Testing a candidate meiotic drive locus identified by pool sequencing

Daniel A. Barbash,[1,*] Bozhou Jin,[1] Kevin H-C Wei,[2] Anne-Marie Dion-Côté[1,3,*]

[1]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA
[2]Department of Zoology, The University of British Columbia, Vancouver, British Columbia V6T1Z3, Canada
[3]Département de Biologie, Université de Moncton, Moncton, NB E1A 3E9, Canada

*Corresponding author: Department of Molecular Biology and Genetics, Cornell University, 401 Biotechnology Building, Ithaca, NY 14853, USA. Email: barbash@cornell.edu;
*Corresponding author: Département de Biologie, Université de Moncton, 18 Antonine-Maillet Ave., Moncton, NB E1A 3E9, Canada. Email: anne-marie.dion-cote@umoncton.ca

Meiotic drive biases the transmission of alleles in heterozygous individuals, such that Mendel's law of equal segregation is violated. Most examples of meiotic drive have been discovered over the past century based on causing sex ratio distortion or the biased transmission of easily scoreable genetic markers that were linked to drive alleles. More recently, several approaches have been developed that attempt to identify distortions of Mendelian segregation genome wide. Here, we test a candidate female meiotic drive locus in *Drosophila melanogaster*, identified previously as causing a ~54:46 distortion ratio using sequencing of large pools of backcross progeny. We inserted fluorescent visible markers near the candidate locus and scored transmission in thousands of individual progeny. We observed a small but significant deviation from the Mendelian expectation; however, it was in the opposite direction to that predicted based on the original experiments. We discuss several possible causes of the discrepancy between the 2 approaches, noting that subtle viability effects are particularly challenging to disentangle from potential small-effect meiotic drive loci. We conclude that pool sequencing approaches remain a powerful method to identify candidate meiotic drive loci but that genotyping of individual progeny at early developmental stages may be required for robust confirmation.

Keywords: meiotic drive; Drosophila; segregation distortion

Introduction

Mendel's first law that heterozygous individuals transmit each of their 2 alleles at equal frequency is well established. But rare cases of meiotic drivers that generate exceptions to this law have been found in many organisms, with some alleles biasing their own transmission almost completely. While meiotic drive has become a coverall term for a range of mechanisms causing transmission ratio distortion, we distinguish here 2 distinct processes that cause such distortion: "True" meiotic drive in females and gamete competition in males.

"True" meiotic drive can only occur in an animal or plant that has an asymmetric meiosis where 1 meiotic product becomes an egg or ovum while the other meiotic products are discarded as polar bodies (Novitski 1967; Pardo-Manuel de Villena and Sapienza 2001a; Chmátal et al. 2017). If an allele in a heterozygote can bias its transmission to the egg vs a polar body, then it will have a direct advantage and increase in frequency. Examples include knobs in maize, Robertsonian translocations in animals, structurally variant chromosomes in Drosophila, and centromeric alleles in Mimulus and mice (Rhoades 1942; Novitski 1967; Pardo-Manuel de Villena and Sapienza 2001b; Fishman and Saunders 2008; Iwata-Otsubo et al. 2017).

In contrast, male meiosis is typically symmetric, with all 4 meiotic products becoming gametes. There is thus no straightforward opportunity for alleles to directly bias their transmission during meiosis. However, 1 genotype of spermatocyte or spermatid can become nonfunctional during meiosis, or competition can occur postmeiotically between gametes carrying different alleles. Such processes would best be described as segregation distortion but are also often termed as meiotic drive (Sandler and Novitski 1957; Lindholm et al. 2016). It has been most readily detected as causing sex ratio distortion when driving alleles are located on sex chromosomes, and several autosomal distorters have also been characterized (Jaenike 2001; Herrmann and Bauer 2012; Larracuente and Presgraves 2012). Analogous systems also occur in fungi where they manifest as spore killers (Zanders and Johannesson 2021).

On the one hand, meiotic drivers bias their own transmission and will quickly go to fixation if left unchecked. On the other hand, if meiotic drive alleles have pleiotropic fitness costs, they will induce selective pressure for suppressors, potentially leading to recurrent cycles of new drive alleles arising followed by host evolution (Sandler and Novitski 1957; Crow 1991). Recurrent cycles of meiotic drive and suppression have been proposed to be a major contributor to karyotype evolution, speciation, sex chromosome turnover, and host–protein evolution (Buckler et al. 1999; Zwick et al. 1999; Henikoff et al. 2001; Pardo-Manuel de Villena and Sapienza 2001b; Meiklejohn and Tao 2010).

Most meiotic drivers have been discovered fortuitously, in crosses that happened to have linked markers present that showed very large deviations or by causing significant skewing of sex ratios. This leaves important questions open of how common meiotic drive is in natural populations and whether moderate strength drive alleles may be more prevalent than currently appreciated. Detection of small or moderate strength alleles can be particularly challenging to distinguish from viability effects, which are almost certainly more prevalent than meiotic drive.

New methods are needed to address these questions, particularly approaches that provide genome-scale detection. For male meiotic drive, sequencing large pools of motile sperm or pollen is a promising way to directly type gametes and look for unequal prevalence of alleles (Corbett-Detig et al. 2015, 2019). Sequencing large pools of progeny is another approach applicable to inferring either male or female meiotic drive or transmission ratio distortion in general (Bélanger et al. 2016; Seymour et al. 2019; Ren et al. 2021). We previously developed one such method, where F1 progeny are made between 2 fully sequenced inbred strains of Drosophila and then backcrossed to either one of the parental strains (Wei et al. 2017). Assuming Mendelian segregation, any SNPs that distinguish the 2 strains will either be heterozygous or homozygous in first-generation backcross (BC1) progeny for SNPs derived from the parent used to backcross. If many progeny are pooled together and sequenced, then one expects to detect a 75:25 ratio of parental alleles, with deviations indicating potential meiotic drive.

We performed several sets of crosses and sequenced pools of thousands of BC1 progeny to screen for female meiotic drive loci in crosses between several *Drosophila melanogaster* strains (Wei et al. 2017). In crosses with strains DGRP-129 and DGRP-882, we identified a locus mapping broadly to the chromosome 3 centromere region that confers an approximately 54:46 ratio in favor of the DGRP-882 allele. This result was replicated in both embryonic and adult progeny, suggesting that it is unlikely to be due to post-embryonic viability effects. The allele skew was only observed in the progeny of heterozygous females, not males, providing further evidence against viability effects and also strongly suggesting that it reflects female-specific meiotic drive.

As noted in that study, pooling has its caveats. Most importantly, any pooling strategy depends on the assumption that all individuals contribute equally to the DNA pool that is sequenced. This assumption would be violated if certain genotypes have more cells or have higher levels of polytenized nuclei; if so, they will disproportionately contribute DNA to the pool in a way that mimics the effects of meiotic drive.

The most direct way to distinguish true inference of meiotic drive is to genotype individual progeny, which provides an independent test not relying on pooling. Here, we do so for progeny of the same cross between DGRP-129 and DGRP-882.

Materials and methods
Construction of dfd-3XP3 marker plasmids
A schematic of the marker plasmid is shown in Fig. 1a. All oligonucleotide primers used in this study are listed in Supplementary Table 1. We amplified the 3xP3 promoter from pHD-DsRed-attP-w+ using oligos #1827 (contains a XhoI site) and #1828 (contains a BglII site) and cloned it into pdfd-GMR-nvYFP that had been digested with BamHI and XhoI to remove the GMR promoter (Le et al. 2006). pHD-DsRed-attP-w+ was a gift from Kate O'Connor-Giles (Addgene plasmid # 80898; http://n2t.net/addgene:80898; RRID:Addgene_80898). We then removed the Hsp70 promoter by digesting with PmeI and XhoI, forming blunt ends with Klenow

enzyme treatment, gel purifying the fragment, phosphorylating with T4 polynucleotide kinase, self-ligating the product, and confirming the promoter region by sequencing. We next added an attB site by PCRing from pTA-attB (Groth et al. 2000) with oligos #502 and #503 (each of which contains a NotI site), digesting the product with NotI, and cloning into a unique NotI site that is 5′ to the *dfd* promoter, to create the plasmid pAttB-dfd-3xP3-nvYFP. The plasmid was tested for expression by transforming into the strain P{nos-phiC31\int.NLS}X, P{CaryP}attP2.

We then replaced nvYFP with eGFP because the GFP signal in the eye shows less quenching by wild-type eye pigmentation. We PCR amplified eGFP from the plasmid pBS-KS-attB1-2-PT-SA-SD-0-EGFP-FlAsH-StrepII-TEV-3xFlag (Venken et al. 2011) using primers GFP-fwd and GFP-rev and PCR amplified attB-dfd-3XP3 from pAttB-dfd-3XP3-nvYfp with primers #1831 and #1832 and then used Gibson assembly to make plasmid #758 = pAttB-dfd-3xP3-GFP.

To replace nvYFP with mCherry, we PCR amplified mCherry from pQC NLS mCherry IX (Addgene plasmid 37354) using primers #1833 and #1834 and PCR amplified attB-dfd-3XP3 from pAttB-dfd-3XP3-nvYfp with primers #1831 and #1832 and performed Gibson assembly to generate plasmid #795 = pAttB-dfd-3xP3-mCherry.

Construction of targeting plasmids
The plasmid #779 = pHD-AttP-dfd-3xP3-mCherry-3R was generated by Gibson assembly of the vector backbone from pHD-DsRed digested with EcoRI and XhoI and the following 4 PCR fragments: attP, amplified from p697 using oligos #1835 and #1836; promoter and reporter sequences amplified from pAttB-dfd-3xP3-mCherry using oligos #1842 and #1843; left homology arm, amplified from DGRP-129 genomic DNA with oligos #1838 and #1839; and right homology arm, amplified from DGRP-129 genomic DNA with oligos #1840 and #1841. The full sequence of p779 is in Supplementary File 1.

Plasmid #778 = pHD-AttP-dfd-3xP3-eGFP-3R was similarly made except using pAttB-dfd-3xP3-eGFP as the template for the reporter sequence and DGRP-882 genomic DNA as the template for the homology arms. The full sequence of p778 is in Supplementary File 2.

Choosing target site and gRNA
We searched on Gbrowse for a region in the chromosome 3R pericentromere that is relatively free of repetitive DNA and contains transgenes with expressed visible markers, settling on 3R:4,150,200..4,150,600 (Flybase Release 6; Gramates et al. 2022). We chose a gRNA target site within this region at 4,150,207–4,150,226, with left and right homology arms at 4,148,824..4,150,224 and 4,150,224..4,151,624, respectively. The gRNA was cloned into pU6-BbsI-chiRNA (Gratz et al. 2013) using annealed oligos 1852 and 1853 to generate plasmid #773.

Microinjection and strain construction
pHD-AttP-dfd-3xP3-eGFP-3R plasmid along with Cas9-expressing plasmid pBS-Hsp70-Cas9 (Gratz et al. 2014) was injected into DGRP-882 by Genetivision. From 87 fertile G0 flies, we obtained 5 candidate transformants from which 1 was confirmed to express *Gfp* and have the correct integration site. We refer to this strain as DGRP-882 *Gfp*.

pHD-AttP-dfd-3xP3-mCherry-3R plasmid along with Cas9 protein was injected into DGRP-129 by Genetivision. From 131 fertile G0 flies, we obtained 3 candidate transformants from which 1 was
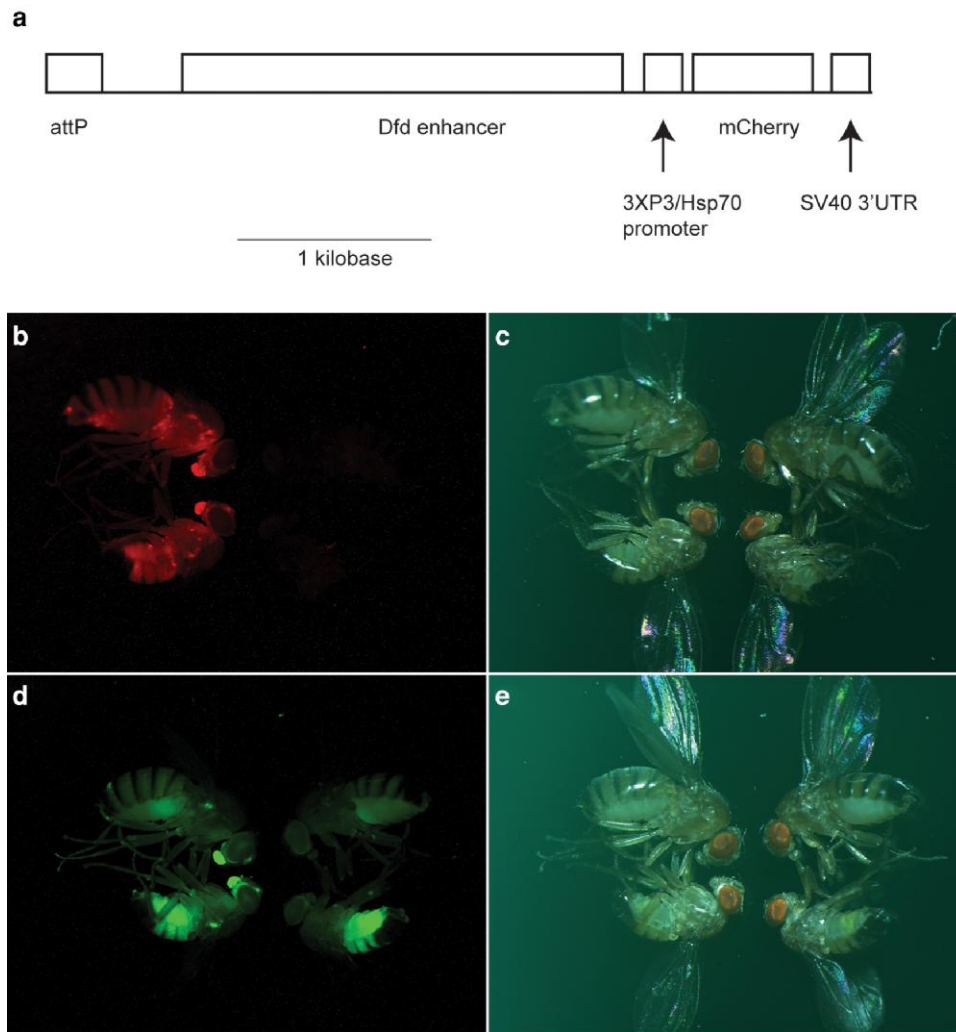
Fig. 1. Strong expression of Gfp and mCherry in DGRP strains heterozygous for transgenes in the 3R pericentromere. a) Diagram of the mCherry reporter driven by *dfd* regulatory sequences and the 3XP3 promoter. Full sequences of the targeting constructs that include this marker and the similar Gfp reporter are provided in Supplementary Files 1 and 2. b–e) Each panel shows a female (top) and male (bottom) carrying the transgene on the left side. On the right side in each panel, we show a female (top) and male (bottom) that do not carry the transgene in order to illustrate autofluorescent background, which is extensive in the Gfp channel but minimal in the mCherry channel. b) Image of mCherry signal and c) image under bright light of flies from a cross with DGRP-129 *mCherry*. d) Image of Gfp signal and e) image under bright light of flies from a cross with DGRP-882 *Gfp*. Wild-type females (Zambia line 379) were crossed separately to either DGRP-129 *mCherry* (b, c) or DGRP-882 *Gfp* (d, e) males at 25°C. F1 progeny were collected within 24 hours of eclosion, aged for 4 days, and then frozen at −80°C. Flies were thawed at room temperature and imaged using an Olympus SZX7 stereo microscope connected to an Olympus America S97809 camera.

confirmed to express *mCherry* and have the correct integration site. We refer to this strain as DGRP-129 *mCherry*.

Images of the transformed flies are shown in Figs. 1b-e. We confirmed the identity of the following strains prior to performing the experiments in Table 1 and immediately after performing the experiments in Table 2: DGRP-129, DGRP-882, DGRP-129 *mCherry*, and DGRP-882 *Gfp*. We used the RFLPs 2L5 and 3R2 that are diagnostic for these genotypes (MacKay et al. 2012).

Generation of DGRP-882 *w* mutant

We designed 2 gRNAs to the *white* gene using the w1 and w2 sequences from (Ren et al. 2013), cloned into the plasmid pU6-BbsI-chiRNA. These 2 plasmids along with in vitro-synthesized Cas9 mRNA were injected into DGRP-882 embryos by Rainbow Transgenic Flies. Two out of 17 fertile G0 embryos produced white mutant progeny, which were brother–sister mated to produce a homozygous DGRP-882 *w* stock.

Fly crosses

For the Gfp control crosses in Table 1, ~15 DGRP-882 *w* virgin females were crossed to ~15 DGRP-882 *w; Gfp* males. Two replicates of ~15–20 DGRP-882 *w; Gfp/+* virgin daughters were then crossed to ~15–20 DGRP-882 *w* males at 25°C. Vials were flipped every 1–2 days for a total of 6 flips. Similar crosses were done for the mCherry control, with DGRP-129 *mCherry* females crossed to DGRP-129 males, and then, F1 daughters crossed to DGRP-129 males.

For the crosses in Table 2 (diagrammed in Fig. 2a), 2 biological replicates of the crosses to generate F1s were performed (called 1350 and 1351 in Supplementary Table 2). Approximately 15–20 DGRP-882 *Gfp* females were crossed to ~15–20 DGRP-129 *mCherry* males for each replicate. F1 virgin females were confirmed to express both Gfp and mCherry and then crossed to males of either DGRP-129 or DGRP-882. Three sets (A, B, and C) were done with females aged 3–5 days and crosses set with 5

Table 1. Testing markers for potential viability effects.

Marker	No. with marker	No. without marker	No. with marker/total	P-value
Gfp	3463	3678	0.485	0.01132
mCherry	1693	1691	0.500	0.9863

DRGP-882 *w* females were crossed to DGRP-882 *Gfp* males. F1 DGRP-882 *w/Y*; *Gfp/+* males were mated to DRGP-882 *w* females. F2 DGRP-882 *w*; *Gfp/+* females were then mated to DRGP-882 *w* males and progeny scored. DGRP-129 *mCherry* females were mated to DGRP-129 males. F1 DGRP-129 *mCherry/+* females were mated to DGRP-129 males and progeny scored. Significance of the ratio was tested using binom.test of the number of progeny carrying the marker relative to the total, given an expectation of 0.5 (R Core Team 2017). Data from each individual vial are shown in Supplementary Table 2.

females and 5 males, with vials flipped every 1–2 days. As we found that many parents died by the third vial, we increased the number of parents to ~10–14 of each sex for 2 more sets; the females for these sets (D and E) were aged 8–11 days by happenstance. Only female progeny were scored, as was done in Wei et al. (2017). mCherry[+] females were first separated out and counted. Subsets were checked to confirm that were Gfp[−], but this was not done systematically. Then, all females scored as mCherry[−] were examined to confirm that they were Gfp[+].

Results

We wished to insert easily scorable fluorescent markers that express both early in development and in adults, near the putative meiotic drive region of the chromosome 3 centromeres of both candidate strains. A *dfd-GMR* construct provides expression in embryos and in the adult proboscis via the *dfd* enhancer and in adult eyes via the *GMR* promoter (Le et al. 2006). We replaced the *GMR* promoter with a 3XP3 promoter to obtain higher expression in the eyes and made versions expressing either Gfp or mCherry.

We introduced *dfd-3XP3-Gfp* into DGRP-882 and *dfd-3XP3-mCherry* into DGRP-129 at a pericentromeric site on chromosome 3 using CRISPR/Cas9-mediated recombination. Both transgenes expressed well (Fig. 1). Signal in the eye driven by 3XP3 was difficult to detect at low magnification due to wild-type eye pigmentation. We additionally observed high expression of mCherry in the abdomen, which is driven by the 3XP3 promoter (Champer et al. 2017). A similar signal cannot be detected with Gfp due to the high background in abdominal tissues in non-Gfp flies (Fig. 1d). We therefore relied on expression in the proboscis to score Gfp in adults.

We were able to establish and maintain lines that are viable and fertile as homozygotes for each of the 2 resulting strains. It was important though to determine whether either marker has any viability effect, as that could impair its use in accurately assessing a candidate meiotic driver of modest effect.

We therefore tested for potential dominant effects of each marker within its strain background, using a *white* mutant background for DGRP-882 in order to more easily score 3XP3-Gfp. We found that mCherry had no effect on viability in DGRP-129 but that Gfp caused a small but significant reduction in DGRP-882 (Table 1).

We next tested for potential meiotic drive in heterozygous DGRP-129-mCherry[+]/DGRP-882-Gfp[+] F1 females (Table 2; Fig. 2a). We performed 2 sets of crosses, backcrossing the F1 females to DGRP-129 and DGRP-882 males, respectively. By scoring 6,027 and 4,672 progeny in the 2 sets, respectively, we had power to detect deviations from Mendelian expectations as low as ~51.8%–52% (Fig. 2b). While we found no significant differences in progeny ratios between the 2 sets of crosses, both sets gave

Table 2. Crosses to test for meiotic drive in DGRP-129/DGRP-882 heterozygotes.

Sire	No. Gfp+	No. mCherry+	Ratio Gfp+/total	P-value
DGRP-129	2931	3096	0.486	0.0346
DGRP-882	2216	2456	0.474	0.00047

DGRP-882 *Gfp* females were crossed to DGRP-129 *mCherry* males. F1 virgin females were then crossed to males of the genotype listed in column 1 ("Sire"). Significance of the ratio was tested using binom.test of the number of *Gfp*[+] relative to the total, given an expectation of 0.5 (R Core Team 2017). Data from each individual vial are shown in Supplementary Table 2, including a small number of ambiguous flies that are not included in this table.

small but significant deficits in the expected number of Gfp[+] progeny, with ratios of 48.6 and 47.4%, respectively. Note that this deviation is opposite in direction to our hypothesis that DGRP-882 contains a mild female meiotic driver when heterozygous to DGRP-129.

This small deficit of Gfp[+] progeny in our experimental crosses is very similar to the 48.5% value of Gfp[+] progeny in our control crosses (Table 1). Assuming that the expression of Gfp does not have variable effects on viability in different genetic backgrounds, the most straightforward conclusion is that the small but significant deviations we observed in the experimental crosses are due to viability effects, not meiotic drive.

Discussion

What then accounts for the different results here compared to Wei et al. (2017)? Even subtle effects could have a strong impact, given the relatively low magnitude of the putative meiotic drive locus reported in Wei et al. (2017). We consider 4 possibilities.

1) Environmental effects. The experiments here were performed several years after those in Wei et al. (2017), with possible differences such as batch effects of components used for Drosophila media and building environment occurring.

2) Genetic drift. We confirmed the identity of the DGRP stocks during this study using diagnostic RFLPs, but that does not preclude the possibility that modifier alleles could have evolved over the several years in which these stocks were maintained between the 2 studies.

3) Marker variability. The fluorescent markers used here to genotype the individual flies had strong signals that we found straightforward to score. Given that they are inserted in pericentric heterochromatin, it is nevertheless possible that their expression might be variable or even suppressed in a small proportion of flies. It is also possible that expression is variable in some genotypes but not others. Variable expression in the experimental progeny that are heterozygous, relative to expression within the pure-strain homozygous backgrounds, would lead to misinferences. We note, however, that only 5 flies from the crosses in Table 2 were ambiguous and could not be scored (Supplementary Table 2), suggesting that marker expression is highly penetrant.

4) Background-dependent viability and developmental effects. Viability and developmental effects that vary across genetic backgrounds could cause discrepancies similar to those described above for marker variability. In this study, we found that the *dfd-3XP3-Gfp* marker causes a slight reduction in viability in a pure DGRP-882 background (Table 1). Because we saw nearly the same deficit of Gfp[+] progeny in the experimental crosses (Table 2), we concluded that there is no meiotic drive effect. This conclusion, however, assumes
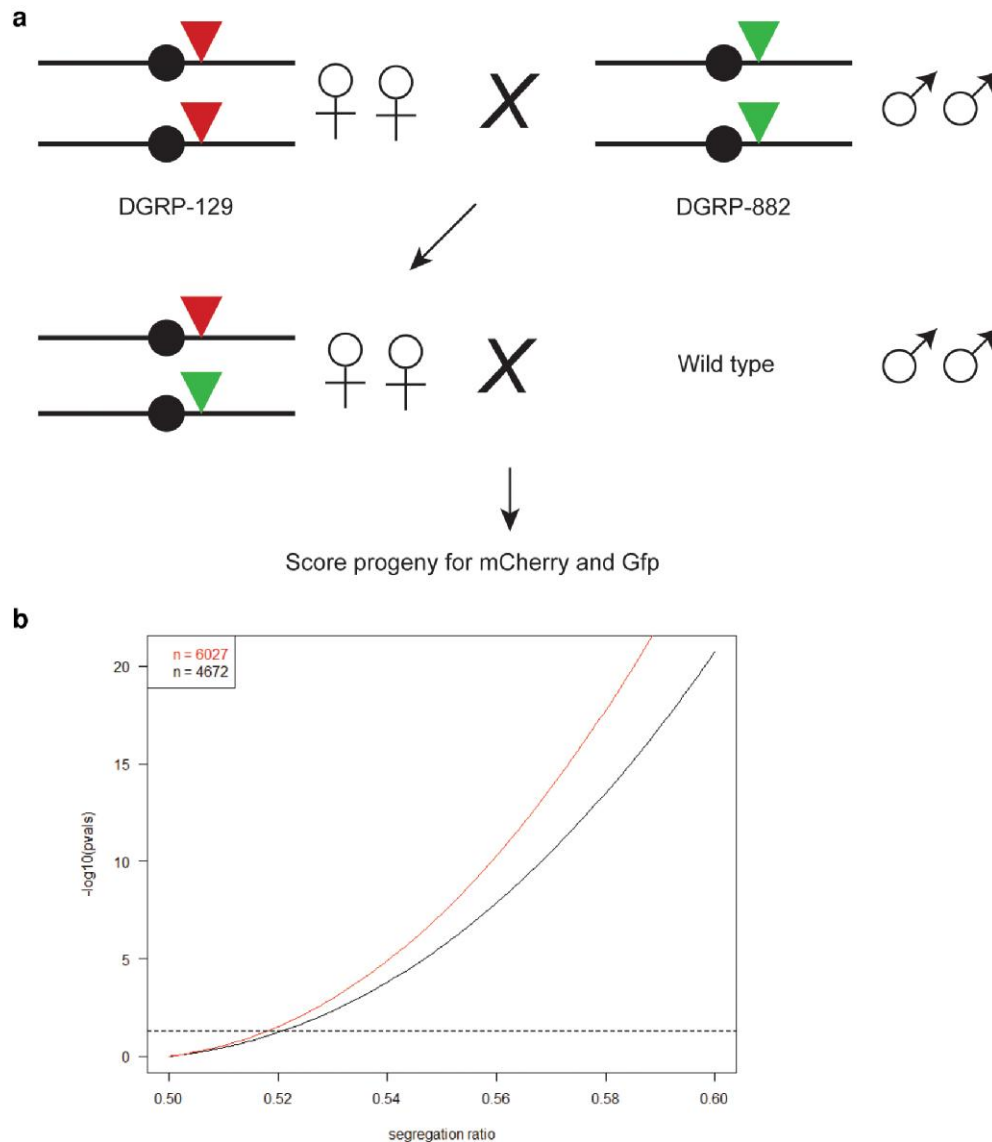
Fig. 2. Diagram and power calculations of crosses to detect meiotic drive performed in Table 2. a) Red and green triangles represent dfd-3XP3 transgenes inserted in the chromosome 3 pericentromere, expressing mCherry and Gfp, respectively. Wild-type males were either DGRP-129 or DGRP-882. b) Statistical power simulation of non-Mendelian segregation rates at different population sizes. Given the number of individuals in the 2 collections (n = 4,672 or 6027), we simulated chi-square statistics at different levels of segregation distortion (x-axis) and calculated the resulting P-value (y-axis, plotted in negative log scale) using the chisq.test function in R v4.2.2 (R Core Team 2017). Horizontal dotted line demarcates a P-value cutoff of 0.05.

that any viability effect of the marker is constant regardless of background. Swapping the markers by creating new DGRP-129-Gfp[+] and DGRP-882-mCherry[+] strains and repeating the experiment would help to understand potential marker effects.

A broader range of potential developmental effects needs to be considered with the pooling approach used in Wei et al. (2017). Differences between genotypes in their number of cells and/or degree of polytenization could cause skews in allele frequencies that resemble meiotic drive effects. Wei et al. (2017) found that the allele skew only occurred in crosses where female parents were heterozygous but not in the reciprocal cross where male parents were heterozygous. This result was an important piece of evidence arguing for female meiotic drive rather than developmental effects. But as Wei et al. (2017) noted, it assumes that any developmental effects are constant across maternal and paternal genotypes. If

instead developmental effects interact with or are caused by, for example, maternal effects, they could lead to misinference of meiotic drive.

Any potential effects that vary among genotypes are challenging to control for. We suggest that the least biased approach would be to genotype individual embryos, as viability effects are best minimized by genotyping as early in development as possible. Developing high-throughput, robust, and inexpensive methods to genotype large numbers of embryos (at the earliest possible developmental stage) may provide the means to reliably screen for and test moderate-strength meiotic drive loci.

Nevertheless, we suggest that pooled sequencing methods remain a potentially powerful approach to identify loci causing meiotic drive, and more generally, transmission ratio distortion, due to their ability to screen the entire genome. It will though be important to confirm any candidates identified using a distinct method.

Data availability

Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Supplemental material available at G3 online.

Acknowledgments

We are grateful to Greg Beitel for providing the dfd-GMR-nvYFP plasmid, Shuqing Ji for the help performing crosses, and Dean Castillo and Sarah E. Lower for technical advice.

Funding

Conflicts of interest

The authors declare no conflict of interest.

Literature cited

Bélanger S, Esteves P, Clermont I, Jean M, Belzile F. 2016. Genotyping-by-sequencing on pooled samples and its use in measuring segregation bias during the course of androgenesis in barley. Plant Genome. 9(1):1–13. doi:10.3835/plantgenome 2014.10.0073.

Buckler ES, Phelps-Durr TL, Buckler CS, Dawe RK, Doebley JF, Holtsford TP. 1999. Meiotic drive of chromosomal knobs reshaped the maize genome. Genetics. 153(1):415–426. doi:10.1093/genetics/153.1.415.

Champer J, Reeves R, Oh SY, Liu C, Liu J, Clark AG, Messer PW. 2017. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. PLoS Genet. 13(7):e1006796. doi:10.1371/journal.pgen.1006796.

Chmátal 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. doi:10.1007/978-3-319-58592-5_16.

Corbett-Detig R, Jacobs-Palmer E, Hartl D, Hoekstra H. 2015. Direct gamete sequencing reveals no evidence for segregation distortion in house mouse hybrids. PLoS One. 10(6):e0131933. doi:10.1371/journal.pone.0131933.

Corbett-Detig R, Medina P, Frérot H, Blassiau C, Castric V. 2019. Bulk pollen sequencing reveals rapid evolution of segregation distortion in the male germline of Arabidopsis hybrids. Evol Lett. 3(1): 93–103. doi:10.1002/evl3.96.

Crow JF. 1991. Why is Mendelian segregation so exact? Bioessays. 13(6):305–312. doi:10.1002/bies.950130609.

Fishman L, Saunders A. 2008. Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. Science. 322(5907):1559–1562. doi:10.1126/science.1161406.

Gramates LS, Agapite J, Attrill H, Calvi BR, Crosby MA, dos Santos G, Goodman JL, Goutte-Gattat D, Jenkins VK, Kaufman T, *et al.* 2022.

Fly Base: a guided tour of highlighted features. Genetics. 220(4): iyac035. doi:10.1093/genetics/iyac035.

Gratz SJ, Cummings AM, Nguyen JN, Hamm DC, Donohue LK, Harrison MM, Wildonger J, O'Connor-Giles KM. 2013. Genome engineering of Drosophila with the CRISPR RNA-guided Cas9 nuclease. Genetics. 194(4):1029–1035. doi:10.1534/genetics.113.152710.

Gratz SJ, Ukken FP, Rubinstein CD, Thiede G, Donohue LK, Cummings AM, O'Connor-Giles KM. 2014. Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in Drosophila. Genetics. 196(4):961–971. doi:10.1534/genetics.113.160713.

Groth AC, Olivares EC, Thyagarajan B, Calos MP. 2000. A phage integrase directs efficient site-specific integration in human cells. Proc Natl Acad Sci U S A. 97(11):5995–6000. doi:10.1073/pnas.090527097.

Henikoff S, Ahmad K, Malik HS. 2001. The centromere paradox: stable inheritance with rapidly evolving DNA. Science. 293(5532):1098–1102. doi:10.1126/science.1062939.

Herrmann BG, Bauer H. 2012. Evolution of the House Mouse. Cambridge University Press. p. 297–314.

Iwata-Otsubo A, Dawicki-McKenna JM, Akera T, Falk SJ, Chmátal LS, 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. Curr Biol. 27(15):2365–2373.e8. doi:10.1016/j.cub.2017.06.069.

Jaenike J. 2001. Sex chromosome meiotic drive. Annu Rev Ecol Syst. 32(1):25–49. doi:10.1146/annurev.ecolsys.32.081501.113958.

Larracuente AM, Presgraves DC. 2012. The selfish Segregation Distorter gene complex of *Drosophila melanogaster*. Genetics. 192(1):33–53. doi:10.1534/genetics.112.141390.

Le T, Liang Z, Patel H, Yu MH, Sivasubramaniam G, Slovitt M, Tanentzapf G, Mohanty N, Paul SM, Wu VM, *et al.* 2006. A new family of Drosophila balancer chromosomes with a w- dfd-GMR yellow fluorescent protein marker. Genetics. 174(4):2255–2257. doi:10.1534/genetics.106.063461.

Lindholm AK, Dyer KA, Firman RC, Fishman L, Forstmeier W, Holman L, Johannesson H, Knief U, Kokko H, Larracuente AM, *et al.* 2016. The ecology and evolutionary dynamics of meiotic drive. Trends Ecol Evol. 31(4):315–326. doi:10.1016/j.tree.2016.02.001.

Mackay TFC, Richards S, Stone EA, Barbadilla A, Ayroles JF, Zhu D, Casillas S, Han Y, Magwire MM, Cridland JM, *et al.* 2012. The Drosophila melanogaster genetic reference panel. Nature. 482(7384):173–178. doi:10.1038/nature10811.

Meiklejohn CD, Tao Y. 2010. Genetic conflict and sex chromosome evolution. Trends Ecol Evol. 25(4):215–223. doi:10.1016/j.tree.2009.10.005.

Novitski E. 1967. Nonrandom disjunction in Drosophila. Annu Rev Genet. 1(1):71–86. doi:10.1146/annurev.ge.01.120167.000443.

Pardo-Manuel de Villena F, Sapienza C. 2001a. Nonrandom segregation during meiosis: the unfairness of females. Mamm Genome. 12(5):331–339. doi:10.1007/s003350040003.

R Core Team. 2017. R: a Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Pardo-Manuel de Villena F, Sapienza C. 2001b. Female meiosis drives karyotypic evolution in mammals. Genetics. 159(3):1179–1189. doi:10.1093/genetics/159.3.1179.

Ren P, Deng F, Chen S, Ran J, Li J, Yin L, Wang Y, Yin H, Zhu Q, Liu Y. 2021. Whole-genome resequencing reveals loci with allelic transmission ratio distortion in F1 chicken population. Mol Genet Genomics. 296(2):331–339. doi:10.1007/s00438-020-01744-z.

Ren X, Sun J, Housden BE, Hu Y, Roesel C, Lin S, Liu L-P, Yang Z, Mao D, Sun L, *et al.* 2013. Optimized gene editing technology for Drosophila melanogaster using germ line-specific Cas9. Proc Natl Acad Sci U S A. 110(47):19012–19017. doi:10.1073/pnas.1318481110.

Rhoades MM. 1942. Preferential segregation in maize. Genetics. 27(4): 395–407. doi:10.1093/genetics/27.4.395.

Sandler L, Novitski E. 1957. Meiotic drive as an evolutionary force. Am Nat. 91(857):105–110. doi:10.1086/281969.

Seymour DK, Chae E, Arioz BI, Koenig D, Weigel D. 2019. Transmission ratio distortion is frequent in Arabidopsis thaliana controlled crosses. Heredity (Edinb). 122(3):294–304. doi:10.1038/s41437-018-0107-9.

Venken KJT, Schulze KL, Haelterman NA, Pan H, He Y, Evans-Holm Martha, Carlson Joseph W, Levis Robert W, Spradling AC, Hoskins RA, *et al.* 2011. MiMIC: a highly versatile transposon insertion resource for engineering *Drosophila melanogaster* genes. Nat Methods. 8(9):737–743. doi:10.1038/nmeth.1662.

Wei KH-C, 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(1):451–465. doi:10.1534/genetics.116.197335.

Zanders S, Johannesson H. 2021. Molecular mechanisms and evolutionary consequences of spore killers in ascomycetes. Microbiol Mol Biol Rev. 85(4):e0001621. doi:10.1128/MMBR.00016-21.

Zwick ME, Salstrom JL, Langley CH. 1999. Genetic variation in rates of nondisjunction: association of two naturally occurring polymorphisms in the chromokinesin nod with increased rates of nondisjunction in Drosophila melanogaster. Genetics. 152(4): 1605–1614. doi:10.1093/genetics/152.4.1605

Editor: E. Betran