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Centromere drive: model systems and experimental progress

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Abstract

Centromeres connect chromosomes and spindle microtubules to ensure faithful chromosome segregation. Paradoxically, despite this conserved function, centromeric DNA evolves rapidly and centromeric proteins show signatures of positive selection. The centromere drive hypothesis proposes that centromeric DNA can act like a selfish genetic element and drive non-Mendelian segregation during asymmetric female meiosis. Resulting fitness costs lead to genetic conflict with the rest of the genome and impose a selective pressure for centromeric proteins to adapt by suppressing the costs. Here, we describe experimental model systems for centromere drive in yellow monkyflowers and mice, summarize key findings demonstrating centromere drive, and explain molecular mechanisms. We further discuss efforts to test if centromeric proteins are involved in suppressing drive-associated fitness costs, highlight a model for centromere drive and suppression in mice, and put forth outstanding questions for future research.

Keywords

meiosis; non-Mendelian chromosome segregation; centromere; centromere drive; molecular evolution; positive selection

Centromeres – a battleground between selfish DNA and the rest of the genome

Female meiosis is an asymmetric division where pairs of homologous chromosomes form bivalents and segregate either into a single future gamete (egg) or die in a polar body (Fig. 1a). According to Mendel's Law of Segregation, each homologous chromosome in diploid genomes has an equal chance to segregate into the egg. However, across phylogenies, selfish genetic loci find ways to increase their odds of inheritance during female meiosis in a process called "meiotic drive", leading to a transmission ratio distortion (for a comprehensive overview of meiotic drive systems see (Clark and Akera 2021; Kruger and Mueller 2021). Other selfish loci can drive in male meiosis, by different mechanisms beyond

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the scope of this review. A common denominator for meiotic drive systems is a fitness cost to individuals, often by decreasing fertility (Zanders and Unckless 2019). In response, the rest of the genome evolves rescue mechanisms (meiotic drive suppressors) that reduce the fitness cost. Thus, selfish genetic elements are in a conflict with the rest of the genome and can cheat in numerous ways. One is to hijack the mechanisms regulating chromosome attachment to the meiotic spindle (Fig. 1b).

The centromere is a part of the chromosome specialized in connecting it to the mitotic and meiotic spindle microtubules. Centromeres are typically composed of repetitive satellite DNA, which poses a challenge for replication forks and may be subject to uneven meiotic recombination, leading to rapid centromere DNA evolution observed as sequence divergence and satellite expansion (reviewed in (Thakur et al. 2021). Indeed, centromeric regions are extremely variable: from simple “point” centromeres in yeast, and more complex “regional” centromeres in mammals and plants, to holocentromeres spanning entire chromosomes in some insects and worms (Balzano and Giunta 2020). Moreover, there are several examples of centromere chromatin forming on transposable elements (Chang et al. 2019; Fang et al. 2020; Hartley et al. 2021). Centromeric DNA is typically packaged by a specific histone H3 variant, CENP-A or CenH3. This epigenetic mark triggers the formation of a kinetochore, a multi-protein complex that directly binds the spindle microtubules (Kixmoeller et al. 2020). Despite their ancient role in chromosome segregation, many centromeric proteins evolve rapidly, under positive selection across taxa with female meiosis (Henikoff et al. 2001; Talbert et al. 2002; Talbert et al. 2004; Schueler et al. 2010; Finseth et al. 2015; Kumon et al. 2021). Since centromeres are ubiquitously used for faithful chromosome segregation, and the core function of connecting to spindle microtubules is conserved, rapid evolution of the centromeric DNA and the centromeric proteins is paradoxical.

The centromere drive hypothesis aims to explain this paradox by making two predictions. First, rapidly expanding centromeres act like selfish genetic elements, achieving non-Mendelian segregation by hijacking the regulation of kinetochore-microtubule attachments (centromere drive). Second, centromere drive-associated fitness costs impose a selective pressure on centromeric proteins to restore fitness by evolving suppression mechanisms. Since centromeric DNA constantly changes, the rest of the genome is under recurrent pressure in an ongoing genetic conflict leading to divergence. Therefore, centromere drive might also contribute to hybrid incompatibilities between divergent parental centromere proteins or between centromere proteins and centromere DNA, resulting in genetic isolation and speciation (Henikoff et al. 2001).

Currently there are two experimental model systems that have been used to test aspects of the centromere drive hypothesis. In this review, we describe these models and discuss the key findings that collectively demonstrate that centromeres can drive in female meiosis. We also discuss the challenges of testing if recurrent evolution of centromeric proteins is adaptive. Finally, we outline future directions for centromere drive research, highlighting the need for an interdisciplinary approach.

Experimental models of centromere drive

Yellow monkeyflowers pave the way for centromere drive research

Centromere drive is difficult to catch in the act. Meiotic drivers are predicted to either fix quickly in the population, promote selection for suppressors, or lead to extinction of driver-carrying populations if fitness costs are too great (Sandler and Novitski 1957). In order to violate Mendelian segregation, drivers rely on the heterozygosity of their locus and the naiveté of the genomic suppression loci. Therefore, fixed drivers will be missed unless introduced into a closely related naïve population – forming hybrids (Hurst and Werren 2001). Indeed, the first observation that expanded centromeric satellites can drive in female meiosis came from crossing two closely related species of yellow monkeyflowers. Genetic linkage mapping identified a "distorter locus" (D) in *Mimulus guttatus*, which showed a whopping 98:2 segregation bias in an interspecific *M. guttatus/M. nasutus* (D/d) hybrid (Fishman et al. 2001; Fishman and Willis 2005). Cytological analysis further showed that the D locus on chromosome 11 carries an expansion of the centromere-associated Cent278 satellite, demonstrating that D is linked to a centromere (Fishman and Saunders 2008). Consistent with the centromere drive hypothesis, the D locus imposes a fitness cost, with reduced seed (female) and pollen (male) production in plants homozygous for D (Fishman and Saunders 2008; Fishman and Kelly 2015).

Interestingly, the D locus drives much more weakly against a homologous chromosome with a non-driving "D^{-}" locus in a conspecific cross between two *M. guttatus* populations (58:42 in D/D^{-}; 98:2 in D/d; (Fishman and Saunders 2008); Fig. 2b). Weaker drive in the native genetic background suggested the presence of unlinked suppressors. Most recently, an elegant experiment compared drive strength of the D locus introduced into a genomic background naïve (*M. nasutus*) or native (*M. guttatus*) to D, showing stronger drive in the naïve genetic background (73:27 vs 58:42; (Finseth et al. 2021); Fig. 2c). Quantitative locus analysis revealed a potential unlinked drive modifier on chromosome 14 – home to the centromeric histone CenH3 gene, originally proposed as a drive suppressor (Henikoff et al. 2001). Consistently, molecular evolution and population genomics analyses show that CenH3 duplicated in monkeyflowers and the CenH3A paralog evolves rapidly under positive selection (Finseth et al. 2015; Finseth et al. 2021). Future work may reveal if different CenH3A alleles can indeed modify drive strength in *guttatus*.

As a model system for centromere drive research, monkeyflowers leverage variation of natural populations, powerful population genetics, and scalable breeding techniques. Indeed, work on the D driver provided the first evidence that expanded centromeres act as selfish genetic elements, impose a fitness cost, and might drive evolution of unlinked loci. One important question going forward is how common are driving centromeres? The D driver may be a rare example where the driving centromere, the fitness cost, and the unlinked suppressor loci operate at the same time, maintaining D without fixation.

Moreover, D appeared in the population very recently (1500 years ago; (Finseth et al. 2021), and in the future the suppressive loci may reduce the reproductive cost associated with D or the drive itself. D is expected to fix in the population if suppressive loci reduce the fitness cost without suppressing drive. Another outstanding question is how does D drive? The D

locus constitutes a large area of reduced recombination, which encompasses over 300 genes (Finseth et al. 2021); Fig. 2a). It is plausible that D-linked genes encode drivers abetting the responder (centromere) to violate Mendelian segregation. Examples of such drive modifiers are the kinesin driver (Kindr) and TR-1 kinesin (Trkin) motor proteins encoded by loci linked to the driving non-centromeric knob on the Ab10 chromosome in maize (Dawe et al. 2018; Swentowsky et al. 2020). Genome editing in monkeyflowers (Ding et al. 2020) might allow disruption of D-linked genes to address this question. However, understanding detailed mechanisms of centromere drive and suppression will likely require in-depth cell biology research. While a “shooting” model for evo-devo and plant genetics (Yuan 2019), monkeyflower cell biology research is yet to sprout. The exciting work on the D driver may motivate future efforts to study the cell biology of centromere drive.

House mice pioneer centromere drive cell biology research

House mouse (*Mus musculus; Murinae*) is one of the most experimentally tractable model systems for mammalian biology. Early chromosome painting techniques revealed dramatic karyotype rearrangements and chromosome number variation in *Murinae* (reviewed in (Romanenko et al. 2012), suggesting dynamic karyotype evolution. Chromosomal plasticity in *M. musculus* was further evidenced by a striking observation that natural populations isolated for less than 1000 years can carry different chromosome numbers (Britton-Davidian et al. 2000) due to fixation of Robertsonian translocations, which are a common cause of karyotype evolution. These translocations form when the centromeric regions of two telo/acrocentric chromosomes (centromere close to the telomere) fuse, forming a single metacentric chromosome (centromere in the middle) (reviewed in (Garagna et al. 2014). Although widespread in western *M. musculus* populations (Piálek et al. 2005), Robertsonian fusions are associated with reduced fertility (Garagna et al. 2014). Indeed, in heterozygotes for Robertsonian fusions, homologous chromosomes form meiotic trivalents (instead of bivalents), which likely lead to chromosome segregation errors. Despite this initial fitness cost, Robertsonian fusions might fix in a population if they drive against their telocentric homologs in female meiosis (Pardo-Manuel de Villena and Sapienza 2001). Why would fusions fix in some mouse populations while others remain telocentric (Piálek et al. 2005)? A mechanistic explanation for this phenomenon required a cytological approach, taking advantage of natural variation in mouse karyotypes.

In one case a Robertsonian fusion, Rb(6.16), was preferentially excluded from the egg when heterozygous, and immuno-staining of CENP-A and the kinetochore protein Hec1 showed bigger kinetochores on the homologous telocentric chromosomes compared to the fusion. In contrast, in a strain (CHPO) derived from a natural population that has fixed multiple Robertsonian fusions, kinetochores are bigger on the fusions compared to the non-homologous telocentrics. Further experiments showed that differences in kinetochore size yield a functional asymmetry in female meiosis I, with bivalents positioned off center on the spindle when kinetochore size differs between homologous chromosomes. Overall, these observations suggest that newly formed metacentrics would drive and fix in natural populations if they form larger kinetochores than the homologous telocentrics. Indeed, in predominantly metacentric wild populations, the remaining telocentrics have smaller

kinetochores than the metacentrics in the same cell, suggesting that metacentrics fixed by forming larger kinetochores (Chmatal et al. 2014; Chmatal et al. 2017).

The work with Robertsonian fusions provided the first direct evidence supporting the idea that recruiting more centromeric proteins and forming a bigger kinetochore can drive biased segregation in female meiosis, consistent with the centromere drive hypothesis. Further sequencing and fluorescence in situ hybridization analyses revealed low abundance of minor satellite DNA, the dominant site of CENP-A nucleosome assembly in mouse, at CHPO centromeres compared to standard lab strains. In hybrids between CHPO and these other strains, the CHPO centromeres have less CENP-A, suggesting that expansion of minor satellite DNA promotes formation of more centromere chromatin. Moreover, larger centromeres preferentially orient toward the future egg cell when paired with smaller centromeres from CHPO in bivalents in hybrid oocytes. Therefore, a difference in the amount of minor satellite DNA can bias chromosome orientation on the spindle in meiosis I (Iwata-Otsubo et al. 2017); Fig. 3a and 3b).

As a clue to how larger centromeres preferentially orient on the spindle to bias transmission into the egg, cytological observations showed that female metaphase I spindles in *M. musculus* are asymmetric, with the cortical half-spindles enriched in tubulin tyrosination (Akera et al. 2017). Chromosomes are positioned near the cortex so that the cell division is ultimately asymmetric, producing a large egg and small polar body. As part of this process, cortex polarization is directed by a Ras-related nuclear protein (RAN-GTP) signal produced by chromatin, leading to cell division cycle 42 (CDC42) enrichment on the cortex near the chromosomes (reviewed in (Verlhac and Dumont 2008). CDC42 signaling regulates tubulin tyrosination, through mechanisms that are still unclear, to generate the observed spindle asymmetry. Therefore, the tyrosinated half-spindle always faces the cortex, where CDC42 is enriched, and the future polar body. Furthermore, larger centromeres in CHPO hybrid bivalents detach from spindle microtubules more often when facing the tyrosinated cortical half-spindle, allowing re-orientation toward the future egg (Akera et al. 2017; Akera et al. 2019) Fig. 4a). Asymmetric tyrosination is crucial for biased orientation, as abolishing asymmetry also abolished the bias (Akera et al. 2017). In a parallel study, another type of spindle asymmetry was observed, with higher density of microtubules in the cortical half-spindle in the conspecific *M. musculus* hybrid C57BL6/SJL (Wu et al. 2018). Like in the CHPO hybrid, centromeres with more minor satellite DNA biased their orientation toward the future egg by preferentially detaching from the cortical half-spindle (Fig. 4b). Together these studies show that functional spindle asymmetries allow centromeres with more minor satellite DNA to bias their transmission to the egg in female meiosis I.

The remaining unknown was how larger centromeres with more minor satellite DNA preferentially detach and re-orient on the spindle. To correct erroneous kinetochore-microtubule attachments in any cell division, kinetochores detach due to microtubule destabilizing proteins recruited to centromeres (Lampson and Grishchuk 2017). One mechanism to recruit destabilizers is through kinetochore-bound budding uninhibited by imidazole (BUB1) kinase, which phosphorylates histone H2A to form a docking site for shugoshin-2 (SGO2), which brings a microtubule destabilizing kinesin-13, mitotic centromere-associated kinesin (MCAK), to the centromere. SGO2 also brings the Aurora

B kinase to centromeres, which destabilizes the kinetochore-microtubule attachments by phosphorylating kinetochore substrates (Hindriksen et al. 2017). Larger centromeres in the CHPO hybrid build larger kinetochores and recruit more of the same microtubule destabilizers used for error correction (Fig. 4b). Equalizing the destabilizers by tethering BUB1 kinase to major satellite DNA, which is equal across the hybrid bivalents, abolished the bias (Akera et al. 2019). Similarly, inhibition of Aurora B kinase prevented biased orientation in the C57BL6/SJL hybrid (Wu et al. 2018). Unequal destabilizers are also present across the bivalents of an inter-specific hybrid between *M. musculus* and *M. spretus*. In this case *M. spretus* centromeres have more minor satellite DNA and more microtubule destabilizing proteins and bias their orientation toward the future egg. Kinetochores are equal across these hybrid bivalents, but *M. spretus* centromeric chromatin is more condensed, suggesting greater accessibility of BUB1 kinase to pericentromeric histone substrates to recruit microtubule destabilizers (Akera et al. 2019). Selfish centromeric DNA can therefore achieve biased segregation by recruiting more microtubule destabilizers in various ways. As a potential link to spindle asymmetry, MCAK is more active on tyrosinated microtubules (Peris et al. 2009; Sirajuddin et al. 2014), which might facilitate the preferential detachment of selfish centromeres from the tyrosinated cortical half-spindle. Overall, these findings indicate that selfish centromeres cheat by hijacking the essential error-correction machinery and exploiting intrinsic meiotic spindle asymmetries (Fig. 4b).

The work on different mouse hybrids suggests a variable “cheating window” during meiosis I. Mouse oocytes take at least 8 hours for spindle formation and migration toward the cortex, and silencing of the spindle assembly checkpoint before anaphase segregation. In the C57BL6/SJL hybrid, the spindle assembles asymmetrically early in the process and migrates directionally with the denser half-spindle facing the cortex (Wu et al. 2018). This process allows for a long cheating window, as selfish centromeres can bias their orientation at any point before anaphase (Fig. 4c). In contrast, CHPO hybrid oocytes initially assemble symmetric spindles, which become tyrosinated asymmetrically only after migration to the cortex. Therefore, the “cheating window” shortens, and selfish centromeres can bias their orientation only after spindle migration. However, some of the homologous chromosomes in the CHPO hybrid make trivalents, which might trigger the spindle assembly checkpoint and allow extra time for re-orientation before anaphase (Fig. 4c). Therefore, shortening the time between spindle migration and anaphase onset could be a mechanism to shrink the “cheating window” to prevent drive. Indeed, *M. musculus/M. spretus* hybrid oocytes divide faster than the CHPO hybrid, which does not allow time for biased orientation unless anaphase onset is delayed (Akera et al. 2019); Fig. 4c). Altogether, these studies established functional asymmetries within both the meiotic spindle and the hybrid bivalents, provided a cell biological framework for understanding mechanisms of centromere drive.

Mechanisms to suppress costs of centromere drive

The centromere drive hypothesis predicts that fitness cost associated with driving centromeres would elicit a genomic response by selecting for suppression mechanisms. Despite significant progress in understanding the genetics and cell biology of centromere drive, we have only just started conceptualizing what the suppression mechanisms might be. A fitness cost associated with centromere drive in monkeyflower is the reduction in pollen

and seeds in plants homozygous for the D driver (Fishman and Saunders 2008; Fishman and Kelly 2015). While the mechanism underlying this cost is unclear, a potential drive suppressor was mapped to a locus encoding the CenH3 protein (Finseth et al. 2015; Finseth et al. 2021). In mice, costs of centromere drive have not yet been reported, but a likely cost is unequal interactions of homologous centromeres with the spindle due to asymmetry in microtubule destabilizers, which might interfere with chromosome segregation mechanisms (Akera et al. 2019). Equalizing microtubule-destabilizing factors recruited by divergent centromeres would abolish this cost (Akera et al. 2019; Kumon et al. 2021). Therefore, both drive models point at centromeric proteins as the likeliest suppressors. In this section, we discuss efforts to experimentally test the second prediction of the centromere drive hypothesis: adaptive evolution of centromeric proteins in response to costly expansion of selfish centromere DNA.

Molecular evolution analyses suggest adaptation of centromeric proteins

How can we tell that a protein might be evolving adaptively? Purifying selection eliminates most mutations in protein-coding sequences to preserve function, and accumulating mutations might reflect either genetic drift or adaptation (Pal et al. 2006). To detect mutations reflective of adaptation, current molecular evolution methods test for recurrent changes in the same codon in orthologous sequences. Multiple sequence alignments from closely related species are used to capture putatively beneficial non-synonymous substitutions that fix fast under positive selection, while reducing noise from neutral changes (van der Lee et al. 2017). Additionally, comparing the ratio of non-synonymous to synonymous substitutions between and within species can help distinguish beneficial fixed mutations from non-adaptive polymorphisms (McDonald and Kreitman 1991; Booker et al. 2017).

Signatures of adaptive evolution were first reported in Cid (centromere identifier/*Drosophila* CENP-A orthologue; (Malik and Henikoff 2001; Malik et al. 2002)), and subsequently in CENP-C, a key centromeric scaffold protein and CENP-A binding partner (Talbert et al. 2004). More recent molecular evolution analyses benefit from the increasing number of available genomes, powerful molecular evolution models, and online resources for rigorous testing of adaptive protein evolution (e.g. Hypothesis Testing using Phylogenies/HyPhy package (Pond et al. 2005); Phylogenetic Analysis by Maximum Likelihood/PAML package (Yang 2007)). Indeed, signatures of positive selection have been detected in CENP-A or CENP-C in primates (Schueler et al. 2010), rodents (Kumon et al. 2021), and plants (Talbert et al. 2002; Talbert et al. 2004; Ravi et al. 2010; Finseth et al. 2015; Zedek and Bures 2016; Kratka et al. 2021). The CENP-A orthologue does not evolve adaptively in yeast, however, which lack the meiotic asymmetry that provides the opportunity to drive (Talbert et al. 2004; Baker and Rogers 2006). CENP-A also does not evolve adaptively in *Tetrahymena*, which has asymmetric meiosis typical for females but lacks symmetric “male” meiosis, suggesting that drive costs might be associated with male meiosis (Elde et al. 2011).

Genome sequencing and molecular evolution analyses in *Murinae* identified signatures of positive selection in multiple centromeric proteins, including those close to the centromeric DNA (centromere proteins/CENPs: CENP-C, CENP-I and CENP-T), kinetochore proteins

more distant from the DNA (KNL1, DSN1, NDC80), scaffold proteins involved in recruiting microtubule destabilizers (SGO2, inner centromere protein/INCENP), and components of the centromere chromatin assembly pathway (MIS18 binding protein 1/MIS18BP1 and the chaperone, Holliday junction recognizing protein/HJURP; (Kumon et al. 2021). Another survey reported that mouse and human kinetochore proteins evolve on average 4 times faster than conserved spindle assembly checkpoint proteins (van Hooff et al. 2017). These analyses suggest that both protein-DNA and protein-protein interactions at centromeres are under recurrent selective pressure and might be involved in suppressing costs associated with centromere drive.

Molecular evolution analyses gave rise to the centromere drive hypothesis and have since guided models for centromere drive suppression mechanisms. *M. guttatus* lines carrying the centromere-associated D driver show elevated linkage disequilibrium and low nucleotide diversity around the CenH3A locus, which is typical for a selective sweep and suggests CenH3A adaptation. This *in silico* analysis supports co-evolution of the CenH3A locus with the expanded D centromere because the sweep occurred around the time D appeared in wild *M. guttatus* populations (Finseth et al. 2021). Centromeric proteins govern chromosome segregation in every dividing cell, however, and some also regulate post-mitotic processes (e.g. KNL1 in neurons; (Cheerambathur et al. 2019; Zhao et al. 2019), so the selective pressures shaping their evolution might have nothing to do with drive in female meiosis. Moreover, molecular evolution analyses might yield false positives, for example due to errors in multiple sequence alignment (Fletcher and Yang 2010). Therefore, developing experimental models and frameworks to test the phenotypic output of protein divergence remains an important challenge.

Cell biology offers a glance into centromere drive suppression mechanisms

As a conceptually simple approach to determine the phenotypic consequences of rapid evolution, an orthologous version of a protein can be expressed in a tractable model system. Taking the well-documented divergence of *Drosophila* Cid as an example (Malik et al. 2002), *D. bipectinata* Cid does not localize to centromeres when expressed in *D. melanogaster* tissue culture cells. Localization is rescued by replacing the rapidly evolving L1 loop (part of the histone fold domain) of *bipectinata* Cid with the equivalent region of *melanogaster* Cid, suggesting tight co-evolution of *melanogaster* Cid with its own centromere (Vermaak et al. 2002). Furthermore, chimeric *D. melanogaster* Cid with the *D. bipectinata* L1 loop localizes to *D. melanogaster or D. simulans* centromeres if co-expressed with the *bipectinata* Cid chaperone (chromosome alignment defect 1/Cal1). Therefore, rapid evolution of Cid likely regulates the interaction with its chaperone. In addition, ectopic localization of the divergent Cid partially supported the recruitment of the kinetochore protein Ndc80 (Rosin and Mellone 2016), suggesting that divergent evolution of Cid impacts its interaction with the centromeric chromatin rather than its capacity to build a kinetochore.

Parallel experiments in plants showed that CenH3 (Cenp-A orthologue) from *Zea mays* can functionally substitute for CenH3 in the distant *Arabidopsis thaliana* (Maheshwari et al. 2015). Therefore, evolutionarily distant CenH3 variants can build functional kinetochores on divergent centromeric repeats (Maheshwari et al. 2017). Moreover, in a cross between

wild type *A. thaliana* and *A. thaliana* expressing *Z. mays* CenH3, partial or complete loss of the chromosomes with *Z. mays* CenH3 is observed in the hybrid embryo. These findings suggest that divergent CenH3 variants may compete for binding to *A. thaliana* interacting partners (such the CenH3 chaperone) necessary for epigenetic propagation of centromere chromatin, leading to loss of centromeres marked with the divergent CenH3. Together, these orthologue-swapping studies indicate that centromeric proteins are not simply co-evolving with the underlying DNA, and selection favors changes that modulate protein-protein interactions, which might drive the evolution of centromeric proteins that do not bind the centromeric DNA at all. An example is the widespread rapid evolution of the essential kinetochore protein KNL1 (Tromer et al. 2015), suggesting that any protein regulating kinetochore-microtubule attachments could contribute to suppression of drive-associated fitness costs. Indeed codons under positive selection are found in rodent KNL1 motifs regulating kinetochore-microtubule attachments (Fig. 5, (Kumon et al. 2021).

As an important mechanism for regulating kinetochore-microtubule attachments, microtubule destabilizers are recruited via the kinetochore (reviewed in (Marston 2015; Kitajima 2018; Funabiki 2019) and via the pericentromeric heterochromatin (reviewed in (Marston 2015; Higgins and Prendergast 2016). In the hybrid mouse models, selfish centromeres drive by recruiting more destabilizers via the kinetochore pathway, which is asymmetric between the paired homologous chromosomes within a meiotic bivalent, while pericentromeric heterochromatin is symmetric and therefore expected to equalize the paired centromeres (Chmatal et al. 2014; Iwata-Otsubo et al. 2017; Kumon et al. 2021). In our parallel pathway model, drive therefore depends on the relative contributions of the two pathways. One model prediction is that weakening the asymmetric kinetochore pathway would make the symmetric heterochromatin pathway more dominant, so that paired centromeres become functionally more similar. As an experimental manipulation to test this prediction, mouse CENP-C and a divergent variant from rat localize similarly to centromeres when overexpressed in mouse oocytes, but rat CENP-C weakens the kinetochore pathway based on reduced recruitment of the SGO2 component (Kumon et al. 2021). This result suggests that CENP-C has co-evolved with protein-interacting partners in the kinetochore pathway, such that a divergent allele partially disrupts these interactions. Furthermore, bivalents in CHPO hybrid oocytes expressing rat CENP-C are positioned less off-center on the spindle, a hallmark of more equal microtubule destabilizing activity and force generation across the bivalent, consistent with the prediction. Another experimental manipulation, knockout of the CENP-B protein, has the opposite effect, with hybrid bivalents positioned more off-center. CENP-B binds to minor satellite DNA (Masumoto et al. 1989) and contributes to the kinetochore pathway via CENP-C recruitment (Fachinetti et al. 2015) and also to formation of pericentromere heterochromatin (Okada et al. 2007; Otake et al. 2020). The bivalent position assay indicates that the dominant effect of CENP-B knockout is to weaken the heterochromatin pathway, however, as paired centromeres become functionally more different (Kumon et al. 2021). Overall, this work established a comprehensive model for centromere drive and suppression via two parallel pathways for recruiting microtubule destabilizers to regulate kinetochore-microtubule attachments in mouse oocytes (Fig. 6). According to this model, fitness costs associated with functional differences between centromeres can be suppressed by weakening the asymmetric kinetochore pathway

relative to the symmetric heterochromatin pathway, while maintaining essential centromere functions. Changes in multiple different proteins could have such effects, consistent with widespread signatures of positive selection in centromere proteins.

An important next step will be to ask if phenotypic changes observed by expressing divergent proteins are due to regions under positive selection. In contrast to mutations of conserved sites under purifying selection, which are expected to have strong phenotypes, mutations in recurrently changing sites are expected to yield mild phenotypes that do not strongly impair essential functions. A key goal is to test the role of positive selection in suppressing fitness costs associated with centromere drive. While the cost in mice remains to be determined, functional differences between centromeres are likely involved. Therefore, established assays measuring bivalent position and orientation on the spindle will likely continue to prove valuable.

Outstanding questions and future directions

Two tractable centromere drive model systems have been established since the centromere drive hypothesis was proposed over 20 years ago. While they provided evidence supporting the hypothesis, it remains unknown how widespread is the centromere drive phenomenon. Furthermore, we have only taken initial steps in testing the key prediction that rapid evolution of centromeric proteins plays a role in suppressing fitness costs (Finseth et al. 2021; Kumon et al. 2021). In this section we highlight three outstanding questions that may guide future experimental directions.

Can we find more centromere drive model systems?

Centromeric proteins evolve under positive selection in many taxa with asymmetric female meiosis, suggesting that centromere drive is ubiquitous. The details of asymmetric female meiosis differ between species, and so might the drive mechanisms. Leveraging well-established experimental model systems may lead to exciting discoveries, deepening our understanding of the centromere drive phenomenon. Taking *Drosophila* as an example, centromeric sequences change during karyotype evolution (Bracewell et al. 2019), and Cid-positive chromatin varies in size between different chromosomes, suggesting a dynamic range of centromere sizes (Chang et al. 2019). Furthermore, a recent survey in *Drosophila* detected a potential centromere-associated driver with a predicted transmission ratio distortion of 54:46 via female meiosis (Wei et al. 2017). Female meiosis in flies (reviewed in (Hughes et al. 2018) is asymmetric but otherwise has important differences from mice, so selfish centromeres likely cheat by different mechanisms. New centromere drive model systems will be paramount to establish that centromere drive is indeed ubiquitous and to reveal the diversity of drive mechanisms.

Who pays the price for centromere drive?

Fitness costs associated with centromere drive may vary with the biology of the organism. Monkeyflowers' D locus is associated with a reproductive cost in homozygous males and females (Fishman and Saunders 2008; Fishman and Kelly 2015). While the underlying mechanism is not yet clear, one possibility is that D is associated with deleterious, dose-

dependent alleles that become harmful only in homozygotes. Indeed there are many alleles associated with D (Finseth et al. 2021), some of which might impact the reproductive machinery in homozygotes. On the other hand, the fitness cost is unknown in mice. Based on the cell biology, heterozygotes might suffer from chromosome mis-segregation in the germ line because hybrid bivalents with unequal centromeres fail to align at the metaphase plate. There is also evidence that the mammalian chromosome segregation machinery struggles with big kinetochores due to increased microtubule attachment errors (Drpic et al. 2018). Thus, centromere expansion could lead to increased aneuploidy resulting from somatic cell divisions. Finally, in any inter-species hybrid model system, genetic variation of centromere-unlinked loci might contribute to fitness costs, complicating the interpretation of observed phenotypes. Therefore, introgressing centromeres of different sizes into an isogenic strain may help decipher the who, when, and how of the price of centromere drive.

Does recurrent evolution of centromeric proteins impact drive?

Existing experimental evidence supports the idea that divergence of centromeric proteins has functional consequences, based on ectopic expression of divergent variants or chimeras (Vermaak et al. 2002; Rosin and Mellone 2016; Kumon et al. 2021). However, positively selected residues identified in molecular evolution analyses have not been specifically tested. Established gene editing methods make it possible to swap recurrently changing regions or codons in the endogenous locus and ask where (germ line or soma) and when (embryo or adult) phenotypic changes occur. Finally, the impact on biased chromosome segregation can be tested by introgression of mutated alleles into hybrids with unequal centromeres. Unlike classical “ON/OFF” cell biology experiments, in which mutations are designed to have strong effects (such as complete loss of an enzymatic activity or a post-translational modification site), mutations guided by natural variation are likely to elicit more incremental phenotypic changes. Although likely impactful at evolutionary time scales, more nuanced phenotypes might be difficult to detect experimentally or easy to overlook. For instance, adaptive changes in centromeric proteins may have modest effects on regulation of microtubule attachments while preserving error-correction mechanisms. Another consideration is that recurrent changes do not occur in a void and are likely permitted or restricted by epistatic interactions elsewhere in the protein (reviewed in (Domingo et al. 2019). One approach to probe the evolutionary landscapes is to examine recurrently changing residues or regions in the context of protein structure predictions at the level of individual proteins (Baek et al. 2021; Jumper et al. 2021) or larger complexes (Humphreys et al. 2021). Studying centromere protein evolution may yield insights beyond the centromere drive hypothesis and help us understand functional domains that have been neglected due to their high divergence.

Does the evolution of centromeric DNA sequence impact drive?

The monkeyflower and mouse model systems provide evidence that centromeric DNA repeats abundance impacts centromere drive, but the significance of repeat monomer sequence divergence remains unclear. In most organisms that have been studied, the centromere is defined epigenetically by CENP-A nucleosomes rather than by specific centromeric DNA sequences. Although polymorphisms of centromeric DNA are observed

within species, for example in human (Maloney et al. 2012) their functional significance is unclear. Future studies may reveal how distinct centromeric sequences affect meiotic segregation, and whether the evolution of these sequences drives adaptive evolution of DNA-binding domains of proteins such as CENP-A or CENP-T.

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Abbreviations:

BUB1	budding uninhibited by imidazole
Cal1	chromosome alignment defect 1
CDC42	cell division cycle 42
CenH3	centromeric histone 3 (CENP-A orthologue in monkeyflowers)
CENP-A	centromere protein A
CENP-B	centromere protein B
CENP-C	centromere protein C
CENP-I	centromere protein I
CENP-T	centromere protein T
Cid	centromere identifier (CENP-A orthologue in *Drosophila*)
DSN1	kinetochore-associated protein DSN1 homolog
HJURP	Holliday junction recognition protein
HP1	heterochromatin protein 1
HyPhy	Hypothesis Testing using Phylogenies
INCENP	inner centromere protein
Kindr	Kinesin driver
KNL1	kinetochore scaffold 1
MCAK	mitotic centromere-associated kinesin
MELT	Met-Glu-Leu-Thr motif
MIS12	minichromosome instability-12
MIS18BP1	MIS18 binding protein 1
NDC80	kinetochore protein NDC80 homolog

PAML	Phylogenetic Analysis by Maximum Likelihood
RAN-GTP	Ras-related nuclear protein-guanosine-5’-triphosphate
SGO2	shugoshin-2
Trkin	TR-1 kinesin

References:

Akera T, Chmatal L, Trimm E, Yang K, Aonbangkhen C, Chenoweth DM, Janke C, Schultz RM, Lampson MA (2017) Spindle asymmetry drives non-Mendelian chromosome segregation. Science 358: 668–672. 10.1126/science.aan0092 [PubMed: 29097549]

Akera T, Trimm E, Lampson MA (2019) Molecular Strategies of Meiotic Cheating by Selfish Centromeres. Cell 178: 1132–1144 e1110. 10.1016/j.cell.2019.07.001 [PubMed: 31402175]

Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, Wang J, Cong Q, Kinch LN, Schaeffer RD et al. (2021) Accurate prediction of protein structures and interactions using a three-track neural network. Science 373: 871–876. 10.1126/science.abj8754 [PubMed: 34282049]

Baker RE, Rogers K (2006) Phylogenetic analysis of fungal centromere H3 proteins. Genetics 174: 1481–1492. 10.1534/genetics.106.062794 [PubMed: 17028330]

Balzano E, Giunta S (2020) Centromeres under Pressure: Evolutionary Innovation in Conflict with Conserved Function. Genes (Basel) 11. 10.3390/genes11080912

Booker TR, Jackson BC, Keightley PD (2017) Detecting positive selection in the genome. BMC Biol 15: 98. 10.1186/s12915-017-0434-y [PubMed: 29084517]

Bracewell R, Chatla K, Nalley MJ, Bachtrog D (2019) Dynamic turnover of centromeres drives karyotype evolution in Drosophila. eLife 8. 10.7554/eLife.49002

Britton-Davidian J, Catalan J, da Graca Ramalhinho M, Ganem G, Auffray JC, Capela R, Biscoito M, Searle JB, da Luz Mathias M (2000) Rapid chromosomal evolution in island mice. Nature 403: 158. 10.1038/35003116 [PubMed: 10646592]

Chang CH, Chavan A, Palladino J, Wei X, Martins NMC, Santinello B, Chen CC, Erceg J, Beliveau BJ, Wu CT et al. (2019) Islands of retroelements are major components of Drosophila centromeres. PLoS Biol 17: e3000241. 10.1371/iournal.pbio.3000241 [PubMed: 31086362]

Cheerambathur DK, Prevo B, Chow TL, Hattersley N, Wang S, Zhao Z, Kim T, Gerson-Gurwitz A, Oegema K, Green R et al. (2019) The Kinetochore-Microtubule Coupling Machinery Is Repurposed in Sensory Nervous System Morphogenesis. Dev Cell 48: 864–872 e867. 10.1016/i.devcel.2019.02.002 [PubMed: 30827898]

Chmatal L, Gabriel SI, Mitsainas GP, Martinez-Vargas J, Ventura J, Searle JB, Schultz RM, Lampson MA (2014) Centromere strength provides the cell biological basis for meiotic drive and karyotype evolution in mice. Current biology : CB 24: 2295–2300. 10.1016/i.cub.2014.08.017 [PubMed: 25242031]

Chmatal L, Schultz RM, Black BE, Lampson MA (2017) Cell Biology of Cheating-Transmission of Centromeres and Other Selfish Elements Through Asymmetric Meiosis. Prog Mol Subcell Biol 56: 377–396. 10.1007/978-3-319-58592-516 [PubMed: 28840246]

Clark FE, Akera T (2021) Unravelling the mystery of female meiotic drive: where we are. Open Biol 11: 210074. 10.1098/rsob.210074 [PubMed: 34465214]

Dawe RK, Lowry EG, Gent JI, Stitzer MC, Swentowsky KW, Higgins DM, Ross-Ibarra J, Wallace JG, Kanizay LB, Alabady M et al. (2018) A Kinesin-14 Motor Activates Neocentromeres to Promote Meiotic Drive in Maize. Cell 173: 839–850 e818. 10.1016/j.cell.2018.03.009 [PubMed: 29628142]

Ding B, Patterson EL, Holalu SV, Li J, Johnson GA, Stanley LE, Greenlee AB, Peng F, Bradshaw HD Jr., Blinov ML et al. (2020) Two MYB Proteins in a Self-Organizing Activator-Inhibitor System Produce Spotted Pigmentation Patterns. Current biology : CB 30: 802–814 e808. 10.1016/j.cub.2019.12.067 [PubMed: 32155414]

Domingo J, Baeza-Centurion P, Lehner B (2019) The Causes and Consequences of Genetic Interactions (Epistasis). Annu Rev Genomics Hum Genet 20: 433–460. 10.1146/annurev-genom-083118-014857 [PubMed: 31082279]

Drpic D, Almeida AC, Aguiar P, Renda F, Damas J, Lewin HA, Larkin DM, Khodjakov A, Maiato H (2018) Chromosome Segregation Is Biased by Kinetochore Size. Current biology : CB 28: 1344–1356 e1345. 10.1016/j.cub.2018.03.023 [PubMed: 29706521]

Elde NC, Roach KC, Yao MC, Malik HS (2011) Absence of positive selection on centromeric histones in Tetrahymena suggests unsuppressed centromere: drive in lineages lacking male meiosis. J Mol Evol 72: 510–520. 10.1007/s00239-011-9449-0 [PubMed: 21643829]

Fachinetti D, Han JS, McMahon MA, Ly P, Abdullah A, Wong AJ, Cleveland DW (2015) DNA Sequence-Specific Binding of CENP-B Enhances the Fidelity of Human Centromere Function. Dev Cell 33: 314–327. 10.1016/j.devcel.2015.03.020 [PubMed: 25942623]

Fang Y, Coelho MA, Shu H, Schotanus K, Thimmappa BC, Yadav V, Chen H, Malc EP, Wang J, Mieczkowski PA et al. (2020) Long transposon-rich centromeres in an oomycete reveal divergence of centromere features in Stramenopila-Alveolata-Rhizaria lineages. PLoS Genet 16: e1008646. 10.1371/journal.pgen.1008646 [PubMed: 32150559]

Finseth FR, Dong Y, Saunders A, Fishman L (2015) Duplication and Adaptive Evolution of a Key Centromeric Protein in Mimulus, a Genus with Female Meiotic Drive. Mol Biol Evol 32: 2694–2706. 10.1093/molbev/msv145 [PubMed: 26104011]

Finseth FR, Nelson TC, Fishman L (2021) Selfish chromosomal drive shapes recent centromeric histone evolution in monkeyflowers. PLoS Genet 17: e1009418. 10.1371/journal.pgen.1009418 [PubMed: 33886547]

Fishman L, Kelly AJ, Morgan E, Willis JH (2001) A genetic map in the Mimulus guttatus species complex reveals transmission ratio distortion due to heterospecific interactions. Genetics 159: 1701–1716. 10.1093/genetics/159.4.1701 [PubMed: 11779808]

Fishman L, Kelly JK (2015) Centromere-associated meiotic drive and female fitness variation in Mimulus. Evolution 69: 1208–1218. 10.1111/evo.12661 [PubMed: 25873401]

Fishman L, Saunders A (2008) Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. Science 322: 1559–1562. 10.1126/science.1161406 [PubMed: 19056989]

Fishman L, Willis JH (2005) A novel meiotic drive locus almost completely distorts segregation in mimulus (monkeyflower) hybrids. Genetics 169: 347–353. 10.1534/genetics.104.032789 [PubMed: 15466426]

Fletcher W, Yang Z (2010) The effect of insertions, deletions, and alignment errors on the branch-site test of positive selection. Mol Biol Evol 27: 2257–2267. 10.1093/molbev/msq115 [PubMed: 20447933]

Funabiki H (2019) Correcting aberrant kinetochore microtubule attachments: a hidden regulation of Aurora B on microtubules. Current opinion in cell biology 58: 34–41. 10.1016/j.ceb.2018.12.007 [PubMed: 30684807]

Garagna S, Page J, Fernandez-Donoso R, Zuccotti M, Searle JB (2014) The Robertsonian phenomenon in the house mouse: mutation, meiosis and speciation. Chromosoma 123: 529–544. 10.1007/s00412-014-0477-6 [PubMed: 25053180]

Hartley GA, Okhovat M, O'Neill RJ, Carbone L (2021) Comparative Analyses of Gibbon Centromeres Reveal Dynamic Genus-Specific Shifts in Repeat Composition. Mol Biol Evol 38: 3972–3992. 10.1093/molbev/msab148 [PubMed: 33983366]

Henikoff S, Ahmad K, Malik HS (2001) The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293: 1098–1102. 10.1126/science.1062939 [PubMed: 11498581]

Higgins JM, Prendergast L (2016) Mitotic Mysteries: The Case of HP1. Dev Cell 36: 477–478. 10.1016/j.devcel.2016.02.019 [PubMed: 26954539]

Hindriksen S, Lens SMA, Hadders MA (2017) The Ins and Outs of Aurora B Inner Centromere Localization. Front Cell Dev Biol 5: 112. 10.3389/fcell.2017.00112 [PubMed: 29312936]

Hughes SE, Miller DE, Miller AL, Hawley RS (2018) Female Meiosis: Synapsis, Recombination, and Segregation in Drosophila melanogaster. Genetics 208: 875–908. 10.1534/genetics.117.300081 [PubMed: 29487146]

Humphreys IR, Pei J, Baek M, Krishnakumar A, Anishchenko I, Ovchinnikov S, Zhang J, Ness TJ, Banjade S, Bagde SR et al. (2021) Computed structures of core eukaryotic protein complexes. Science 374: eabm4805. 10.1126/science.abm4805 [PubMed: 34762488]

Hurst GD, Werren JH (2001) The role of selfish genetic elements in eukaryotic evolution. Nature reviews Genetics 2: 597–606. 10.1038/35084545

Iwata-Otsubo A, Dawicki-McKenna JM, Akera T, Falk SJ, Chmatal L, Yang K, Sullivan BA, Schultz RM, Lampson MA, Black BE (2017) Expanded Satellite Repeats Amplify a Discrete CENP-A Nucleosome Assembly Site on Chromosomes that Drive in Female Meiosis. Current biology: CB 27: 2365–2373 e2368. 10.1016/j.cub.2017.06.069 [PubMed: 28756949]

Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek A, Potapenko A et al. (2021) Highly accurate protein structure prediction with AlphaFold. Nature 596: 583–589. 10.1038/s41586-021-03819-2 [PubMed: 34265844]

Kitajima TS (2018) Mechanisms of kinetochore-microtubule attachment errors in mammalian oocytes. Dev Growth Differ 60: 33–43. 10.1111/dgd.12410 [PubMed: 29318599]

Kixmoeller K, Allu PK, Black BE (2020) The centromere comes into focus: from CENP-A nucleosomes to kinetochore connections with the spindle. Open Biol 10: 200051. 10.1098/rsob.200051 [PubMed: 32516549]

Kratka M, Smerda J, Lojdova K, Bures P, Zedek F (2021) Holocentric Chromosomes Probably Do Not Prevent Centromere Drive in Cyperaceae. Front Plant Sci 12: 642661. 10.3389/fpls.2021.642661 [PubMed: 33679859]

Kruger AN, Mueller JL (2021) Mechanisms of meiotic drive in symmetric and asymmetric meiosis. Cellular and molecular life sciences : CMLS 78: 3205–3218. 10.1007/s00018-020-03735-0 [PubMed: 33449147]

Kumon T, Ma J, Akins RB, Stefanik D, Nordgren CE, Kim J, Levine MT, Lampson MA (2021) Parallel pathways for recruiting effector proteins determine centromere drive and suppression. Cell 184: 4904–4918 e4911. 10.1016/i.cell.2021.07.037 [PubMed: 34433012]

Lampson MA, Grishchuk EL (2017) Mechanisms to Avoid and Correct Erroneous Kinetochore-Microtubule Attachments. Biology (Basel) 6. 10.3390/biology6010001

Maheshwari S, Ishii T, Brown CT, Houben A, Comai L (2017) Centromere location in Arabidopsis is unaltered by extreme divergence in CENH3 protein sequence. Genome Res 27: 471–478. 10.1101/gr.214619.116 [PubMed: 28223399]

Maheshwari S, Tan EH, West A, Franklin FC, Comai L, Chan SW (2015) Naturally occurring differences in CENH3 affect chromosome segregation in zygotic mitosis of hybrids. PLoS Genet 11: e1004970. 10.1371/journal.pgen.1004970 [PubMed: 25622028]

Malik HS, Henikoff S (2001) Adaptive evolution of Cid, a centromere-specific histone in Drosophila. Genetics 157: 1293–1298. https://www.ncbi.nlm.nih.gov/pubmed/11238413 [PubMed: 11238413]

Malik HS, Vermaak D, Henikoff S (2002) Recurrent evolution of DNA-binding motifs in the Drosophila centromeric histone. Proceedings of the National Academy of Sciences of the United States of America 99: 1449–1454. 10.1073/pnas.032664299 [PubMed: 11805302]

Maloney KA, Sullivan LL, Matheny JE, Strome ED, Merrett SL, Ferris A, Sullivan BA (2012) Functional epialleles at an endogenous human centromere. Proceedings of the National Academy of Sciences of the United States of America 109: 13704–13709. 10.1073/pnas.1203126109 [PubMed: 22847449]

Marston AL (2015) Shugoshins: tension-sensitive pericentromeric adaptors safeguarding chromosome segregation. Mol Cell Biol 35: 634–648. 10.1128/MCB.01176-14 [PubMed: 25452306]

Masumoto H, Masukata H, Muro Y, Nozaki N, Okazaki T (1989) A human centromere antigen (CENP-B) interacts with a short specific sequence in alphoid DNA, a human centromeric satellite. The Journal of cell biology 109: 1963–1973. 10.1083/icb.109.5.1963 [PubMed: 2808515]

McDonald JH, Kreitman M (1991) Adaptive protein evolution at the Adh locus in Drosophila. Nature 351: 652–654. 10.1038/351652a0 [PubMed: 1904993]

Okada T, Ohzeki J, Nakano M, Yoda K, Brinkley WR, Larionov V, Masumoto H (2007) CENP-B controls centromere formation depending on the chromatin context. Cell 131: 1287–1300. 10.1016/i.cell.2007.10.045 [PubMed: 18160038]

Otake K, Ohzeki JI, Shono N, Kugou K, Okazaki K, Nagase T, Yamakawa H, Kouprina N, Larionov V, Kimura H et al. (2020) CENP-B creates alternative epigenetic chromatin states permissive for CENP-A or heterochromatin assembly. Journal of cell science 133. 10.1242/jcs.243303

Pal C, Papp B, Lercher MJ (2006) An integrated view of protein evolution. Nature reviews Genetics 7: 337–348. 10.1038/nrg1838

Pardo-Manuel de Villena F, Sapienza C (2001) Female meiosis drives karyotypic evolution in mammals. Genetics 159: 1179–1189. 10.1093/genetics/159.3.1179 [PubMed: 11729161]

Peris L, Wagenbach M, Lafanechere L, Brocard J, Moore AT, Kozielski F, Job D, Wordeman L, Andrieux A (2009) Motor-dependent microtubule disassembly driven by tubulin tyrosination. The Journal of cell biology 185: 1159–1166. 10.1083/icb.200902142 [PubMed: 19564401]

Piálek J, Hauffe HC, Searle JB (2005) Chromosomal variation in the house mouse. Biol J Linn Soc 84: 535–563. 10.1111/j.1095-8312.2005.00454.x

Pond SL, Frost SD, Muse SV (2005) HyPhy: hypothesis testing using phylogenies. Bioinformatics 21: 676–679. 10.1093/bioinformatics/bti079 [PubMed: 15509596]

Ravi M, Kwong PN, Menorca RM, Valencia JT, Ramahi JS, Stewart JL, Tran RK, Sundaresan V, Comai L, Chan SW (2010) The rapidly evolving centromere-specific histone has stringent functional requirements in Arabidopsis thaliana. Genetics 186: 461–471. 10.1534/genetics.110.120337 [PubMed: 20628040]

Romanenko SA, Perelman PL, Trifonov VA, Graphodatsky AS (2012) Chromosomal evolution in Rodentia. Heredity (Edinb) 108: 4–16. 10.1038/hdy.2011.110 [PubMed: 22086076]

Rosin L, Mellone BG (2016) Co-evolving CENP-A and CAL1 Domains Mediate Centromeric CENP-A Deposition across Drosophila Species. Dev Cell 37: 136–147. 10.1016/i.devcel.2016.03.021 [PubMed: 27093083]

Sandler I, Novitski E (1957) Meiotic drive as an evolutionary force. Am Nat 91: 105–110.

Schueler MG, Swanson W, Thomas PJ, Program NCS, Green ED (2010) Adaptive evolution of foundation kinetochore proteins in primates. Mol Biol Evol 27: 1585–1597. 10.1093/molbev/msq043 [PubMed: 20142441]

Sirajuddin M, Rice LM, Vale RD (2014) Regulation of microtubule motors by tubulin isotypes and post-translational modifications. Nature cell biology 16: 335–344. 10.1038/ncb2920 [PubMed: 24633327]

Swentowsky KW, Gent JI, Lowry EG, Schubert V, Ran X, Tseng KF, Harkess AE, Qiu W, Dawe RK (2020) Distinct kinesin motors drive two types of maize neocentromeres. Genes Dev 34: 1239–1251. 10.1101/gad.340679.120 [PubMed: 32820038]

Talbert PB, Bryson TD, Henikoff S (2004) Adaptive evolution of centromere proteins in plants and animals. J Biol 3: 18. 10.1186/ibiol11 [PubMed: 15345035]

Talbert PB, Masuelli R, Tyagi AP, Comai L, Henikoff S (2002) Centromeric localization and adaptive evolution of an Arabidopsis histone H3 variant. Plant Cell 14: 1053–1066. https://www.ncbi.nlm.nih.gov/pubmed/12034896 [PubMed: 12034896]

Thakur J, Packiaraj J, Henikoff S (2021) Sequence, Chromatin and Evolution of Satellite DNA. Int J Mol Sci 22. 10.3390/iims22094309

Tromer E, Snel B, Kops GJ (2015) Widespread Recurrent Patterns of Rapid Repeat Evolution in the Kinetochore Scaffold KNL1. Genome Biol Evol 7: 2383–2393. 10.1093/gbe/evv140 [PubMed: 26254484]

van der Lee R, Wiel L, van Dam TJP, Huynen MA (2017) Genome-scale detection of positive selection in nine primates predicts human-virus evolutionary conflicts. Nucleic Acids Res 45: 10634–10648. 10.1093/nar/gkx704 [PubMed: 28977405]

van Hooff JJ, Tromer E, van Wijk LM, Snel B, Kops GJ (2017) Evolutionary dynamics of the kinetochore network in eukaryotes as revealed by comparative genomics. EMBO reports 18: 1559–1571. 10.15252/embr.201744102 [PubMed: 28642229]

Verlhac MH, Dumont J (2008) Interactions between chromosomes, microfilaments and microtubules revealed by the study of small GTPases in a big cell, the vertebrate oocyte. Mol Cell Endocrinol 282: 12–17. 10.1016/i.mce.2007.11.018 [PubMed: 18191018]

Vermaak D, Hayden HS, Henikoff S (2002) Centromere targeting element within the histone fold domain of Cid. Mol Cell Biol 22: 7553–7561. 10.1128/MCB.22.21.7553-7561.2002 [PubMed: 12370302]

Wei KH, Reddy HM, Rathnam C, Lee J, Lin D, Ji S, Mason JM, Clark AG, Barbash DA (2017) A Pooled Sequencing Approach Identifies a Candidate Meiotic Driver in Drosophila. Genetics 206: 451–465. 10.1534/genetics.116.197335 [PubMed: 28258181]

Wu T, Lane SIR, Morgan SL, Jones KT (2018) Spindle tubulin and MTOC asymmetries may explain meiotic drive in oocytes. Nature communications 9: 2952. 10.1038/s41467-018-05338-7

Yang Z (2007) PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol 24: 1586–1591. 10.1093/molbev/msm088 [PubMed: 17483113]

Yuan YW (2019) Monkeyflowers (Mimulus): new model for plant developmental genetics and evo-devo. New Phytol 222: 694–700. 10.1111/nph.15560 [PubMed: 30471231]

Zanders SE, Unckless RL (2019) Fertility Costs of Meiotic Drivers. Current biology : CB 29: R512–R520. 10.1016/i.cub.2019.03.046 [PubMed: 31163165]

Zedek F, Bures P (2016) CenH3 evolution reflects meiotic symmetry as predicted by the centromere drive model. Sci Rep 6: 33308. 10.1038/srep33308 [PubMed: 27629066]

Zhao G, Oztan A, Ye Y, Schwarz TL (2019) Kinetochore Proteins Have a Post-Mitotic Function in Neurodevelopment. Dev Cell 48: 873–882 e874. 10.1016/i.devcel.2019.02.003 [PubMed: 30827899]

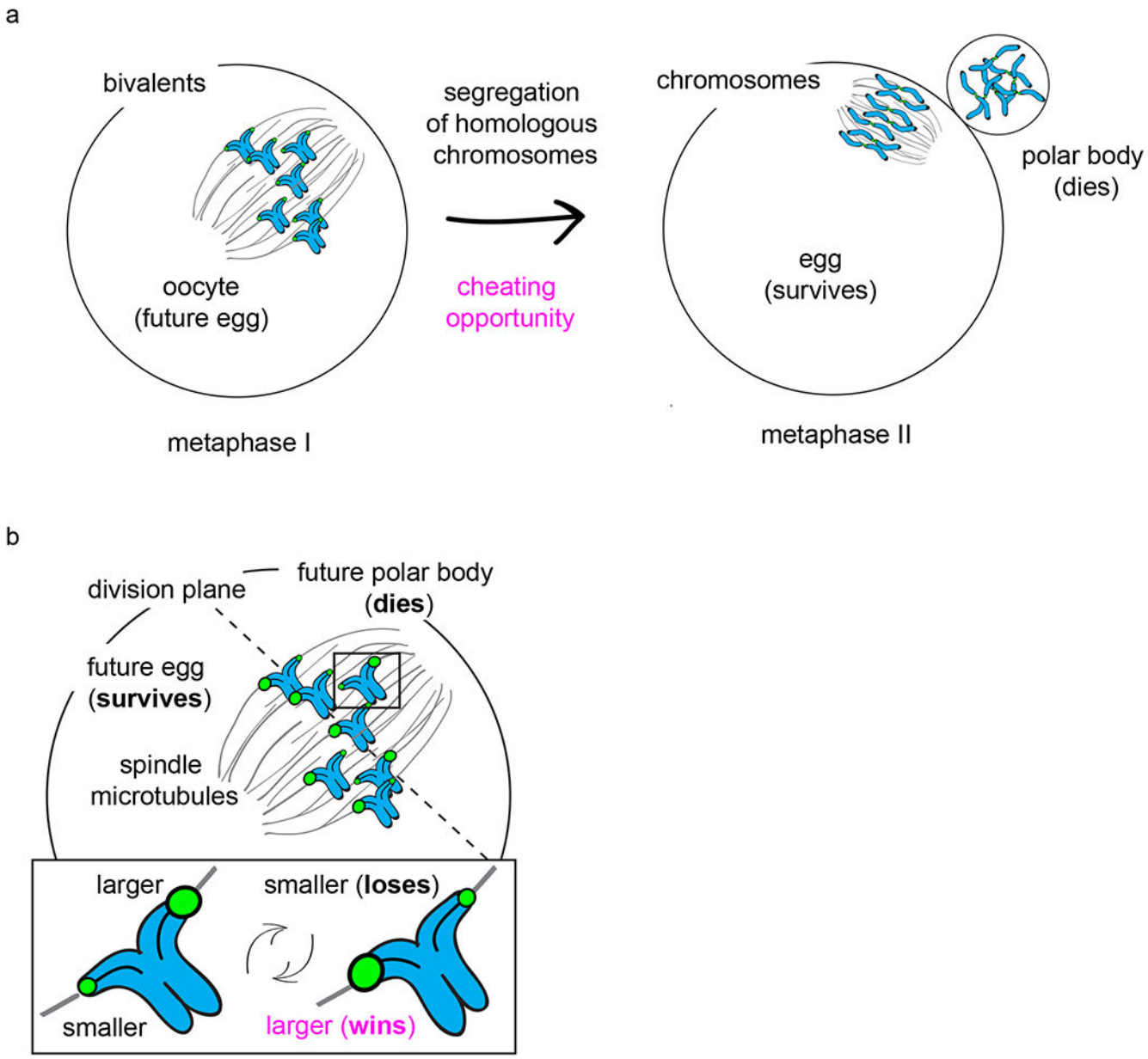

Fig. 1. Asymmetric female meiosis I is hijacked by selfish centromeres

a) Female meiosis I (MI, left): homologous chromosomes form bivalents and segregate into the future egg or into the polar body, creating an opportunity for non-Mendelian segregation of selfish centromeres. Female meiosis II (MII, right): winning chromosomes from meiosis I align at metaphase II, while losing chromosomes die in the polar body. **b**) In MI selfish centromeres (larger green circles) hijack the machinery regulating microtubule-kinetochore attachments to re-orient toward the future egg side of the spindle. Non-centromeric selfish elements can also cheat in MII (not shown) depending on the crossover position

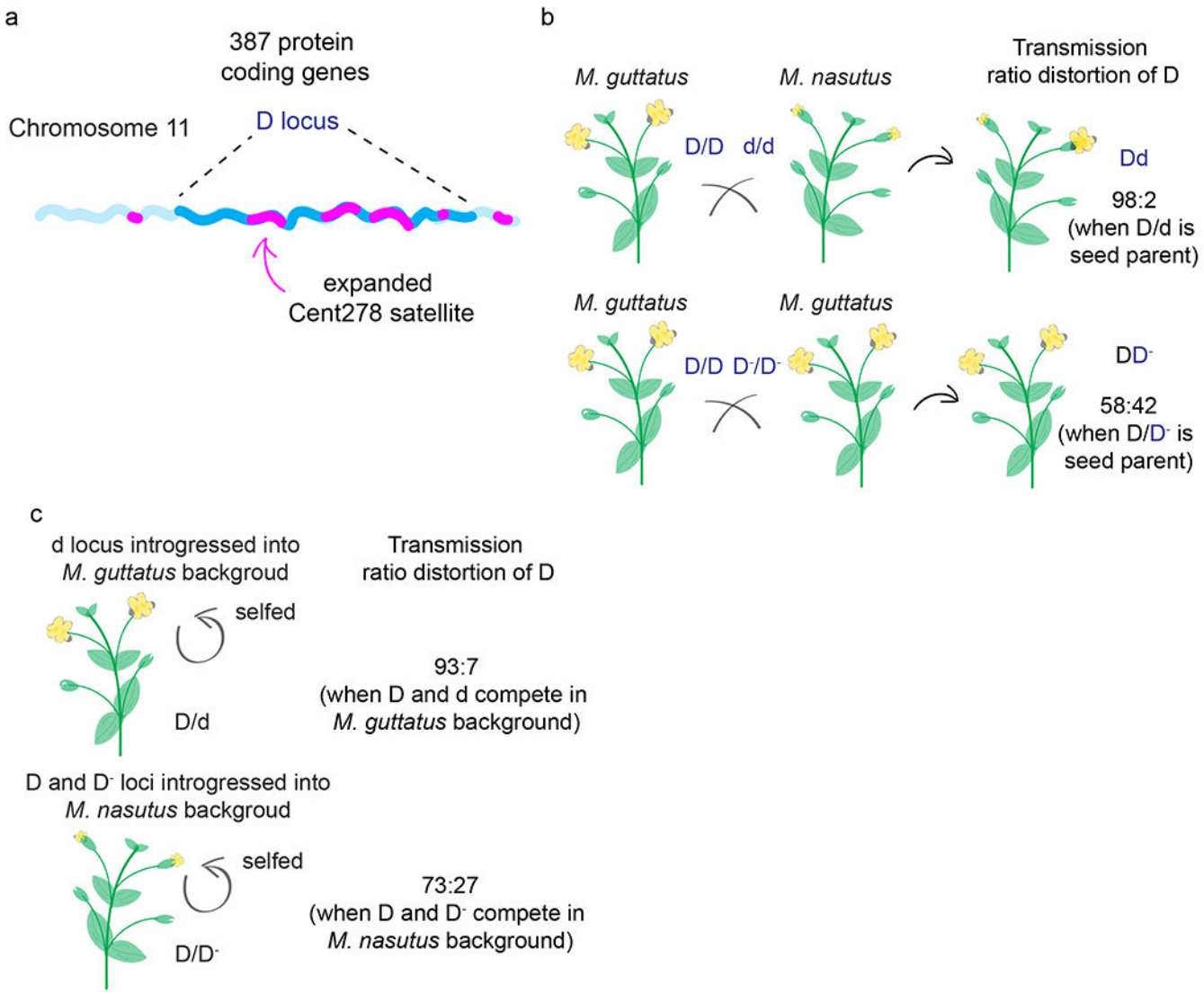

Fig. 2. Centromere drive in yellow monkeyflowers

a) The D locus in Mimulus guttatus is defined as a low recombination region on chromosome 11, including expanded Cent278 centromere-associated satellite repeats and over 300 protein coding genes. **b**) Transmission ratio distortion in monkeyflowers. In an inter-specific hybrid, D drives strongly (98:2) in competition with the d locus from M. nasutus in female meiosis. In a conspecific hybrid, D drives less strongly when competing with the D- locus from M. guttatus. A fitness cost is observed only in the D/D genotype. **c**) Strength of D locus drive depends on the genomic background. Top: the native M. guttatus genome reduces the drive strength (93:7) compared to the M. guttatus/M. nasutus hybrid background (98:2, b) in D/d plants. Bottom: the naive M. nasutus genomic background allows stronger drive (73:27) compared to the M. guttatus background (58:42, b) in D/D- plants

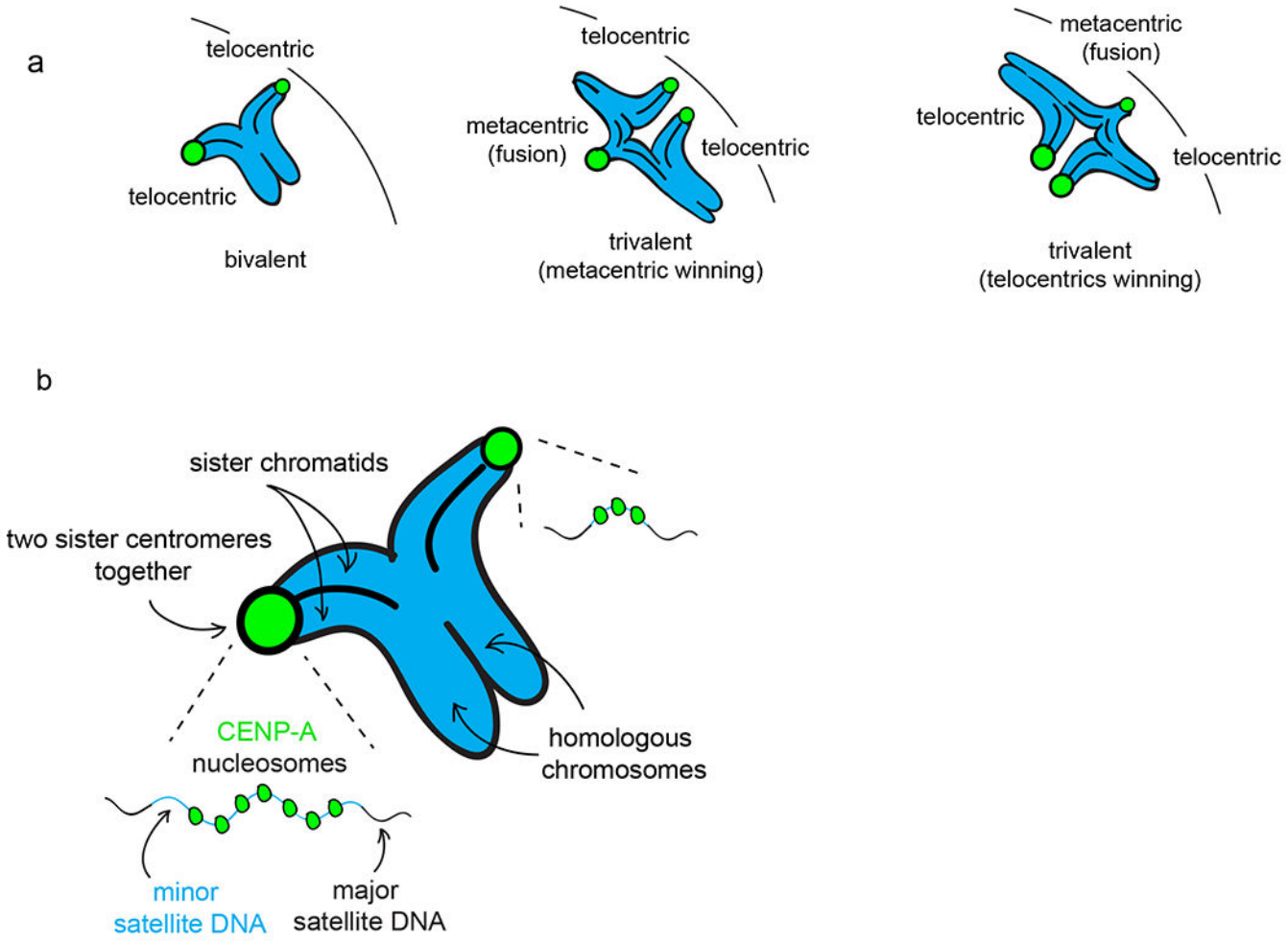

Fig. 3. Asymmetry in minor satellite DNA leads to centromere drive in mice

a) Centromere size biases chromosome orientation at metaphase I. In both bivalents and trivalents, telocentric or metacentric chromosomes with larger centromeres are predicted to face the future egg more often than the polar body. **b**) A bivalent in meiosis I is composed of two homologous chromosomes, each with two sister chromatids. CENP-A nucleosomes decorate the minor satellite DNA (blue) and define the centromeres. Selfish centromeres contain more minor satellite DNA and more CENP-A chromatin compared to the homologous chromosome

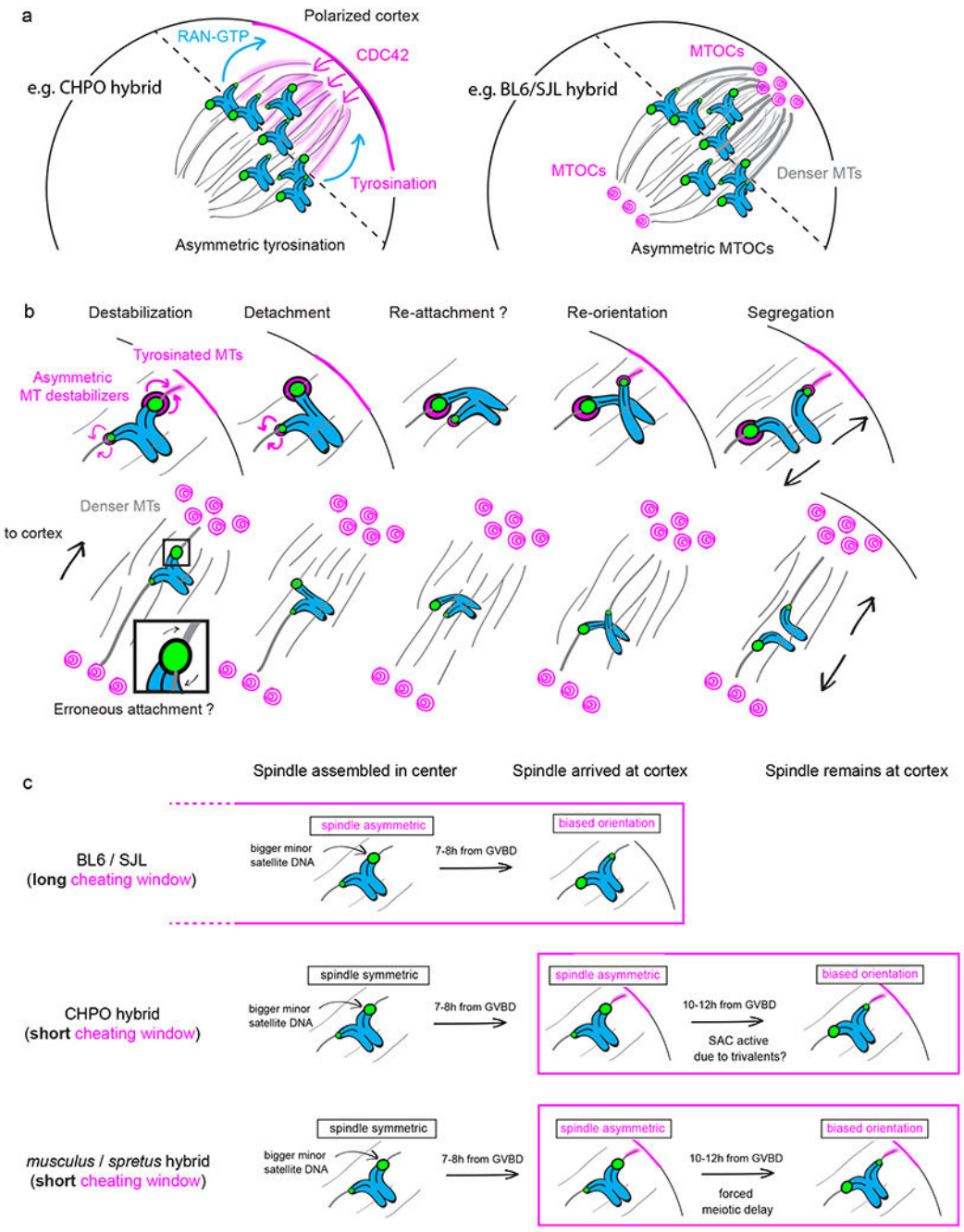

Fig. 4. Molecular mechanisms of centromere drive in mouse oocytes

a) Microtubule asymmetry defines the winning and the losing sides of the spindle. The meiosis I spindle can be asymmetric in tyrosination levels (left) and/or in microtubule density across the spindle (right). **b**) Microtubule destabilization allows bivalent re-orientation. Predicted steps leading to biased segregation of selfish centromeres are depicted, due to asymmetric microtubule destabilizers and unequal microtubule tyrosination (CHPO hybrid, top) or erroneous attachments (proposed for C57BL6/SJL hybrid, bottom). **c**) Selfish

centromeres can bias their orientation on an asymmetric spindle during a variable ”cheating window” (magenta frame) in meiosis I

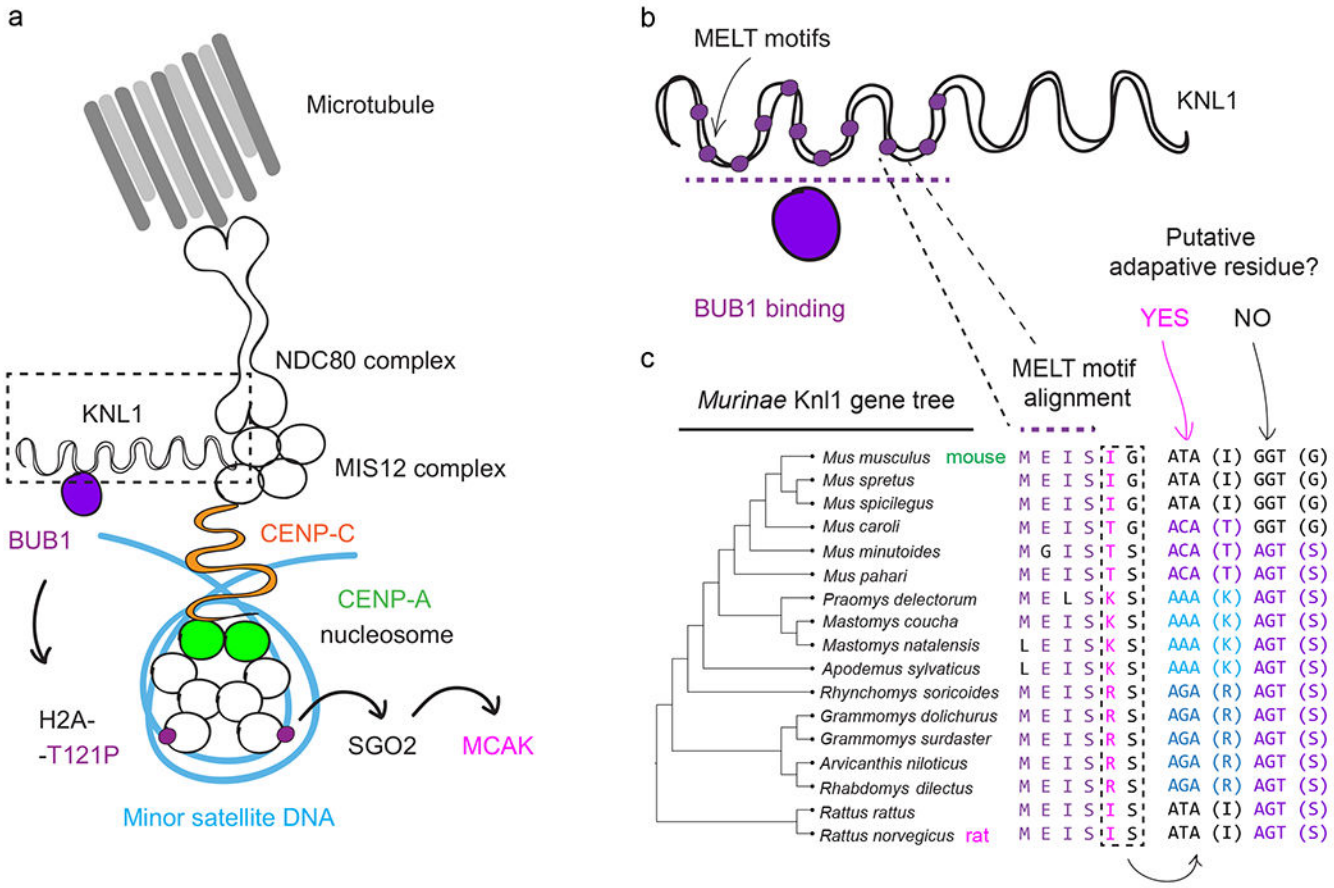

Fig. 5. Positive selection in a kinetochore protein that regulates microtubule-destabilizing activity at centromeres

a) CENP-A nucleosomes assemble a kinetochore by recruiting CENP-C, the MIS12 complex, KNL1, and the NDC80 complex that binds to microtubules. KNL1 recruits BUB1 kinase to kinetochores, which phosphorylates histone H2A to recruit SGO2 and microtubule destabilizers such as MCAK. **b**) KNL1 is an example of a rapidly evolving centromeric protein, which does not bind centromeric DNA. Met-Glu-Leu-Thr (MELT) motifs bind BUB1 kinase. **c**) Phylogenetic gene tree for Murinae KNL1 and an alignment of one MELT motif with an adjacent recurrently changing codon under positive selection detected using PAML software (modified from Kumon et al., 2021)

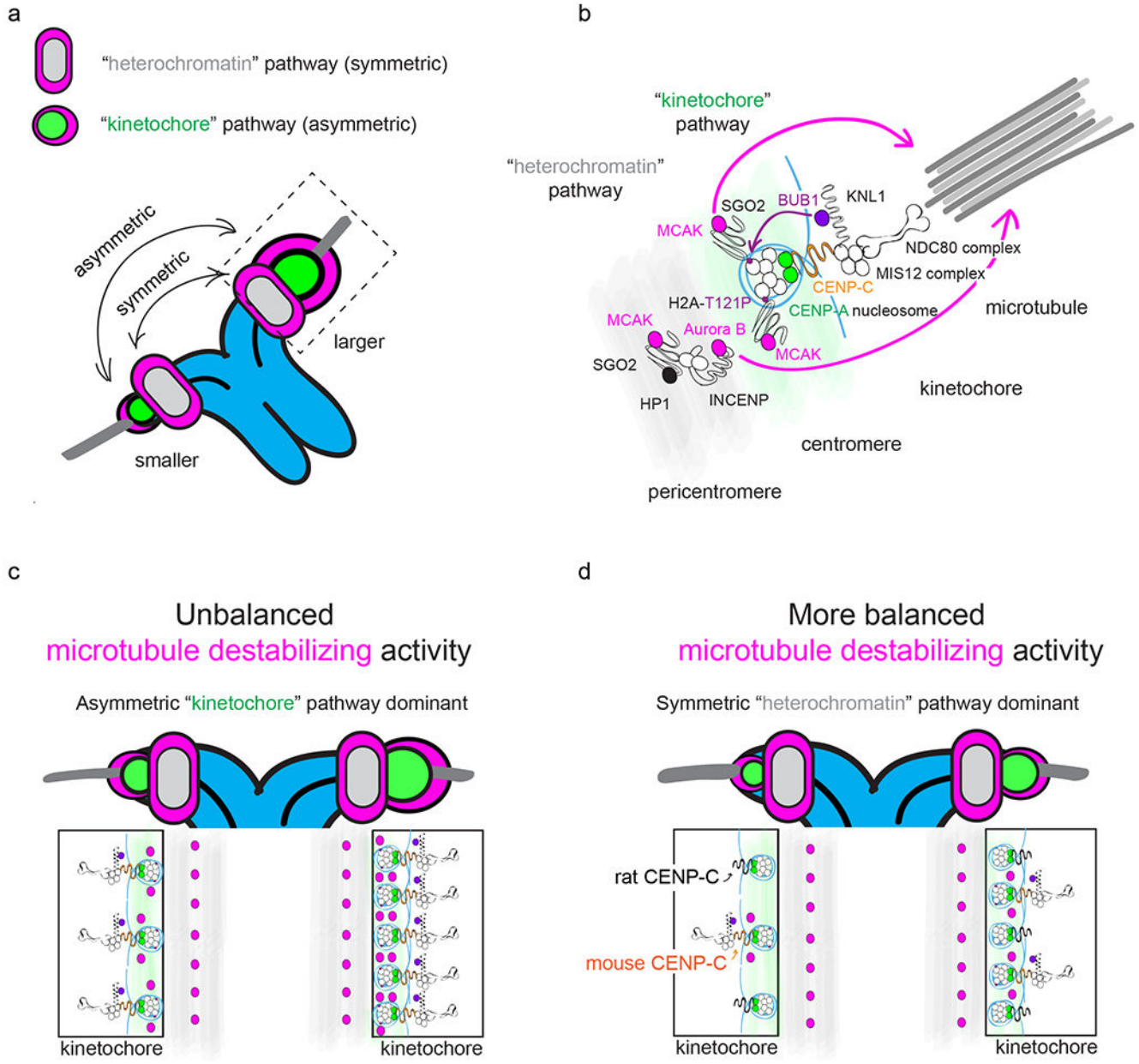

Fig. 6. Parallel pathway model for centromere drive and suppression

a) Two parallel pathways recruit microtubule destabilizers (magenta) via pericentromeric heterochromatin (gray) and the kinetochore (green). **b**) Microtubule destabilizers (e.g. MCAK and Aurora-B kinase) are recruited via BUB1 (kinetochore pathway) or heterochromatin protein 1 (HP1; heterochromatin pathway). **c**) Kinetochore pathway asymmetry across the bivalent leads to unbalanced microtubule destabilizing activity. **d**) Weakening the asymmetric kinetochore pathway makes the heterochromatin pathway relatively more dominant, providing more balanced microtubule destabilizing activity across the bivalent. For example, the two sides of the bivalent are functionally more equal after

expression of rat CENP-C, which binds mouse centromeres but is less effective in recruiting destabilizers (Kumon et al., 2021)