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Plant neocentromeres: fast, focused, and driven

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Abstract

Plant neocentromeres are large heterochromatic domains that associate with microtubules and move rapidly poleward during meiotic cell division. In maize, neocentromeres are part of a process that leads to the preferential recovery (meiotic drive) of knobs in progeny. These 'classical' plant neocentromeres differ from animal neocentromeres by their morphology, inability to mediate sister chromatid cohesion, and their rates of movement on the spindle. We provide a comprehensive review of classical neocentromeres with emphasis on their origin and mechanisms of motility. The data support the view that most, if not all, classical neocentromeres are the outcome of selection by meiotic drive. In addition, we compare and contrast neocentromere-mediated meiotic drive with a recently proposed meiotic drive model for centromere evolution.

Introduction

Among the many intriguing phenomena documented by early maize geneticists is a now-classic example of non-kinetochore chromosome movement known as neocentromere activity. First described by Marcus Rhoades (Rhoades & Vilkomerson 1942), maize neocentromeres occur at large subterminal heterochromatic domains known as knobs (Figures 1A and 1B); are observed only at meiosis; and occur only in the presence of a chromosome variant known as abnormal chromosome 10 (Ab10). Further, maize neocentromeres are associated with skewed Mendelian segregation ratios that favor knobs and knob-linked loci. Test cross ratios (expected to be 50:50) can be skewed as severely as 80:20, with the knobs/neocentromeres always in the over-represented class (Rhoades 1952). This effect, referred to as meiotic drive (Sandler & Novitski 1957), is inextricably entwined with the cell biology of neocentromeres. During the heyday of plant cytogenetics, neocentromeres were identified in at least 12 other flowering plant genera and a moss (Table 1), although the effects on Mendelian segregation were never documented. Many years later a different form of neocentromere was discovered in humans (du Sart *et al.* 1997). Information on human neocentromeres has dominated the literature in the past several years, though a substantial amount of information on plant neocentromeres has also accumulated. The result has been two parallel bodies of literature based on structures with the same name, but with little else in common.

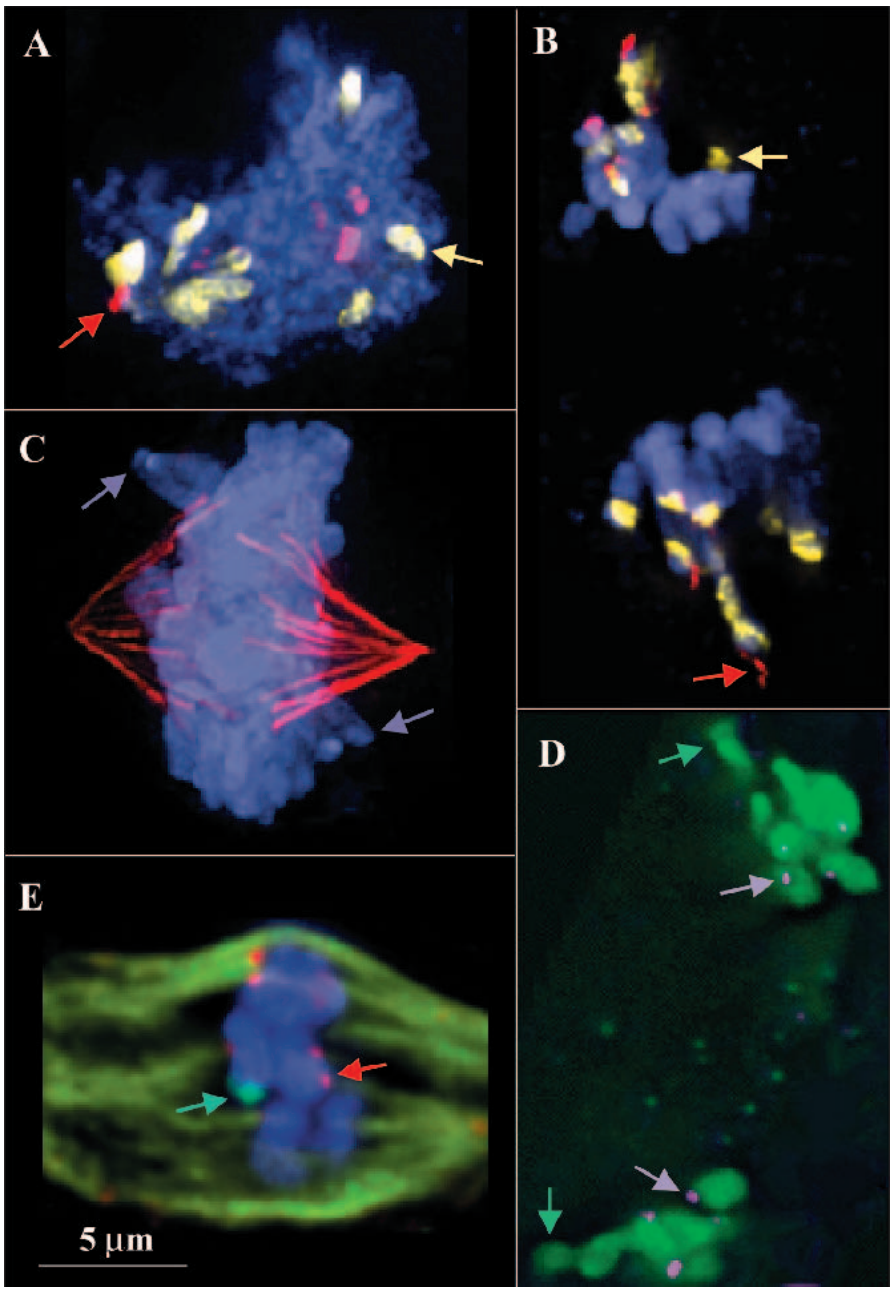

Figure 1. Neocentromeres and chromosome arm motility. (**A**) Maize knobs in early pachytene. The 180 bp repeats are labeled in yellow, and TR-1 repeats in red. (**B**) Neocentromeres in maize at anaphase II. TR-1 repeats (red) lead the 180 bp repeats (yellow) towards the poles. (**C**) Natural chromosome arm motility at metaphase I in *Clematis jackmanii*. The chromosomes are shown in purple and the spindle in red. The chromosome arms (arrows) in this large-genome species, as in most if not all other angiosperms, orient towards the pole during mitosis and meiosis. This image was generously provided by Dr Carolyn Lawrence. (**D**) The kinetochore protein CENH3 (Zhong *et al.* 2002) does not localize to neocentromeres. Chromosomes are shown in green and CENH3 in purple. The true centromeres stained brightly for CENH3 (purple arrows) whereas there was no evidence of CENH3 staining at neocentromeres (green arrows). (**E**) The kinetochore protein MAD2 (Yu *et al.* 1999, Yu 2000) does not localize to neocentromeres when Ab10 is present. In this four-color image the chromosomes are shown in purple, the spindle in yellow, the 180 bp repeat in green, and MAD2 in red. Note that there is no MAD2 staining on the knob (green arrow), though MAD2 staining (red arrow) is pronounced at this stage of prometaphase II. This image was generously provided by Dr Hong-Guo Yu.

Table 1. Plant species with documented neocentromere activity.

Species	*Meiotic stages*[2]	*Heterochromatin (Knob)*	*Chromosomal location*	*Special circumstances*	*References (earliest citations)*
Pleurozium schreberi	MI, AI, AII	Heterochromatin	Subterminal	None known	Vaarma 1954
Lilium formosanum	MII	Not mentioned	Subterminal	2 plants X-ray treated	Zohary 1955
Pennisetum orientale[1]	MI, AI	Heterochromatin (suggested)	Terminal knobs (suggested)	Allotriploid	Vardhan & Lakshmi 1983
Zea mays ssp. *mays*	preMI, MI, AI MII, AII	Knobs	Subterminal-knobs	Requires Ab10, affected by genetic background	Rhoades & Vilkomerson 1942
Aegilops umbellulata[1]	AI	Not mentioned	"Distal ends"	Wheat – *Aegilops* addition line Neo's on *Aegilops* univalents	Noronha-Wagner 1956
Bromus pitensis × *B. marginatus*[1]	AI	Not mentioned	Terminal regions	Interspecific hybrids Neo's on univalents	Walters 1952a,b
Bromus marginatus × *B. pseudolaevipes*[1]					
Elymus wiegandi	MI, AI, MII, AII	Heterochromatin	Terminal and subterminal	None known	Vilkomerson 1950
Festuca pratensis	AI	Heterochromatin	Ends	Accessory chromosome univalents	Bosemark 1956
Lolium perenne	Diplotene	Not mentioned	Ends	Heat stress	Jain 1960
Phalaris coerulescens × *P. minor*[1]	AI, AII AI, AII	No evidence for knobs	Subterminal rounded ends	Interspecific hybrids Neo's on univalents	Hayman 1955
Phalaris tuberose × *P. minor*[1]					
Secale cereale	preMI, MI, AI MII, AII	Heterochromatin (C bands)	Terminal and subterminal	Varies with genetic background	Kattermann 1939, Prakken & Müntzing 1942
Secale dighoircum × *S. turkestanicum*[1]	AI, AII	Heterochromatin	Terminal	Interspecific hybrid	Jones 1969

[1]In these seven cases, neocentromeres were found in interspecific crosses.
[2]MI = metaphase I, MII = metaphase II, AI = anaphase I, AII = anaphase II.

The neocentromeres in animals are fully functional, apparently 'normal' centromeres (Choo 2001). Animal neocentromeres tend to arise in cancer cell lines (Choo 2001) or under other unusual circumstances (Williams *et al.* 1998), and once established are entirely stable throughout mitosis and meiosis. At least 20 known kinetochore proteins are present on one human neocentromere (Saffrey *et al.* 2000); the only difference between a true centromere and the neocentromere is the underlying DNA (Choo 2001). In contrast, the neocentromeres of plants bear little if any resemblance to normal centromeres and probably have an entirely different biology and origin (Yu *et al.* 1997). Plant neocentromeres do not mediate sister chromatid cohesion, which is a critical aspect of normal centromere function. Secondly, plant neocentromeres are deeply staining heterochromatic domains, not the weakly staining constrictions that are diagnostic of normal centromeres. Finally, at least in maize, neocentromeres move much faster on the spindle than true centromeres and appear to interact with the walls of the microtubule lattice instead of the ends. Given the clear differences between plant and animal neocentromeres, we refer to those originally described in maize as 'classical neocentromeres', reserving the simpler term 'neocentromeres' for the transplanted but otherwise fully functional centromeres of animals.

So what are classical neocentromeres and how did they evolve? We begin with a brief review of spindle dynamics and microtubule-based motors, in an effort to set the stage for how and why classical neocentromeres might have evolved. We move on to chromosome arm motility in particular, and finally discuss the genesis and mode of classical neocentromere activity as it relates to meiotic drive.

Primer on the poleward forces within a spindle

Chromosome movement involves a variety of functionally redundant processes that differ from organism to organism. Proteins within the kinetochore are critical for chromosome movement, but the kinetic properties of microtubules and forces generated at chromosome arms and spindle poles are also involved. The major events can be attributed to microtubule flux as well as several classes of microtubule-based motors.

Microtubule flux describes a rapid movement of tubulin dimers from the plus ends of microtubules (at the kinetochores) to the minus ends (at the spindle poles). Flux is similar in principle to treadmilling, a biochemical property of microtubules, but the rate of flux in spindles is considerably faster than treadmilling *in vitro* (Sawin & Mitchison 1991). The accelerated plus-to-minus end flow of dimers through the spindle is most likely caused by the loss of dimers at the minus ends (Waters *et al.* 1996, Rogers *et al.* 2004). Many have noted that under circumstances where dimers are constantly flowing from chromosome to pole, anaphase could occur spontaneously if the cohesion between chromosomes was destroyed, tubulin addition at kinetochores ceased, and other anti-poleward (plateward) forces were halted (Inoué 1995). The rates of flux closely match the rates of anaphase chromosome movement in *Xenopus* and *Drosophila*, consistent with this view (Desai *et al.* 1998, Maddox *et al.* 2002). The evidence in plants, though indirect, is also consistent with this hypothesis. When a kinetochore fiber is severed the remaining stub of microtubules lengthens in a way that suggests new dimers are added at the kinetochore (Czabab *et al.* 1993). Evidence that the pole contributes to flux comes from the fact that when the spindle pole is irradiated, chromosome movement temporarily stops (Bajer 1969). Perhaps most convincing is the fact that small particles (of undefined origin) in the *Haemanthus* spindle move at the same speed as the chromosomes, such that there is a roughly constant distance between the moving chromosomes and the particles (Bajer & Mole-Bajer, 1963).

Kinetochore-localized, microtubule-based motors are also major factors in anaphase movement. The large microtubule-based motor, dynein, provides part of the force for anaphase motion in some organisms (Goshima & Vale 2003), but appears to be absent in plants (Lawrence *et al.* 2001). Other options include kinesin-like motors such as CENP-E (Lombillo *et al.* 1995) or 'pac-man' depolymerases that can chew away the plus ends of the microtubules to pull chromosomes poleward at the kinetochores (Rogers *et al.* 2004). Pac-man kinesins seem to have major roles in

some species, but not others (Salmon 1989, Desai *et al.* 1998). There is also a class of kinesins known as the chromokinesins that bind specifically to chromosome arms and regulate plateward chromosome movement (e.g. Funabiki & Murray 2000, Goshima & Vale 2003). Plants have representatives of all major classes of kinesins known to function during chromosome movement, suggesting that some or all of these forces are well conserved (Lawrence *et al.* 2002).

The mechanism of chromosome arm movement, and neocentromere activity in particular

A noteworthy feature of plant cell division is that the chromosome arms point poleward throughout metaphase, suggesting a particularly strong affinity of the chromosomes for microtubules (Figure 1C) (Khodjakov *et al.* 1996). The apparent poleward force is strong enough that severed chromosome arms move rapidly poleward at metaphase (Bajer 1958, Khodjakov *et al.* 1996). This effect has been attributed in part to the fact that higher plant cells have anastral spindles (Khodjakov *et al.* 1996); i.e. they lack centrosomes and organized spindle poles throughout their life cycles (Rieder *et al.* 1993). However, the relevance of anastral spindles to chromosome arm movement is unclear. Centrosomes are dispensable in many cell types where they were previously thought to be indispensable, and the same basic set of kinesins and regulatory proteins appear to be required for both types of cell division (Compton 1998). Poleward chromosome arm movement is probably a feature of all species under the appropriate conditions. For example, *Xenopus* chromosome arms generally orient towards the metaphase plate; however, when the chromokinesin Xkid is inhibited the arms move poleward (Funabiki & Murray 2000). Similarly, although mammalian chromosome arms typically orient towards the metaphase plate, when a chromosome arm is severed from its centromere it often moves poleward (Liang *et al.* 1993).

Importantly, microtubules seem to have a higher affinity for meiotic chromosomes than mitotic chromosomes (Rieder *et al.* 1993). A nice demonstration of this phenomenon was published in a study of *Xenopus* oocytes, where injected *E. coli* or phage DNA was shown to promote microtubule polymerization and the formation of rudimentary spindles (Karsenti *et al.* 1984). Meiotic chromosomes in mouse, *Drosophila*, and maize have all been shown to actively initiate spindle formation during meiosis (Church *et al.* 1986, Theurkauf & Hawley 1992, Chan & Cande 1998, Brunet *et al.* 1999). In contrast, mitotic spindles are initiated from microtubule organizing centers outside the nucleus (Mazia 1961, Lambert 1993, Rieder *et al.* 1993), with the chromosomes helping in later stages to ensure that the resulting structure is bipolar (Gruss *et al.* 2002). It is noteworthy that all plant neocentromeres are meiosis-limited (Table 1) and the only known cases of classical neocentromeres in animals were discovered in the meiotic cells of insects; i.e. *Tityus bahiensis* (Rhoades & Kerr 1949), *Pales ferruginea* (Fuge 1975), and *Pascaris univalens* (Goday & Pimpinelli 1989). A plausible scenario is that the proteins involved in chromatin-mediated meiotic spindle formation (or other related proteins) function in some capacity to mediate neocentromere activity.

Unfortunately the mechanism for poleward chromosome arm movement is not yet known in any species. It has been argued that microtubule flux underlies some forms of chromosome arm movement (LaFountain *et al.* 2001). However, maize neocentromeres are not inhibited by the microtubule-stabilizing drug taxol, indicating that flux alone is not the primary source of poleward motility (Hiatt *et al.* 2002). Nor is there any evidence that known kinetochore proteins are involved in neocentromere activity. In all eukaryotes the centromere/kinetochore complex is a large organelle containing centromeric DNA and up to 50 proteins (Cleveland *et al.* 2003, McAinsh *et al.* 2003). At the inner kinetochore are a group of conserved chromatin proteins that are diagnostic of active centromeres (Yu *et al.* 2000). Two particularly well studied inner kinetochore proteins, Centromere Protein C (CENP-C) (Dawe *et al.* 1999) and Centromeric Histone H3 (CENH3) (Zhong *et al.* 2002), are absent from maize neocentromeres (Dawe *et al.* 1999; Figure 1D). There are also a number of kinetochore proteins known as spindle checkpoint proteins that regulate the metaphase-anaphase transition (Cleveland *et al.* 2003). Mitotic Arrest Defective 2 (MAD2) is a

spindle checkpoint protein that has been extensively characterized in maize (Yu *et al.* 1999). Consistent with lack of inner kinetochore proteins at neocentromeres, there is no detectable MAD2 on knobs when Ab10 is present during meiosis (Figure 1E).

The absence of conserved kinetochore proteins at neocentromeres does not rule out the participation of other kinetochore or kinetochore-like proteins. Particularly good candidates are the motors within the kinesin superfamily (Hiatt *et al.* 2002, Forer *et al.* 2003) most of which are poorly understood in plants. A candidate gene approach is hampered by the fact that all known chromosome-localized kinesins are plus-end directed, i.e. they are expected to move cargo plateward, not poleward (Lawrence *et al.* 2002). Minus-end directed kinesins tend to be spindle-associated or cytoplasmic (Ovechkina & Wordeman 2003), but in principle they could interact transiently with chromosome arms. Further complicating matters is the observation that CENP-E, a plus-end directed kinesin (Wood *et al.* 1997) can move objects towards microtubule minus ends under some conditions (Lombillo *et al.* 1995). We are left with the reality that nearly all kinesins are potential motors for neocentromere motility. As more is learned about plant kinesins (Reddy 2001), more informed speculation about which motors, if any, might be involved in driving neocentromeres poleward will be possible.

The meiotic drive model for the genesis of neocentromeres

The mechanism of neocentromere activity is clearly an important question for future research, but an equally important question is *why* did neocentromeres form in the first place? Given the propensity for neocentromeres to evolve in meiotic cells where chromatin has a natural tendency to interact with microtubules (Rieder *et al.* 1993) and in plants, where poleward chromosome arm motion is already pronounced (Figure 1C), it seems likely that neocentromeres arise initially as accidental features of chromatin organization. Some sequences will be more likely than others to get caught up in the flux of the meiotic spindle or bind to motors that mediate poleward movement. The inherent propensity for DNA to propagate itself (Doolittle & Sapienza 1980) and the fact that meiosis is under constant threat of being exploited by meiotic drive (Haig & Grafen 1991) makes it likely that specific chromatin-spindle interactions will persist (Buckler *et al.* 1999, Hiatt *et al.* 2002). As shown below, the biology of megasporogenesis (plant female meiosis) naturally favors selection for effective spindle-binding sequences. Ever more specialized interactions between repeats and repeat-binding proteins are likely to result, and under appropriate conditions could evolve into multi-component meiotic drive systems. Maize illustrates a particularly elaborate example of such a process, but variations on the same theme can be found in a variety of species.

Neocentromere-mediated meiotic drive in maize

Maize neocentromeres are associated with a rare form of the tenth chromosome known as abnormal chromosome 10 (Ab10) (Longley 1937, Rhoades & Vilkomerson 1942). When Ab10 is present, knobs become neocentromeres and move towards the poles ahead of normal centromeres (Yu *et al.* 1997, Hiatt *et al.* 2002). Although the number of knobs varies among varieties, there are only 22 sites where knobs have been found (Kato 1976). Knobs are generally found in subterminal locations in maize (Longley 1939) whereas in teosinte (the presumed progenitor of maize) they are often found at chromosome ends (Kato 1976). At the molecular level knobs are composed primarily of two tandem repeats, a 180 bp repeat and a 350 bp repeat known as TR-1 (Peacock *et al.* 1981, Dennis & Peacock 1984, Ananiev *et al.* 1998a, b). The two repeats tend to be isolated from each other, either in different knobs or in separate domains of the same knob (Figure 1A) (Hiatt *et al.* 2002, Hsu *et al.* 2003). A relative paucity of retroelements in knobs suggests that the repeats are present in long uninterrupted arrays (Ananiev *et al.* 1998b, Mroczek & Dawe 2003). Interestingly, TR-1 arrays move poleward much faster than 180 bp arrays, stretching out along microtubules in long thin 'leaders' (Figure 1B) (Hiatt *et al.* 2002).

The meiotic drive system in maize is confined to a terminal segment of Ab10 that extends the long

arm to about 1.3 times its normal size (Hiatt & Dawe 2003a). Comparative RFLP mapping demonstrates that at least five independent rearrangements are responsible for the current organization of the drive system. These include a translocation from N10, at least two inversions and the insertion of two novel segments of chromatin (Mroczek, Melo & Dawe, in preparation). This complex structural polymorphism essentially excludes crossing over, creating a haplotype that covers at least 45 cM. The haplotype is composed of four distinguishable domains (Figure 2): the differential segment (with three small knobs), the central euchromatin, K10L knob, and distal tip (Figure 2). The three small knobs within the differential segment are composed primarily of the TR-1 repeat while the large K10L knob is composed primarily of the 180 bp knob repeat (Hiatt *et al.* 2002).

In 1952 Rhoades put forth a model to explain meiotic drive that is supported by a variety of empirical evidence (Figure 3A) (Rhoades 1952, Dawe & Cande 1996, Buckler *et al.* 1999). In most angiosperms only the basal cell of the post-meiotic linear tetrad develops into an egg. Rhoades argued that meiotic drive is the result of neocentromeres preferentially drawing the knobs to this basal megaspore and thus increasing the representation of knobs in progeny. The model requires that a single cross over occur between the centromere and the knob to produce a heteromorphic dyad. At anaphase I and II, neocen-

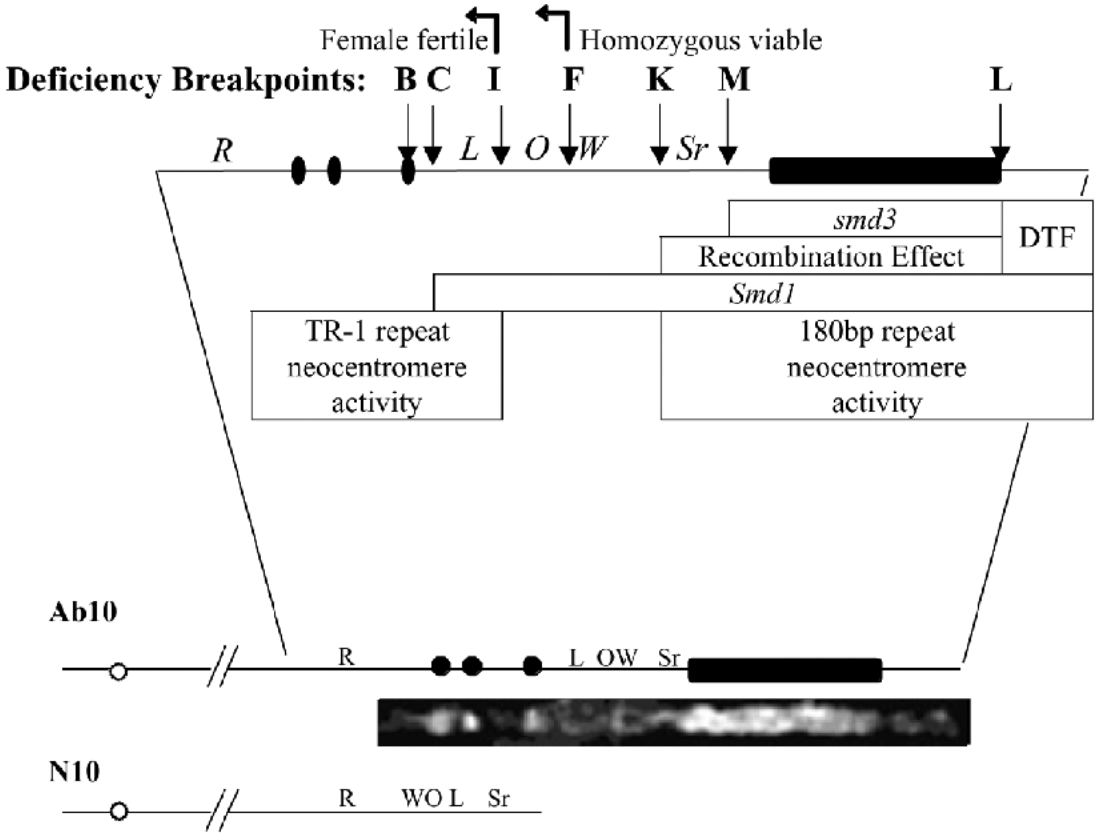

Figure 2. Schematic diagram of abnormal chromosome 10 (Ab10). The lower portion of the figure shows a comparison of N10 to Ab10, along with an actual image of the DAPI stained, computationally straightened long arm Ab10L. The upper portion shows an expanded Ab10 long arm with known loci and deficiency breakpoints indicated. Plants homozygous for Df(F) are feeble but viable; the more severe deletions (Df(I), Df(C), and Df(B)) are male sterile and have reduced female fertility. Boxes indicate the regions of Ab10 to which various Ab10 functions have been mapped. A more detailed description of Ab10 can be found in Hiatt and Dawe (2003a,b).

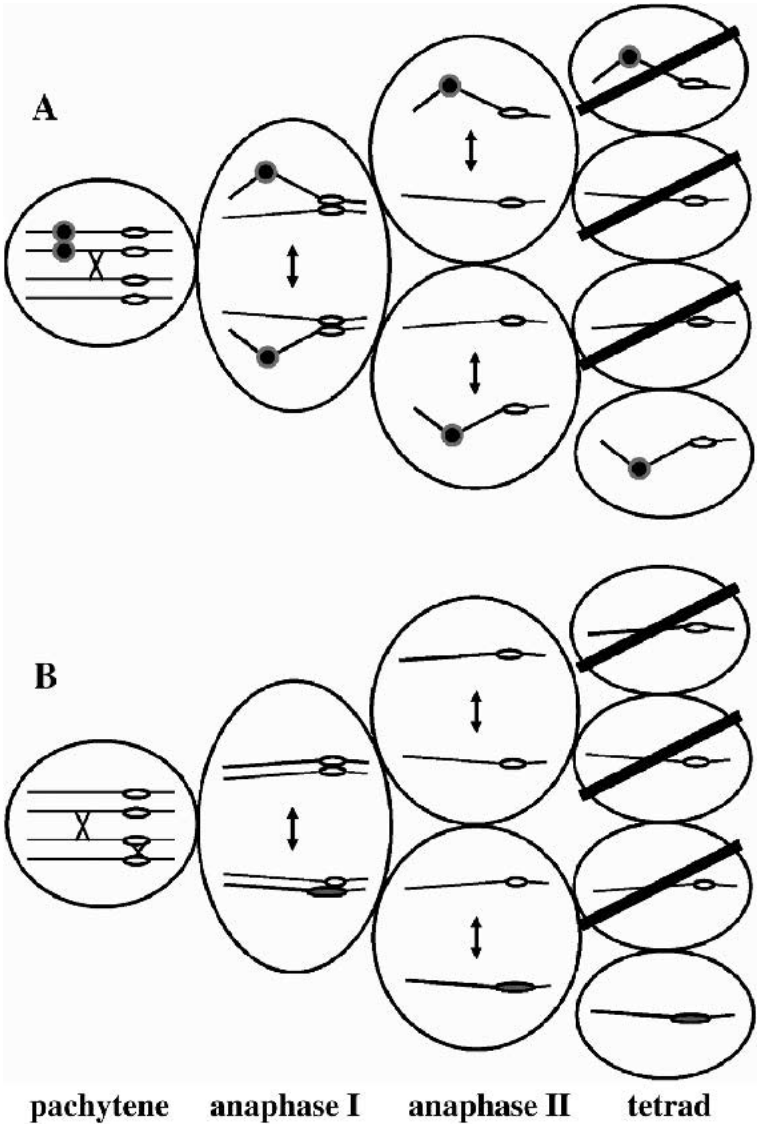

Figure 3. Meiotic drive of knobs and centromeres. (**A**) The Rhoades model for meiotic drive (Rhoades 1952). During pachytene, a crossover must occur between the centromere and a knob (shown in grey) such that each chromosome carries a knobbed chromatid and a non-knobbed chromatid in anaphase I (these are referred to as heteromorphic dyads). Neocentromere activity draws the knobs rapidly poleward, placing them at the outer edges of the telophase I nuclei. Anaphase II again draws the knobs outward, resulting in the preferential recovery of knobs in the basal megaspore. Only the basal cell goes on to become an egg: the upper three products of female meiosis degenerate in most angiosperms. (**B**) An interpretation of the meiotic drive model for centromere evolution (only the first step of the model is shown here, for a full discussion, including the proposed counterselection by CENH3, see the original papers: Henikoff *et al.* 2001, Malik & Henikoff 2002). Unequal sister chromatid exchange during prophase I (Petes & Pukkila 1995) can cause expansions and contractions within centromeric satellite arrays. Larger centromeres (in grey) are likely to attract more microtubules and in principle move faster towards the poles at meiosis I. By virtue of their poleward-most, peripheral positions at prophase II, the larger centromeres might be more likely to arrive in the basal megaspore than the smaller centromeres. Plant megasporogenesis is shown here, but the biology of *Drosophila* female meiosis is very similar.

tromere activity pulls the knobbed homologues toward the outside poles ahead of the centromeres, such that the innermost cells of the tetrad rarely receive a knob. Even though many chromosomes may contain an active centromere as well as an active neocentromere, the expected chromosome bridge and breakage cycle (McClintock 1943) is not observed. Knobs appear to choose a pole very quickly, and the resulting tension swings the linked centromere around to the same pole (Yu *et al.* 1997). The maximum drive expected under the Rhoades model is 83.3% (Buckler *et al.* 1999), which is similar to the highest values observed under field conditions. However we and others have observed significant variation among years, seasons, and genetic backgrounds (from 69–81% meiotic drive) (Hiatt 2000).

Two *suppressor of meiotic drive* mutations (*Smd1* and *smd3*) and 20 deletion derivatives of Ab10 have been isolated and characterized (Figure 2) (Hiatt & Dawe 2003a and references cited therein). A region of Ab10 lying between two deletion derivative breakpoints (Df(K) and Df(L)) is responsible for a marked increase in recombination when Ab10 is present (Rhoades & Dempsey 1966). Since the Rhoades model requires recombination between knobs and centromeres, the recombination effect gene(s) is likely to increase the efficiency of drive. Neocentromere activity maps to two different locations: a region proximal to the Df(I) breakpoint was found to control TR-1-mediated neocentromere activity, and a region distal to the Df(K) breakpoint was found to control 180 bp-mediated neocentromere activity (Hiatt & Dawe 2003b). These data suggest there are two different neocentromere 'cassettes': one promoting TR-1 neocentromeres and the other promoting 180 bp neocentromeres. The *Smd1* mutation causes a reduction in neocentromere activity as well as meiotic drive, providing strong evidence for the Rhoades model (Dawe & Cande 1996). Unfortunately the *smd1* gene has not been cloned or accurately mapped. Other known factors include the SMD3 product and a gene or gene(s) collectively called the Distal Tip Function (DTF; see Figure 2).

All of the known meiotic drive factors act *in trans* to cause meiotic drive at other knobs (Rhoades & Vilkomerson 1942, Longley 1945, Rhoades & Dempsey 1966, Hiatt & Dawe 2003b). The involvement of trans-acting factors provides a strong evolutionary incentive for new knobs to form, as long as they are distant enough from a centromere to ensure a single cross over (Buckler *et al.* 1999). Indeed, an analysis of the frequency, size, and position of the non-Ab10 knobs suggests they arose after the evolution of the drive system to exploit the 'free ride' provided by the trans-acting drive loci (Buckler *et al.* 1999).

These data and general considerations of how meiotic drive systems evolve (Charlesworth & Hartl 1978, Wu & Hammer 1990) suggest the following scenario for the evolution of the maize meiotic drive system and associated neocentromeres. The process seems to have begun with the addition of a novel segment to the end of chromosome 10L, presumably from another grass species by a wide cross (the distal segment of the Ab10 haplotype is not essential for maize growth) (Figure 2) (Hiatt & Dawe 2003b). We see this ancestral event as akin to the wide crosses that activate neocentromeres in other grasses (see below): 'awakening' a suppressed but already existing meiotic drive system. The alien segment as well as other genes on 10L that increased the fidelity of preferential segregation (such as the recombination effect) became tightly linked as structural polymorphisms accumulated in the area. This created a large 45 cM palette from which other modifiers and a new TR-1-based neocentromere system could evolve without being scrambled by recombination (e.g. Charlesworth & Hartl 1978, Haig & Grafen 1991). Over time, knob repeats were seeded to other sites, presumably by some sort of transposition (Ananiev *et al.* 1998a, Buckler *et al.* 1999). When repeats landed in favorable positions, they gradually expanded by unequal recombination until they reached a size sufficient to recruit the trans-acting factors provided by Ab10.

Buckler and colleagues (1999) argued that Ab10 must have strongly influenced the frequency of alleles linked to Ab10 and hundreds of other alleles linked to other knobs; all without regard to their fitness effects. In addition, the Ab10 haplotype itself is expected to accumulate deleterious mutations that cannot be removed by recombination (Charlesworth & Hartl 1978). Under such threats, the organism is expected to fight back in

the form of suppressors of meiotic drive (Charlesworth & Hartl 1978, Wu & Hammer 1990). Suppressors have been identified in populations segregating for other drive systems (Ardlie 1998) and are possibly in *Zea*, since Ab10 has not gone to fixation in any known population of maize or teosinte (Kato 1976). It is also possible that deleterious mutations within the Ab10 haplotype have limited its spread (Ardlie 1998, Buckler *et al.* 1999).

Neocentric activity in rye

Prakken and Müntzing (1942) reported on a 'remarkable meiotic pecularity' in rye (*Secale cereale*) pollen mother cells. Although the initial description of rye neocentromeres came from inbred lines, later research identified neocentromere activity in multiple open pollinated varieties (Prakken & Müntzing 1942, Östergren & Prakken 1946, Kavander & Viinikka 1987, Manzanero & Puertas 2003). Rye neocentromeres are not observed in mitosis but are observed throughout meiosis (Prakken & Müntzing 1942, Viinikka 1985, Manzanero & Puertas 2003). Intercrossing revealed that neocentromeres are genetically controlled (Prakken & Müntzing 1942) but by multiple unlinked loci (Hayward 1962). There appears to be a strong environmental influence on neocentromere activity that makes the genetics difficult to interpret (Östergren & Prakken 1946, Kavander & Viinikka 1987). Rye neocentromeres, like those in maize, are composed of long repeat arrays that appear on chromosomes as deeply staining heterochromatic domains. The blocks of rye heterochromatin are referred to as C bands (for their deep staining pattern). Fluorescent *in situ* hybridization with various repetitive elements revealed that three repeats (pSc34, pSc74 and pSc200) hybridized to the ends of the neocentromeres (Manzanero & Puertas 2003).

The generality of the meiotic drive model for the evolution of subterminal and terminal heterochromatic blocks

The similarities between maize and rye neocentromeres are so striking that it is tempting to speculate that they were derived from a common ancestor. In both species neocentromeres are genetically controlled; in maize by a single long haplotype (Figure 2) and in rye by several apparently unlinked genes (Hayward 1962). In maize the neocentromeres are large heterochromatic knobs composed of two different sequences (Peacock *et al.* 1981, Hiatt *et al.* 2002). Similarly, rye neocentromeres are composed of large heterochromatic C bands composed of three different tandem repeats (Östergren & Prakken 1946, Viinikka 1985, Viinikka & Kavander 1986, Manzanero & Puertas 2003). Acentric fragments containing maize knobs and rye C bands will independently move to a pole as long as trans-acting genetic factors are present, showing that in both species neocentromeres can move poleward independent of a centromere (Jones 1969, Dawe & Cande 1996). Finally, there are undeniable similarities between the cell biology of neocentromere movement in maize and rye; in both species there are interactions between microtubules and neocentromeres (Östergren & Prakken 1946, Yu *et al.* 1997), powerful forces capable of stretching chromosome arms, and diagnostic 'leaders' of repeat arrays that precede the bulk of the knob/C band chromatin (Hiatt *et al.* 2002, Manzanero & Puertas 2003).

Perhaps not surprisingly, since maize and rye span the grass radiation that began some 60 million years ago, the 12 other examples of classical neocentromeres in grasses have many of the same cytological features (Table 1). The fact that most of the information we have is from two subfamilies of the Poacea (Panicoideae and Poiideae) containing the major forage and grain crops suggests a sampling bias. It is reasonable to believe that many other cases of neocentromere activity would be uncovered had there been equal focus on all of the major grass lineages. In addition, clear neocentromeres have been identified in species from *Lilium* (lily) and *Pleurozium* (big red stem moss), suggesting that the classical neocentromeres extend well outside of the grasses. Indeed, Rodionov (1999) described how subterminal and terminal heterochromatic blocks are found throughout the plant and animal kingdoms.

Our view is that the evolution of most if not all heterochromatic blocks are the result of selection

by meiotic drive, but that the neocentromere-promoting genes are usually suppressed by the host due to the associated negative fitness consequences (Hiatt *et al.* 2002). In half of the 14 cases where neocentromere activity has been noted and published, neocentromeres were only observed after an interspecific cross (Table 1). Indeed, Ab10 is probably the result of a fortuitous wide cross that evolved into a relatively stable meiotic system. In two other cases neocentromeres were observed only after an environmental stress, namely extreme heat (Jain 1960) and X-rays (Zohary 1955). Even in maize and rye where neocentromeres can be reliably detected, modifiers of the phenotype (i.e. genetic background effects) are very common (Östergren & Prakken 1946, Kavander & Viinikka 1987, Hiatt 2000). The idea that suppressed phenotypes might be revealed after 'genome shock' was first expressed by McClintock (McClintock 1984) and has since been substantiated many times. For instance, unexpected phenotypes have been uncovered when new genes are introduced from allied species, or when environmental stresses are applied (e.g. Hirochika *et al.* 1996, Kashkush *et al.* 2003, Rieseberg *et al.* 2003). Once a neocentromere system is activated, the same pressures to suppress it will reemerge, putting in motion a recurring cycle of activation, inactivation, and activation of neocentromere-mediated meiotic drive.

Evolutionary similarities between centromeres and neocentromeres

Classical plant neocentromeres are centromeres only in the very broadest definition of a centromere: they move on the spindle. The underlying biology is almost certainly different, with neocentromeres originating from subtle interactions of chromatin within the microtubule-rich environment of the spindle. Interestingly, the clearest tie between centromeres and neocentromeres may be in their mode of evolution. As we have described, maize neocentromeres are thought to accelerate their rates of movement to assure a poleward-most location in the newly formed telophase nuclei, and therefore a place at the base of the linear tetrad. In formulating this model, Rhoades was influenced by several earlier observations in *Drosophila* which has a similar 'one egg takes all' mechanism of female gametogenesis. For instance when a *Drosophila* centromere is slowed down by a chromatin bridge, it is more likely to segregate to an inner position in the tetrad, and as a result is poorly transmitted (Sturtevant & Beadle 1936, Novitski 1967). Similarly, if a chromosome is shorter (by deletion) it will cause less drag in the spindle and as a result lead its sister centromere to the pole during anaphase. In a head-to-head contest between a short and a long chromosome, the shorter one is preferentially segregated (Novitski 1951, Novitski 1967). These and other observations make the case that centromeres/neocentromeres can modulate Mendelian segregation ratios. Further, it is a competitive process: when one centromere wins another must lose.

Chromosome movement polymorphisms are not always as dramatic as the presence or absence of a knob, or a large deletion that reduces chromosome drag. Much more subtle changes in centromere size or structure could have cumulative effects over time. This argument has been used to explain a striking aspect of maize knobs, which is their shear size (Figure 1A) (Buckler *et al.* 1999). Each of these knobs must have begun as single monomers or small arrays, but have since undergone many rounds of amplification. Size polymorphism among knobs at the same locus is well documented (Kato 1976). For instance, while there is only one known knob site on chromosome 7L, the size of the knob can vary from very small to nearly as large as the knob on Ab10, depending on the land race and geographic region. Kikudome (1959) demonstrated that when two different sized knobs are paired and test crossed in an Ab10 background, the larger knob is always preferentially recovered (similar data are available for rye; Manzanero & Puertas 2003). In other words Ab10 makes larger knobs more fit, and molecular events that lengthen knob repeat arrays (e.g. unequal chromatid exchange) (Dimitrov & Georgieva 1994, Petes & Pukkila 1995) will generally provide a competitive advantage to the knob.

Reasoning very similar to what has been used to explain knob evolution has been cited in a recent theory for centromere evolution (Henikoff

et al. 2001). Centromeres have long presented a paradox (Bloom 1993): despite their critical importance in cell division they have a structure that resembles 'junk' DNA, vary in size by several orders of magnitude across species, and evolve faster than any other domain of the chromosome. In the first serious effort to explain this paradox, Henikoff and colleagues (Henikoff *et al.* 2001, Malik & Henikoff 2002) suggested that centromere evolution may be an outcome of a genomic conflict involving meiotic drive. As described by the authors, the first event of the conflict occurs when a centromere increases in size (presumably by unequal sister chromatid exchange) (Figure 3B), which in turn increases its capacity to bind microtubules and its chances of being recovered in progeny. This process would lead to larger and larger centromeres, as is the case for knobs in maize. However, uncontrolled centromere expansion will ultimately come in conflict with organismal fitness, as runaway increases in centromere size cause gross imbalances in Mendelian segregation. To counteract these negative effects, the authors speculate the CENH3 functions as a moderator of sorts, binding to newly generated but rare repeats to restore equality among chromosomes. Unequal recombination, gene conversion and CENH3-mediated selection could rapidly drive new monomers through an array. The fact that *Drosophila* and *Arabidopsis* CENH3s show evidence of adaptive evolution is consistent with this hypothesis (Malik & Henikoff 2001, Talbert *et al.* 2002). The tug of war between the selfish centromeres striving to acquire as many microtubules as possible and CENH3 striving to restore equality among centromeres has been compared to an arms race (Henikoff *et al.* 2001). The outcome, as in other genetic arms races (e.g. host–parasite interactions), would be rapid sequence change (McInerney *et al.* 2003). This argument mirrors other theoretical considerations of meiotic drive (Charlesworth & Hartl 1978) except that it is focused on repeat arrays and their characteristically rapid rates of evolution.

The competition-based 'centromere drive' model also provides a compelling explanation for the evolution of new neocentromere repeats. For instance, we can use the logic of Malik and Henikoff (2002) to explain the evolution of the relatively young TR-1 repeat. The 180 bp repeat is found in *Zea* as well as the close relative *Tripsacum* (Dennis & Peacock 1984, Hsu *et al.* 2003) whereas TR-1 is found only in *Zea* (Ananiev *et al.* 1998a, Hsu *et al.* 2003). A likely explanation for the recent appearance of TR-1 is that it evolved to evade suppression of the 180-bp-mediated meiotic drive system. There is significant sequence homology between TR-1 and the 180 bp repeat (Hsu *et al.* 2003) suggesting that one was derived from the other, and strong genetic evidence for a novel neocentromere activation cassette specific for the TR-1 repeat (Hiatt *et al.* 2002). Further, at least at the cytological level, TR1 appears to be a more effective neocentromere-promoting sequence than the 180 bp repeat (in cultivated maize; Figure 1B). A fortuitous event in the context of a genomic conflict may have given rise to a new neocentromere system, and spawned hundreds of thousands of new TR-1 repeats; all within the short 4.5–4.8 million year time frame that separates *Tripsacum* from maize (Hilton & Gaut 1998). The three major neocentromeric repeats in rye (Manzanero & Puertas 2003) may have had a similar origin.

Recent efforts to integrate meiotic drive with theories of neocentromere and centromere evolution (Malik & Henikoff 2002) required an understanding of evolutionary concepts, cell biology, and genomics. It is clear that the interface of these three disciplines is a particularly fertile area for neocentromere/centromere research. A consideration of all forms and permutations of centromeres, and input from researchers with diverse expertise will be necessary if we are ever to fully understand the 'dark matter' (Schindelhauer & Schwarz 2002) that makes up centromeric DNA.

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