Probing "Selfish" Centromeres Unveils an Evolutionary Arms Race

A more complete understanding of nonrandom segregation will shed light on how speciation occurs.
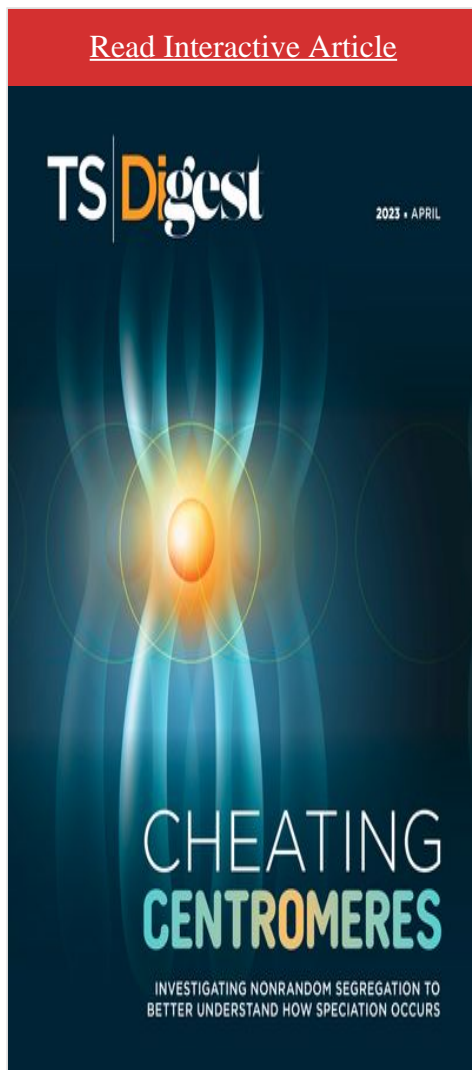
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The Portuguese island of Madeira is home to six different chromosomal races of mice, each with dramatically reduced diploid chromosome numbers compared to mice elsewhere. This striking diversity, first identified at the turn of the 21st century, can be explained by the repeated fusions of separate chromosomes. Each race has a different set of fusions, and a hybrid between two races would likely have reduced fertility or be sterile because of problems with chromosome pairing. Such reproductive isolation among populations is a key step on the road to speciation—and in the mice's case, these chromosomal changes have all occurred within the 1,000 years since their ancestors arrived on the island, possibly on Viking ships.

ABOVE:

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The so-called Robertsonian (Rb) fusions that led to these rapid karyotype changes are relatively common chromosomal rearrangements. But their accumulation in the populations of Madeira Island and in multiple other isolated mouse populations elsewhere is likely due to another influencing factor: the preferential segregation of the Rb fusion into the egg rather than into the discarded polar bodies that form during female meiosis.

We usually think of the chromosome segregation machinery as ensuring unbiased, random segregation. As we learn in high school biology, if a diploid individual carries two different alleles of a gene (i.e., is heterozygous), then either allele is equally likely to end up in a haploid gamete. This law explains the 3:1 ratio of phenotypes that Mendel observed in his classic studies of heredity. Scientists have known for decades, however, that selfish genes can subvert Mendelian segregation to increase their frequency in the next generation, a phenomenon known as meiotic drive. The Madeira mice suggest that fusion chromosomes can also drive unequal inheritance.

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CHEATING CENTROMERES

INVESTIGATING NONRANDOM SEGREGATION TO BETTER UNDERSTAND HOW SPECIATION OCCURS

Because Rb fusions are easy to identify morphologically, and because mouse oocytes are an established model system, studying these fusions in mice provided an entry for my lab at the University of Pennsylvania to investigate the cell biology of meiotic drive, starting in 2010. Focusing on the centromere—the part of each chromosome that interacts with spindle microtubules to direct segregation in mitosis or meiosis—we found that the structure's size determines the direction of biased segregation, with bigger centromeres preferentially segregating into the egg. Centromere DNA is typically highly repetitive, and we found that larger centromeres have more of the satellite repeats characteristic of mouse centromeres and more centromere proteins associated with that DNA. Thus, it seemed that newly formed Rb fusions could result in larger centromeres that would drive and become fixed in natural populations.

Meiotic drive of Rb fusions illustrates an idea proposed more than 50 years ago in a paper by zoologist Michael J.

Centromere drive depends on a combination of

D. White: "It may be that the very few chromosomal rearrangements which play a critical role in speciation through the ability to generate **asymmetries in female meiosis.** powerful isolating mechanisms are precisely those which happen to possess a segregational advantage in the female meiosis." Rb fusions are an example of such a rearrangement that can generate a segregational advantage (i.e., drive) through centromere expansion. The chromosomal races on Madeira Island and elsewhere show how drive can lead to rapid karyotype change and reproductive barriers between populations that have accumulated different sets of fusions.

Early hints of nonrandom segregation

Geneticist Marcus Rhoades introduced the concept of meiotic drive in 1942 based on observations of abnormal chromosome 10 (Ab10) in maize. Ab10 contains an extra DNA segment, termed a knob, that includes a repetitive DNA sequence. Rhoades showed that Ab10 preferentially segregates into the egg in female meiosis. He also proposed a model to explain the phenomenon, involving shifting the position of Ab10 toward the meiotic spindle poles in anaphase. The four products of meiosis are arranged in a linear tetrad, and only the lower cell develops into an egg, so this polar positioning increases the likelihood that Ab10 ends up in the egg. This model turned out to be correct, conceptually, and researchers recently discovered a molecular motor responsible for positioning Ab10.

The maize knobs are not necessary for chromosomal function or even beneficial except in the selfish sense of increasing their own transmission through female meiosis. In contrast, centromeres are ubiquitously used for faithful chromosome segregation during cell division. As stated by pioneering cell biologist Dan Mazia in 1961, "The role in mitosis of the chromosome arms, which carry most of the genetic material, may be compared with that of a corpse at a funeral: they provide the reason for the proceedings but do not take an active part in them." Rather, the action is at the centromere, which mediates the chromosome's interactions with spindle microtubules.

Because the core centromere function of connecting to the spindle is highly conserved across eukaryotes, we expect that centromere components would also be conserved. Contrary to this expectation, many centromeric proteins evolve rapidly in multiple eukaryotic lineages, with patterns of amino acid changes suggesting positive selection. The repetitive DNA at centromeres, which does not code for any proteins, is also highly variable even between closely related species. This rapid evolution of both the protein and DNA components of the centromere, despite the structure's conserved function, appears paradoxical.

Investigating centromeres or other selfish loci as "pathogens" in the context of genetic conflict can provide a unique window into the biology of chromosome segregation and inheritance.

To explain this paradox, in 2001, researchers proposed the idea that centromeres could play a role in meiotic drive. According to the centromere drive hypothesis, centromere DNA sequences (like the maize knobs) can act like selfish genetic elements, promoting their transmission to the next generation by hijacking the chromosome segregation machinery. This centromere drive may impose fitness costs, such as an increased chance of segregation errors that produce aneuploid gametes. These costs impose a selective pressure for adaptive evolution of centromere proteins to suppress the fitness costs.

But the repetitive, noncoding DNA at centromeres constantly changes, putting the rest of the genome, where centromere proteins are encoded, under recurrent pressure to adapt. This continual genetic conflict is analogous to immune factors evolving under pressure from a constantly changing pathogen, but with an essential chromosomal locus as the pathogen. The result is centromeric DNA and proteins that are highly variable even between closely related species. For this reason, the drive theory suggests that proteins adapted to centromeres in one population may not function optimally when confronted with divergent centromeres from another population, leading to hybrid incompatibilities, reproductive isolation, and speciation, analogous to the isolation induced by differences in karyotype.

Initial support for the centromere drive theory came from observations in yellow monkeyflowers (*Mimulus* spp.) published in 2008. In these plants, an expanded centromere, with more copies of the centromeric DNA repeat, exhibits a dramatic transmission bias. When plants are heterozygous for this expanded centromere, it can end up in offspring as much as 98 percent of the time. Plants homozygous for the expanded centromere exhibit reproductive fitness costs, however, in the form of reduced seed and pollen production, although the underlying mechanisms are unclear. Subsequent findings have shown that the magnitude of the transmission bias varies across different genetic backgrounds. Specifically, research points to a variant of the H3 histone protein as a potential suppressor of drive. This variant, known as CENP-A or CenH3, plays a key role in packaging centromeric DNA and serves as the foundation for the kinetochore, a multiprotein complex that binds the spindle microtubules.

These observations are consistent with the centromere drive hypothesis and raise fascinating mechanistic questions for cell biologists: How do selfish centromeres bias their segregation? How might adaptations of centromere proteins prevent drive or otherwise suppress the costs of nonrandom segregation? And what does all this mean for the evolution of populations and species?
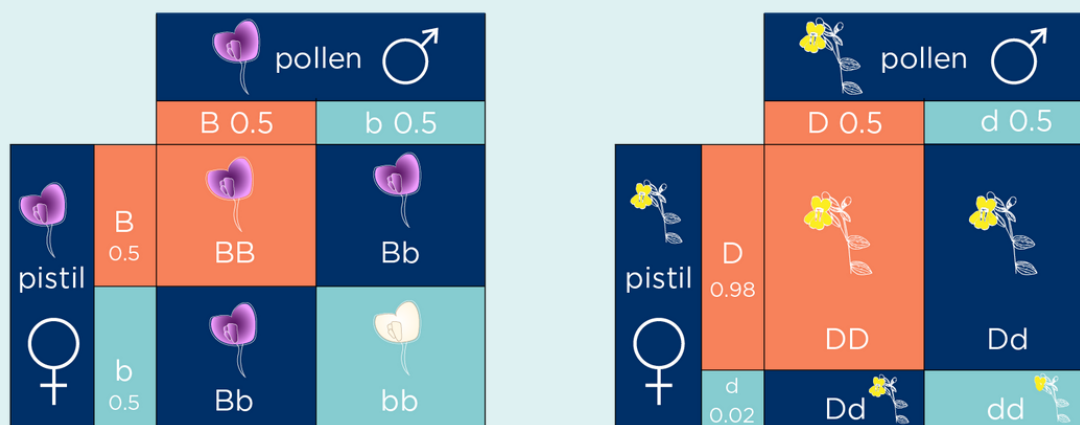
Separating Unequally

Random segregation leads to each of parent's alleles having an equal chance (0.5 probability) of being passed down. This can be visualized in a traditional Punnett square (left), which leads to a 3:1 ratio of offspring phenotypes and a 1:2:1 ratio of offspring genotypes (represented by orange, dark blue, and light blue shading, respectively).

If there is a meiotic drive, those ratios are shifted, sometimes dramatically (right). For example, in hybrid yellow monkeyflowers (*Mimulus guttas* x *M. nasutus*), the "distorter locus" (D) exhibits a whopping 98:2 segregation bias in the seed parent, resulting in an overabundance of DD offspring.

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Mechanisms of biased segregation

Centromere drive depends on a combination of asymmetries in female meiosis. First, there is the cell fate asymmetry that leads to the creation of one functional gamete while the other haploid cells are degraded and are therefore evolutionary dead ends. Second, there is the asymmetric positioning of the spindle close to the cell cortex, a thin layer of actin and other proteins just beneath the plasma membrane, leading to production of a large egg and a small polar body. Half of the chromosomes are

attached to the cortical side of the spindle and are thus destined for the polar body. Third, there is functional asymmetry between the centromeres of homologous chromosomes, with selfish centromeres more likely to remain in the egg. Centromere drive depends on coupling these asymmetries. The spindle provides spatial cues indicating which side leads to the egg versus the polar body, and selfish centromeres interact with the spindle such that they preferentially orient away from the polar body and toward the egg.

For the past eight years, my colleagues and I have used mice to interrogate these dynamics, and have found that spindle asymmetry is indeed coupled with cell fate asymmetry. Previous studies had shown that activation of a GTPase called Ran, by GTP binding, is induced by chromosomes and creates a diffusible signal that the cortex detects, resulting in the cell's polarization. Another GTPase, Cdc42, is enriched on the polarized cortex near the spindle. In 2017, we showed that the combination of spindle positioning, polarization-triggering Ran signaling, and Cdc42 signaling from the cortex back to the spindle leads to asymmetry within the spindle. This spindle asymmetry is based on differences in a post-translational modification of tubulin, the protein that makes up microtubules. The cortical side of the spindle is enriched for tyrosinated α-tubulin, which contains a C-terminal tyrosine, while the egg side is enriched for detyrosinated α-tubulin, from which the tyrosine has been removed by a peptidase. We tested the significance of this asymmetry in a hybrid mouse model made by crossing a strain that has larger centromeres with a strain that has smaller centromeres. When homologous chromosomes pair in female meiosis in the hybrid, larger and smaller centromeres compete for transmission to the egg. We showed that larger, selfish centromeres capitalize on the spindle asymmetry to preferentially orient toward the detyrosinated side destined for the egg.

Preferential orientation depends on the third asymmetry: functional differences between centromeres of homologous chromosomes. Selfish centromeres exploit the well-studied machinery that prevents segregation errors in every cell division. In mitosis, for example, centromeres of sister chromosomes can attach to the same spindle pole, an error that would lead to segregation of both sister chromosomes into one daughter cell. To correct the error before segregation can occur, microtubule destabilizing proteins at centromeres mediate detachment from spindle microtubules, providing an opportunity for one centromere to attach to the opposite pole. In 2019, we showed that selfish centromeres in hybrid mouse models recruit more of these destabilizers relative to the homologous chromosome. From the perspective of a selfish centromere, attachment to the cortical side of the spindle is detrimental because it leads to the polar body. The destabilizers resolve this issue by preferentially detaching the selfish centromere from tyrosinated microtubules and reorienting it toward the egg side.

DRIVEN TO SURVIVE

During oogenesis, only one of the haploid cells created by meiosis survives. The others, called polar bodies, die. This sets up an opportunity for "cheating," or nonrandom segregation, for example during the first round of meiosis when bivalents are split into paired chromosomes, as chromosomes with centromeres facing away from the cell cortex are retained in the future egg cell. One example of this is that larger centromeres hijack the machinery that attaches to the spindle, resulting in them facing away from the cortex preferentially (zoom).
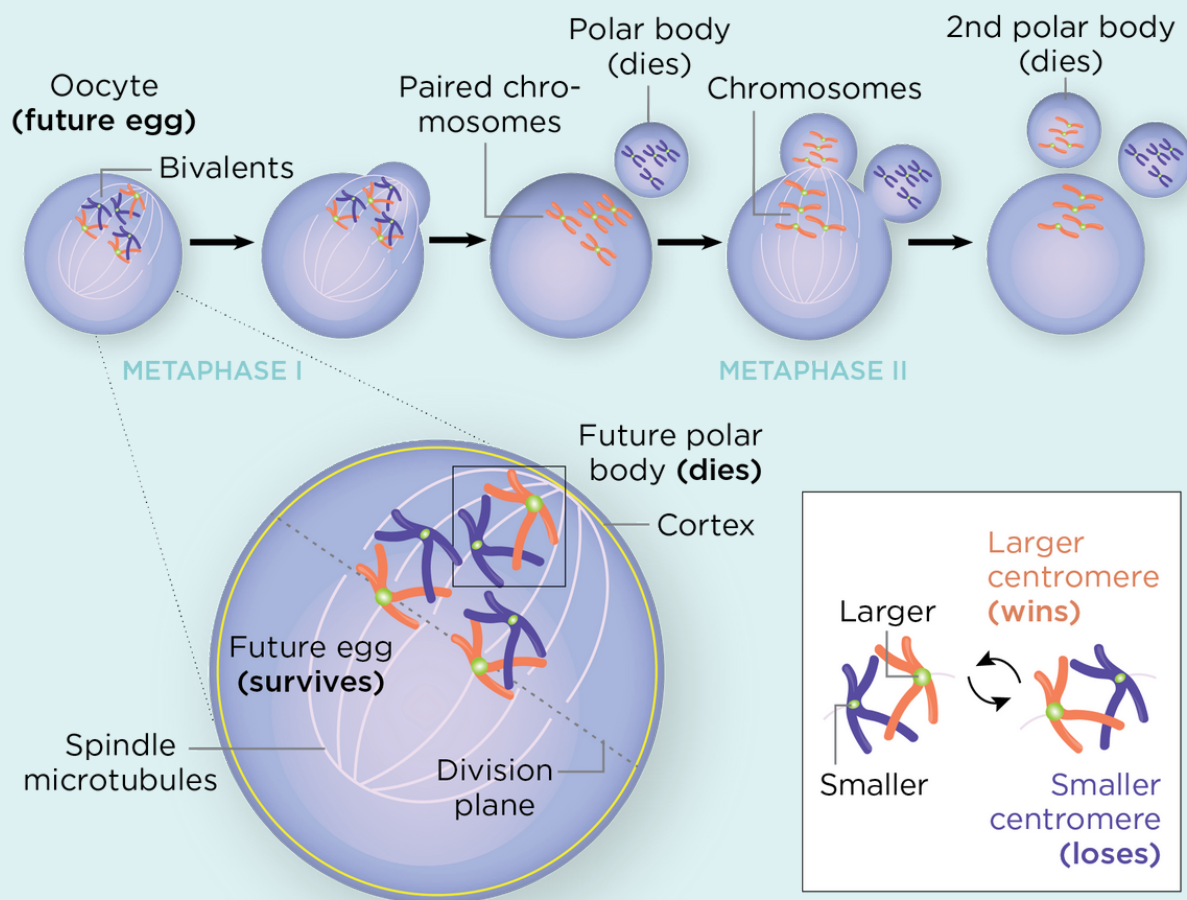
ADAPTED FROM DUDKA AND LAMPSON 2022

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Defending against centromere drive

The fitness costs to organisms of centromere drive are still unclear, but we expect that these costs depend on functional differences between the paired centromeres of homologous chromosomes: differential interaction of these centromeres with spindle microtubules, for example, may lead to segregation errors. Reducing these differences would reduce fitness costs to the organism.

Functional equalization of different centromeres could happen in two ways: by weakening the pathway selfish centromeres exploit to recruit destabilizing proteins, and/or by strengthening another recruitment pathway that is equal at all centromeres. Previous studies had shown that destabilizing proteins can be recruited both through kinetochores and through heterochromatin adjacent to the centromere. Our studies showed that selfish centromeres drive by amplifying the kinetochore pathway to recruit destabilizers, thus increasing functional differences. In contrast, heterochromatin is symmetric between centromeres of homologous chromosomes in our model systems, suggesting that this pathway makes centromeres more similar. These observations suggest that making the heterochromatin pathway dominant relative to the kinetochore pathway would suppress drive.

To test this idea experimentally in our hybrid mouse model system, we introduced a divergent variant of a centromere protein (CENP-C) that is rapidly evolving. We predicted that the divergent variant (taken from rat) would not interact optimally with mouse proteins involved in kinetochore formation, thereby weakening the kinetochore pathway. As a readout for functional asymmetry, we measured the position of the paired homologous chromosomes on the meiotic spindle. Chromosomes are positioned at the spindle equator when centromeres are functionally similar, as in a typical metaphase configuration, and off center when centromeres are functionally different. We found that chromosomes are positioned closer to the spindle equator when the kinetochore pathway is weakened, consistent with our prediction that the centromeres become functionally more similar. Conversely, when we weakened the heterochromatin pathway by knocking out the centromere protein CENP-B, which contributes to formation of heterochromatin near the centromere, we found that centromeres became functionally more different (i.e., more off center).

Thus, there appear to be competing parallel pathways: the kinetochore pathway exploited by selfish centromeres, and the heterochromatin pathway that promotes equal segregation. This means that proteins in both pathways can evolve to suppress drive by either weakening the kinetochore pathway or strengthening the heterochromatin pathway. Consistent with this prediction, by comparing rodent genomes in our study, we found signatures of adaptive evolution in components of both pathways, suggesting that changes in multiple centromere proteins can suppress the costs of drive.

Our and other groups' analyses are just beginning to probe the genetic conflict between selfish centromere DNA and rapidly evolving centromere proteins. We have experimental mouse model systems and a conceptual framework for drive and suppression, and we know which amino acid changes in centromere proteins have signatures of positive selection. We now face the challenge of designing experiments to dissect the functional consequences of these changes, which may be subtle. Meanwhile, other researchers are continuing to use monkeyflowers as a model system to study this conflict, taking advantage of the aforementioned natural variation and powerful population genetics.

And these aren't the only chromosomal loci that can drive: Loci such as the maize knobs provide opportunities to probe centromere-independent mechanisms of cheating in female meiosis and adaptations that suppress the associated fitness costs.

Microbial pathogens have evolved to exploit basic cellular processes, such as cytoskeletal dynamics, membrane trafficking, signal transduction, and the cell cycle, and studies of diverse pathogens have propelled many advances in cell biology. Similarly, investigating centromeres or other selfish loci as "pathogens" in the context of genetic conflict can provide a unique window into the biology of chromosome segregation and inheritance.