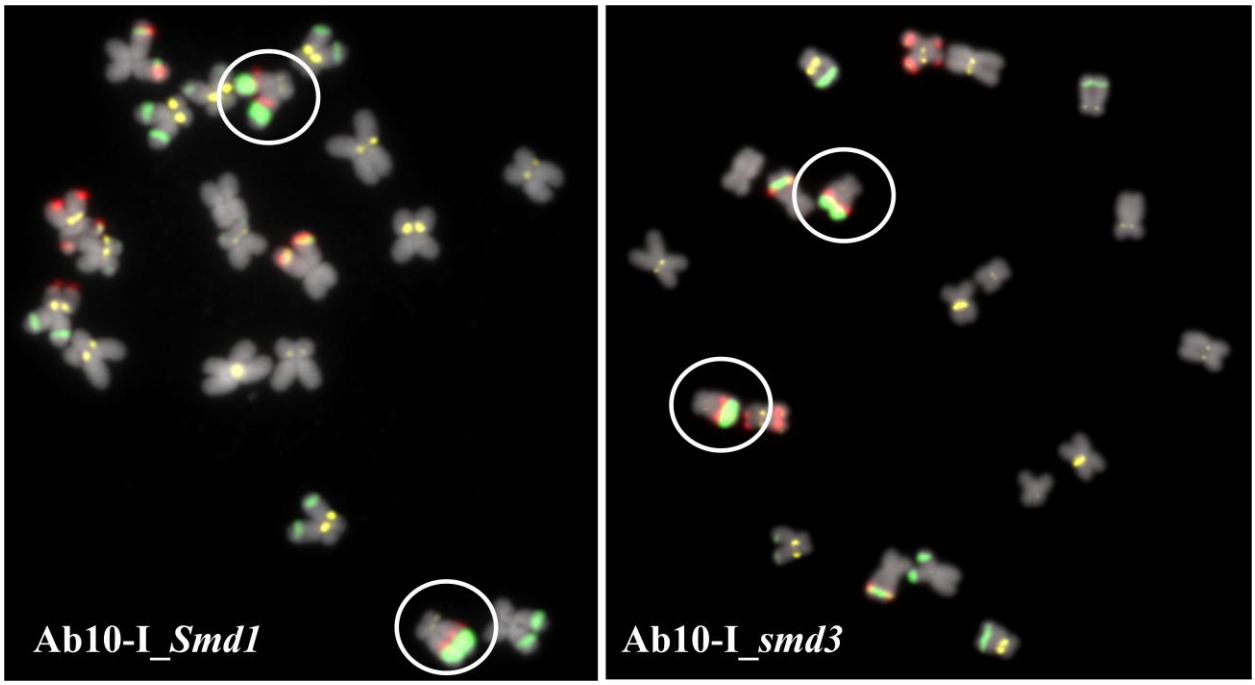

Figure 4.5: Verifying homozygosity of Ab10-I in *Smd1* and *smd3*

Mitotic root tip spreads of a *Smd1* individual and a *smd3* individual. Ab10 is circled in white. Three more individuals from each mutant were verified, and DNA was extracted from all eight individuals. Centromeres (centc) are yellow, TR-1 is red, knob 180 is grey and DNA (DAPI) is grey.

Maize line	Number of Reads
Ameiotic	100,451
Ameiotic wild type sib	162,776
Ab10-I	129,947
Rnj wild type sib of Ab10-I	165,124
Ab10-II	131,226
Ab10-L2	186,649
T1-Ab10-I	145,631
Total	1,021,804

Table 4.1: Number of reads produced per individual from 454 sequencing

The cDNA libraries were tagged, pooled and sequenced on 1.5 454 plates. The average read length was 190 bp.

Genotype of line	Plant1	Plant2	Type of neocentromere
Ab10-I/Ab10-I	25/25	28/28	180 and tr-1
Ab10-II/Ab10-II	26/26	25/25	180
Ab10-L2/Ab10-L2	15/20	20/25	TR-1
Ab10-L2/N10_R-nj	23/26	23/25	TR-1
N10/N10_R-st	0/25	N/A	none
N10/N10_r	0/25	N/A	none
Df(L)/Df(L)	20/25	N/A	TR-1
Df(B)/N10_R-nj	9/17	0/10	TR-1
smd1/smd1	15	N/A	TR-1
smd1/N10_ACR	15/25	15/25	TR-1
smd3/smd3	15/25	N/A	TR-1
smd3/N10_ACR	17/25	9/25	TR-1

Table 4.2: Number of anaphase cells showing neocentromeres

The number of anaphase cells showing neocentromere activity is shown as a ratio of cells with neocentromeres/ total number of observed cells. N/A indicates we could not obtain a properly staged tassel. The R1 allele on N10 is indicated in the heterozygous plants and the N10 negative controls (ACR, r, R-nj, or R-st).

CHAPTER V

CONCLUSIONS AND DISCUSSION

Meiotic drive, the phenomenon by which certain genomic components exhibit non-Mendelian, preferential transmission during meiosis, has been observed in members of all eukaryotic kingdoms [1]. In the classic examples of preferential transmission, including segregation distorter (SD) in *Drosophila melanogaster*, spore killer (SK) in *Neurospora* and, the *t*-haplotype (*t*) in *Mus musculus*, meiosis proceeds normally but the products of meiosis are altered [2]. These three systems cause the abortion of sperm, or spores, that do not house the driving haplotypes [3]. The abnormal chromosome 10 (Ab10) drive system in *Zea mays* is a fourth classical example of meiotic drive, but utilizes different mechanisms than SD, SK and *t*. Ab10 drives through females, by taking advantage of the naturally asymmetric development of female meiocytes in plants, *i.e.* Ab10 segregates to the basal meiocyte that will inherently develop into the egg [4]. Notably, Ab10 drives not only itself, but any maize chromosome that is heterozygous for a knob (heterochromatic repeat locus). The Ab10 drive phenomenon has been studied since the 1940s and necessary drive components have been mapped to a large haplotype located distal to *R1*, the Ab10 haplotype [5,6]. However, little was known about the molecular composition and evolution of this system. The work presented here provides the first sequence data from Ab10 and examines the population dynamics of competing Ab10 variants and their impact on knob repeat abundance.

Through the sequencing and rough mapping of eleven Ab10 specific BACs on Ab10-I we have successfully developed three dominant, Ab10 specific PCR markers that were used to score 355 *Zea mays* accessions for Ab10. These data revealed that Ab10 is present in an estimated 18% of teosinte (*Zea mays* ssp. *mexicana* and ssp. *parviglumis*) individuals distributed across 85% of the natural teosinte populations we examined. These data show that Ab10 is more abundant than previously thought. In past cytological surveys of teosintes Ab10-I and Ab10-II were found in an average of 6% of total individuals and only

35% of the surveyed populations were segregating Ab10 [7]. We also examined Ab10's prevalence in maize (landraces and inbreds). Though we were only able to look at one individual per population in our initial screen, our data show Ab10 in 19% (22/114) examined maize. Previous cytological surveys from landraces show Ab10 in only 14% of landrace populations and only an average of 1% of total individuals contain Ab10 [7].

The discrepancies between our data and the previous data may be explained in a few ways. First, both sample sets are limited in the number of individuals examined. Here we only examined 12 individuals per teosinte population and one individual per landrace, while the previous studies examined about six individuals per teosinte and four individuals per landrace. Thus, it is possible that both samples sets are underestimates of the true prevalence of Ab10 in nature. Second, the location of populations we examined was largely different than the previously surveyed populations. For example, the previous studies examined multiple landrace populations from 18 of the 50 states (USA) and only found Ab10 in Arizona and Louisiana [7]. Notably all seven of the US populations we examined were from the Arizona region. Also, the South American landrace populations we examined were located west of the Andes and north of Argentina, whereas the previously examined South American populations were largely from Brasil and Argentina. A third reason for the discrepancy in frequency is that even in regions in common between our study and the previous surveys, we were not able to use the same populations. Finally, we are using a different screening method (PCR vs. cytology). It is possible that we identified false positives using our PCR markers; for example, there may be forms of N10 that contain one or more of our markers in the landraces and teosintes (although not inbreds). Future work (screening more landraces both cytologically and with our markers) will help to address this question thoroughly. However our initial test of four landraces (Table 2.4) suggests that a single marker is sufficient to identify Ab10 and that N10 chromosomes are not a source of false positives.

Despite the apparent differences in overall Ab10 frequency, the trends identified in our study strongly support the prior results of McClintock and coworkers (Chapter 2, 3) [7]. Namely, Ab10 is more

prevalent and diverse in teosintes than landraces, and in landraces compared to inbreds. In fact, Ab10 appears to be absent from the inbred lines of maize. This indicates that Ab10 underwent a bottleneck along with the rest of the maize genome during domestication and again during the improvement processes. Ab10 is not only more diverse in teosintes than maize, but it is more diverse than previously thought in general. We identified a novel cytological variant of Ab10, Ab10-III through our landrace screen in Chapter 2. Though Ab10-III remains to be tested for neocentromere activity and meiotic drive, the knob staining pattern we observed in pachytene is very similar to Ab10-I, and has not been observed on any other chromosomes. Individuals containing Ab10-III have been planted to assay neocentromere activity in immature tassels. Also, crosses will be made to create heterozygous stocks (Ab10-III/N10) for meiotic drive test crosses.

Ab10 also seems to be highly molecularly diverse. Though we did not recover all three of our markers in a single individual we recovered many different combinations of them in our teosinte and landrace survey. The presence of so many molecular variants suggests that recombination is occurring between different Ab10 types, and in Chapter 3 we show that different Ab10s can recombine with each other. We did find more than one molecular variant of Ab10 within a single teosinte population. In Chapter 3 we show that the different cytological variants of Ab10 compete with each other for transmission when they are paired together. Thus, it is likely that in populations segregating more than one variant a similar competition is occurring. Recombination and competition between Ab10s in nature may lead to the rapid evolution of this otherwise static and genetically isolated haplotype.

The work in chapter three presents a clear case of competition in its extreme: suppression. We characterized what we believe to be the K10L2 knob in an inbred line, CI66, showing that it contains only one of the knob repeats, TR-1. The observation that the CI66 K10L2 confers strong TR-1 neocentromeres and does not activate knob 180 led to several lines of thought. First, it led us to question if this chromosome also exhibits meiotic drive (we showed it only exhibits 51% transmission). Due to its Ab10-like properties, we named this chromosome Ab10-L2. Second, we questioned the origin of this

knob. To address this, we examined pairing and recombination of Ab10-L2 with Ab10-I, Ab10-II and N10. Our data indicate that the genomic regions surrounding the TR-1 knob are more closely related to N10 than to Ab10-I and Ab10-II. Since Ab10-L2 appeared to be more related to N10 than other Ab10s and did not drive itself we examined its affect on the preferential transmission of Ab10-I and Ab10-II. In Chapter 3 we show that Ab10-L2 reduces the preferential transmission of Ab10-I and Ab10-II. In light of these results, we believe that Ab10-L2 acts as a suppressor of meiotic drive in maize.

It was previously reported that Ab10-I and Ab10-II not only drive a knobbed chromosome over an unknobbed chromosome, but if both homologs have a knob the larger of the two will be transmitted. For this reason, knobs containing both the knob 180 and TR-1 repeats have become large in size and very prevalent in the teosintes and maize. If they were not under selection by Ab10, knobs repeats, like other tandem repeats, would expand and contract with equal chance [8,9]. Since the 180 bp repeat is required for meiotic drive (Chapter 3, 4), we expect knobs of this type to spread in accordance with the prevalence of Ab10 [8] (Chapter 2). However, it is more difficult to understand why TR-1 is as abundant as it is. The results in Chapter 3 show that TR-1 alone (as in Ab10-L2) does not exhibit strong drive; rather it is a suppressor of drive, and we believe Ab10-L2 is under direct selection as a suppressor. Moreover, although the ability of Ab10-I to drive TR-1 only knobs on other chromosomes has not been directly tested, Ab10-I confers TR-1 neocentromere activity independently of knob 180 neocentromeres (Chapter 4). Since Ab10-I shows strong drive and activates TR-1 it is possible that Ab10-I has caused the spread of TR-1 knobs to other loci, where they are being continuously selected for as knob 180 suppressors. Fitting with this theory is our observation that Ab10-L2 populations contain less knobs composed only of knob 180 than N10 populations (Chapter 3). Under the scenario that knob 180 and TR-1 are being selected for opposing functions, the two repeats should continuously compete for dominance. Interestingly *Zea perennis* appears to have no 180bp knobs, only TR-1 knobs (Dawe lab, unpublished) [10].

Our molecular analyses leave little doubt that the primary meiotic drive haplotypes Ab10-I and

Ab10-II are also derived from an early form of modern N10, although they have sustained major rearrangements. Ab10 likely evolved before or soon after the ancient tetraploidy event and became genetically isolated through inversions, deletions, and smaller rearrangements or gene conversion events. We show that the central euchromatin is largely syntenous with N10L, but that the key distal segment of the haplotype (containing the knob 180 neocentromere gene) is likely derived from non-N10 portions of the genome (Chapter 2, 4). Since maize is an ancient paleopolyploid with extensive remaining redundancy, a large fraction of the genome can be lost without phenotypic consequence [11], and the loss of one copy vs. another is not consistent when any two lines are compared. Thus, it is possible that the primary neocentromere and meiotic drive genes, including the entire euchromatic distal tip, represent a portion of the genome that has been lost elsewhere as a part of the ongoing genome fractionation process [12]. We were unable to identify clear candidates of drive genes in our cDNA sequence, indicating that the essential drive genes may be fusions or alternative splice variants of known genes that were created during the diploidization and fractionation of the genome.

The data presented here together with previous cytological surveys paint a clear picture of how a large portion of the maize landscape grew to be what it is today. We show that at least five distinct cytological variants and multiple molecular variants of chromosome 10 exist in maize. The different structural variants of chromosome 10 can recombine with each other, likely contributing the high level of molecular and cytological variation of this chromosome. The Ab10 variants are inherently competitive and we show that they compete with not only N10, but with each other for transmission. Moreover, one variant (Ab10-L2) has evolved specifically to suppress meiotic drive. The competition observed between the variants of chromosome 10 explains not only why the different Ab10 haplotypes contain different amounts of the two knob repeats, but also why the two knob repeats are extremely polymorphic through *Zea mays*. The combined data here provide experimental evidence that the evolution of the maize genome has been, and continues to be influenced by the non-Mendelian transmission of large regions of superfluous DNA.

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