

Mimulus finds centromeres in the driver's seat

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Sandler and Novitski first pointed out in 1957 that chromosomes could selfishly exploit meiotic asymmetries to maximize their own transmission, in a process termed 'meiotic drive'. However, since then, only post-meiotic processes of non-Mendelian inheritance have received serious scientific attention in studies of transmission distortion. A recent study by Fishman and Willis puts the focus squarely back on meiotic drive. They found completely biased transmission of a centromere-linked locus from an outcrossing *Mimulus* species over that from an inbred species, providing the first direct evidence that centromeres can act as general, powerful meiotic drivers. This study suggests that, although difficult to detect experimentally, female meiotic drive is a major evolutionary force in nature.

Asymmetries in female meiosis

Chromosome segregation is a faithful process, orchestrated at the centromeric region of every chromosome to minimize any errors introduced during cell division and gametogenesis. It is symmetric in mitosis (in which a diploid cell divides into two diploid daughter cells) and male meiosis (in which a diploid cell divides into four haploid meiotic products, either sperm or pollen). In most plants and animals, however, female meiosis is asymmetric. Similar to male meiosis, four haploid meiotic products are formed, but only one of these is destined to become the egg; the other three form polar bodies that are evolutionary dead-ends. This asymmetry provides opportunities for selfish elements to exploit female meiosis to maximize their own transmission. Examples of such selfish elements include the maize knob elements that comprise blocks of repetitive DNA distal from the centromere that have the ability to attract microtubules [1,2].

It has been previously suggested that even centromeres can participate in this meiotic 'tug-of-war', competing with each other to attain a preferred position in female meiosis and thereby ensuring their own evolutionary dominance [3,4]. However, female meiotic drive is hard to detect. Maize knobs and gross chromosomal rearrangements (e.g. Robertsonian fusions and B chromosomes) are easy to detect cytologically and it is unsurprising that, until recently, these represented all the known examples of female meiotic drive. Conceivably, this kind of meiotic drive could be common but could go undetected in cytologically normal chromosomes, in the absence of detailed genotypic data. The recent study by Fishman

and Willis [5] thus is a major advance by providing just such genotypic data and confirming that even seemingly normal chromosomes can participate in this selfish battle for evolutionary dominance. The findings of Fishman and Willis might have strong implications not only for centromere evolution in plants and animals, but also for the processes by which two reproductively isolated species evolve from a common ancestor.

Female meiotic drive versus male post-meiotic dysfunction: not just semantics

Since the original proposal of meiotic drive [6], most work on transmission ratio distortion (TRD) has concentrated on post-meiotic mechanisms. A celebrated example is the Segregation Distorter (SD) system in *Drosophila melanogaster* [7,8]. First identified by Hiraizumi and colleagues [8,9], SD acts post-meiotically and leads to the reduced condensation and subsequent dysfunction of only those spermatids in the sperm bundle [10] that contain large arrays of a repetitive satellite [7,11]. SD-containing chromosomes are under selection to not carry these satellites, as this would result in self-destruction. Thus, in males heterozygous for SD, as much as 99% of the functional sperm end up containing SD, instead of 50% as would be expected under random Mendelian inheritance.

The eventual outcome of female meiotic drive and male meiotic dysfunction might appear to be the same: the increased propagation of the selfish element. But there are significant differences, perhaps the most important of which is that female meiotic drive does not entail any drop in fertility, or the number of eggs produced, whereas male post-meiotic dysfunction can result in a 50% drop in overall sperm count. In isolation, this might not seem profound. After all, most plants and animals make a significantly larger investment in egg production compared to pollen or sperm production. Thus, they can 'afford to' make more sperm and pollen than they could conceivably need. However, these pollen and sperm face stiff competition from individuals that have not been burdened with such a precipitous drop in fertility. If there was also competition between the X and Y chromosomes (e.g. if the X chromosome were to make the Y dysfunctional [12]) this would lead to a skewed sex ratio, further hampering a population with male post-meiotic dysfunction. Thus, from a population genetics viewpoint, female meiotic drive is considerably more benign and likely to succeed. (One interesting exception is in organisms such as birds, where females are the heterogametic sex; in this case, female meiotic drive can also lead to sex ratio distortion.)

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Mimulus linkage mapping uncovers transmission ratio distortion

Mimulus guttatus and *M. nasutus* are two closely related members of the yellow monkeyflower species complex that differ from each other in several life-history traits. *Mimulus guttatus* is a predominantly outcrossing species (reflecting the ancestral state) and has strikingly beautiful, insect-pollinated flowers, whereas *M. nasutus* is highly self-fertilizing (the derived state) and has reduced flowers (Figure 1a). These two species are only partially isolated and hybrids can be readily observed in the wild. Previously, Fishman, Willis and colleagues had used microsatellite and gene-based markers to construct a linkage map of these two species [13]. Such maps are useful for mapping the genetic basis of a variety of traits, both morphological and behavioral. The authors used this linkage map to analyse *M. nasutus* × *guttatus* F2 hybrids (in all crosses, the female parent is first) and revealed that several genomic regions exhibited TRD, where the inheritance pattern of the markers was strikingly non-Mendelian.

Fishman and Willis investigated the biological basis for the strongest of these TRDs, in Linkage Group 11 [5]. In this group, one of the markers showed a strong deviation from the Mendelian expectation of 1:2:1, instead being 0:2:2 (Figure 1a). Thus, it appeared that in the F1 × F1 cross, there was such a severe transmission bias against the *M. nasutus* allele that the cross became equivalent to one between the F1 and a *M. guttatus* homozygote. Such strong transmission bias is rare and the authors set about identifying the biological basis for this distorter (*D*) locus.

The usual suspects

Fishman and Willis began to test and eliminate alternate biological models for explaining the strong transmission ratio bias. First, using backcrosses of F1s to either parent,

they showed that transmission bias was only evident when the F1 female was heterozygous (Figure 1b). Heterozygous males showed only a slight transmission bias, ruling out the more traditional models of male post-meiotic dysfunction, such as SD. This experiment also excluded the possibility that post-fertilization selection among the zygotes based on nuclear genotype was the cause of TRD: although the same genotypes were generated in male F1 or female F1 backcrosses, only the female backcross was distorted. Second, the authors tested whether the *M. nasutus* cytoplasm had a role in distorting transmission. They found that even F1s containing *M. guttatus* cytoplasm showed the same TRD, ruling out conflict between the nucleus and cytoplasm as the cause of TRD. Third, the authors tested whether ovule–ovule or ovule–endosperm interactions could account for the distortion. Regardless of the physiological means by which this could occur, it would nonetheless involve the loss of all developing ovules or seeds with the disfavored phenotype. On comparing seed development in *D*-heterozygotes (where 50% lethality would be predicted under this model) and *D*-homozygotes (where no lethality is expected), the authors found no difference in either ovule or seed loss.

Finally, the authors constructed a set of near-isogenic lines (NILs) by repeated backcrossing of the F1s with the *M. nasutus* parental line. Under such a scheme, after four backcrossing generations, it was expected that >93% of the genome would be made homozygous for *M. nasutus* alleles (50% heterozygosity remains after one generation, 25% after two and only 6.25% after four). For most markers, the authors found that to be the case. However, for *D*, there still remained >90% heterozygosity, implying that the *D* locus could distort transmission repeatedly and autonomously. Thus, the authors concluded that heterozygosity of the *D* locus in female meiosis was the key to the

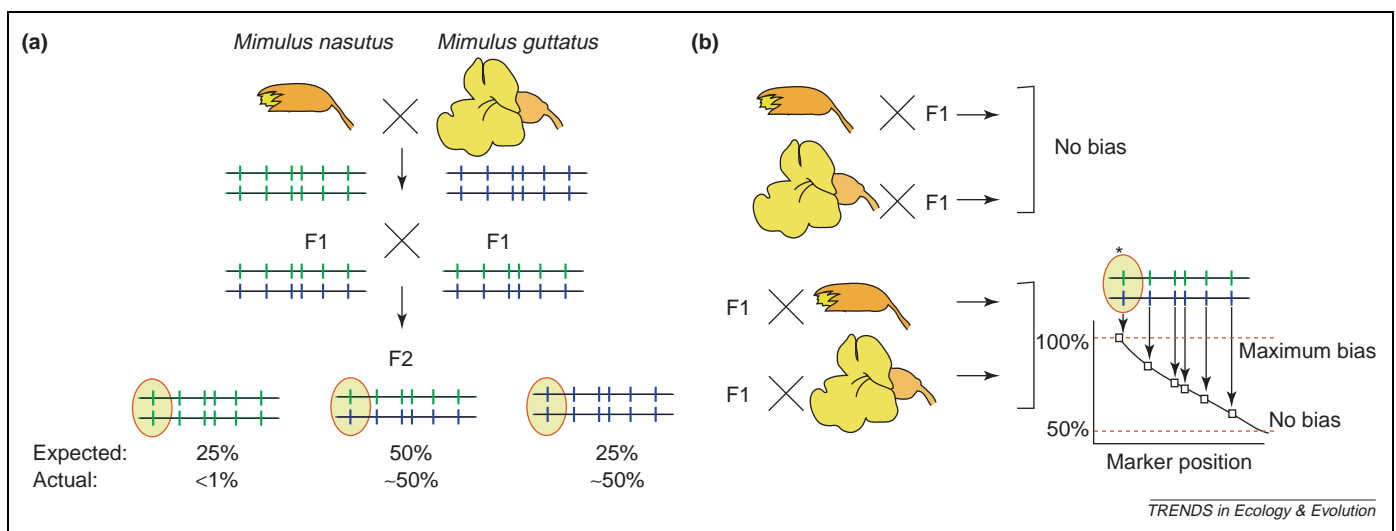


Figure 1. A novel meiotic drive locus in *Mimulus*. (a) Using crosses between *M. nasutus* (a selfer) and *M. guttatus* (an outcrosser), Fishman, Willis and colleagues tracked the inheritance pattern of microsatellite and gene-based amplified fragment length polymorphisms markers [13]. For the *D* locus (marked by the oval), these markers segregated to 0:2:2 (*M. nasutus* homozygotes: heterozygotes: *M. guttatus* homozygotes, respectively) rather than the Mendelian 1:2:1 expectation [5]. This implied that the *D* locus from *M. nasutus* was being selected against. (b) Using backcrosses of the F1 against either parental species (in all crosses, the female parent is shown first), the authors showed that transmission distortion was only evident when the F1 heterozygote was female, was independent of parental line, and was highest in the case of the *D* locus (nearly 100% distortion) but subsided to a more Mendelian pattern of 50% (no bias) as markers got more recombinationally distant. This implied that the *D* locus or a tightly linked locus was principally responsible for all the transmission distortion.

transmission distortion that they saw in Linkage group 11, providing unambiguous support for female meiotic drive.

Why the centromere?

In theory, any chromosomal element could distort female meiosis to its own advantage. However, distal chromosomal elements, such as the knob elements in maize, can only subvert meiosis at a later stage, during Meiosis II [2,14] where the maximal transmission advantage that they could hope to attain is 5/6, or 83.3% [2]. *D* distorted to nearly 100%, implying that it acts earlier, during Meiosis I [14]. The most probable chromosomal element that could distort at this early stage is the centromere, because orientation in Meiosis I is likely to be completely dependent on the centromere, the chromosomal site of microtubule attachments. Thus, Fishman and Willis conclude that the *D* locus of *M. guttatus* was closely linked to the centromere on Linkage group 11, and this was successfully driving against the *M. nasutus* centromere in female meiosis.

How is this drive achieved? One simple possibility is that the *D* centromere in *M. guttatus* is 'stronger' than in *M. nasutus*, in that it is more successful at recruiting microtubule attachments and orienting the meiotic tetrad to favor its own transmission [3,4,14]; thus, *D* is in a perfect position to be the ootid that is chosen to be the egg. The ability of the *D* locus to drive against other centromeres might have arisen *de novo* in *M. guttatus*. Alternatively, the ancestral ability of *D* to drive might have degenerated in the selfing *M. nasutus*, because the *D* locus no longer has adequate opportunities to exploit female meiosis to spread through the species.

Deleterious effects of centromere drive?

Previous studies have suggested that 'centromere drive' occurs, based on the discovery of positive selection on essential, single-copy centromere-binding proteins in plants and animals [3,15,16]. These proteins might act to suppress the predicted deleterious effects of centromere drive on male meiosis, where large differences in centromere strength could lead to meiotic dysfunction [3]. Some of the best evidence of these deleterious effects comes from humans, where 0.14% of the population is a carrier of a Robertsonian translocation (two acrocentric chromosomes fusing at their centromeres to give rise to a metacentric chromosome). Robertsonian fusions are otherwise isogenic with their parental acrocentric chromosomes and differ significantly only in their centromere location, size and strength. In women who are heterozygous for Robertsonian fusions, there is a preferential transmission of the Robertsonian chromosome during meiosis [17], but most men that carry Robertsonians have dysfunctional meiosis, and are sterile [18]. Thus, even in this brief snapshot of human evolution, centromere drive has had a profound impact.

What's in the future for *D*?

Some clear implications emerge from the Fishman and Willis study. Foremost, the authors predict that *D* is tightly linked to the centromere. The ongoing

development of bacterial artificial chromosome libraries will provide the resources to test this prediction cytologically by providing suitable probes for fluorescent *in situ* hybridization. A second prediction is that centromere strength for *D* will be higher in *M. guttatus* than in *M. nasutus*. Centromeres in most plants and animals comprise large arrays of repetitive satellites, and recombination-mediated events can lead to rapid expansions of the satellite arrays. It has been previously suggested that larger satellite arrays attract more centromere-binding proteins, thereby increasing their ability to attract microtubules (centromere 'strength') [19]. It will be interesting to see if different competitive abilities of *D* from these two species translate to different amounts of a particular satellite repeat expansion. Furthermore, different populations of the outcrossing *M. guttatus* might also vary in the amounts of satellite repeats, and possibly *D* ability.

Finally, these findings open up the possibility of genetic screens to identify which *trans*-acting factors can enhance or suppress TRD mediated by *D*. The success of the NILs backcrossing strategy where almost the entire genome could be made a *M. nasutus* homozygote without altering the drive ability of *D* suggests that there are only a few factors that participate in this process. Direct sequencing of genes encoding centromere-binding proteins, such as centromeric histones and CENP-C [3,15,16] in *M. guttatus* and *M. nasutus*, would reveal whether these 'usual suspects' have been subject to positive selection in the brief time since *M. guttatus* and *M. nasutus* separated. If so, the rapid divergence of the centromere (driver) and associated proteins (suppressor) could represent a form of epistatic interaction involving novel alleles of the essential chromosome segregation machinery [3]. Such epistatic interactions (termed Dobzhansky–Muller interactions) between novel species-specific alleles are thought to underlie the process by which one species separates to form two reproductively isolated species [20].

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