**Diverse mating phenotypes impact the spread of *wtf* meiotic drivers in
*Schizosaccharomyces pombe***

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Abstract

Meiotic drivers are genetic elements that break Mendel's law of segregation to be transmitted into more than half of the offspring produced by a heterozygote. The success of a driver relies on outcrossing (mating between individuals from distinct lineages) because drivers gain their advantage in heterozygotes. It is, therefore, curious that *Schizosaccharomyces pombe*, a species reported to rarely outcross, harbors many meiotic drivers. To address this paradox, we measured mating phenotypes in *S. pombe* natural isolates. We found that the propensity for cells from distinct clonal lineages to mate varies between natural isolates and can be affected both by cell density and by the available sexual partners. Additionally, we found that the observed levels of preferential mating between cells from the same clonal lineage can slow, but not prevent, the spread of a *wtf* meiotic driver in the absence of additional fitness costs linked to the driver. These analyses reveal parameters critical to understanding the evolution of *S. pombe* and help explain the success of meiotic drivers in this species.

Introduction

Mating behaviours have long been a focus of art, literature, and formal scientific inquiry. This interest stems, in part, from the remarkable importance of mate choice on the evolution of species. Outcrossing and inbreeding represent distinct mating strategies that both have potential evolutionary benefits and costs (Glemin et al. 2019; Muller 1932; Otto and Lenormand 2002). For example, outcrossing (mating between individuals from distinct lineages) can be beneficial because it can help purge deleterious alleles from a line of descent, but it can also be costly as it can promote the

spread of selfish genes (Burt and Trivers 1998; Crow 1988; Hurst and Werren 2001; McDonald et al. 2016; Zeyl et al. 1996).

Meiotic drivers represent one type of selfish genetic element that relies on outcrossing to persist and spread in a population (Lindholm et al. 2016; Novitski 1957). These loci can manipulate the process of gametogenesis to bias their own transmission into gametes at the expense of the rest of the genome (Burt and Trivers 2006). Meiotic drivers are often considered selfish or parasitic genes because they generally offer no fitness benefits to their hosts and are instead often deleterious or linked to deleterious alleles (Dyer et al. 2007; Higgins et al. 2018; Klein et al. 1984; Rick 1966; Planchart et al. 2005; Taylor et al. 1999; Wilkinson and Fry 2001). As inbreeding is thought to inhibit the spread of selfish genes like drivers, drivers are predicted to be unsuccessful in species that rarely outcross (Burt and Trivers 1998; Hurst and Werren 2001). This assumption appears to be challenged in several fungal species, including the fission yeast *Schizosaccharomyces pombe* (Grognet et al. 2014; Hammond et al. 2012; Svedberg et al. 2021; van der Gaag et al. 2000; Vogan et al. 2021).

Generally, *S. pombe* cells grow as haploids and their mating (fusion of two haploids of opposite mating type) generates diploid zygotes that can then undergo meiosis to generate haploid progeny known as spores (Figure 1-figure supplement 1A). Mating in *S. pombe* is widely thought to preferentially occur between daughter cells clonally derived from a common progenitor via a recent mitotic division, which we refer to as 'same-clone mating' (Figure 1-figure supplement 1B; Egel 1977; Gutz and Doe 1975;

Miyata and Miyata 1981; Billiard et al 2012; Perrin 2011). The support for this idea is described below. Despite this notion that *S. pombe* cells preferentially undergo same-clone mating (leading to inbreeding), *S. pombe* isolates host multiple meiotic drivers (Bravo Nunez et al. 2020b; Eickbush et al. 2019; Farlow et al. 2015; Hu et al. 2017; Nuckolls et al. 2017; Zanders et al. 2014).

In the wild, a minority of *S. pombe* strains are heterothallic, meaning they have a fixed mating type (h^+ or h^- ; Gutz and Doe 1975; Nieuwenhuis and Immler 2016; Schlake 1993). Heterothallic *S. pombe* strains must mate with non-clonally related cells of the opposite mating type to complete sexual reproduction (Egel 1977; Gutz and Doe 1975; Leupold 1949; Miyata and Miyata and Miyata 1981; Nieuwenhuis and Immler 2016; Osterwalder 1924; Schlake 1993). However, most isolates of *S. pombe* are homothallic, i.e., they can switch between the two mating types (h^+ and h^-) during clonal expansion via mitosis (Figure 1-figure supplement 1B; Eie 1987; Gutz and Doe 1975; Klar 1990; Nieuwenhuis et al. 2018; Singh and Klar 2003). Homothallism in *S. pombe* enables mating to occur between cells clonally derived from the same progenitor via mitosis (Billiard et al. 2012). Mating can even occur between two cells produced by a single mitotic cell division (Egel 1977; Gutz and Doe 1975; Miyata and Miyata 1981).

It is often assumed that homothallic fungi, like *S. pombe*, do not generally mate with non-clonally related cells. It is important to note, however, that homothallism in fungi does not inherently lead to mating between between clonally related cells (Giraud et al. 2008). Under some conditions, such as when gametes are dispersed and finding a mate

is costly, homothallism can be a beneficial strategy for outcrossing species as it ensures gamete compatibility (Billiard et al 2012). In addition, population genetic analyses of one homothallic fungus, *Sclerotinia sclerotiorum*, supports that homothallism is compatible with frequent outcrossing (Attanayake et al 2014).

Very little is known about the ecology of *S. pombe* in the wild, including how often *S. pombe* cells outcross (Jeffares et al. 2018). In the lab, the mating propensities of *S. pombe* have mostly been investigated in derivatives of a strain first isolated from French wine in 1921 (Osterwalder 1924). In this work, we will refer to derivatives of this isolate as “*Sp.*” Distinct homothallic *Sp* strains can be induced to mate in the lab (i.e., outcrossed), but such crosses are significantly more challenging to execute than crosses between heterothallic isolates. The relative difficulty associated with outcrossing homothallic strains has been attributed to preferential same-clone mating (Ekwall and Thon 2017; Forsburg and Rhind 2006). Microscopy experiments have supported the idea that homothallic *Sp* haploids tend to undergo same-clone mating (Bendezu and Martin 2013; Egel 1977; Leupold 1949; Miyata and Miyata 1981). However, the precise level of same-clone mating when homothallic *S. pombe* cells are among nonclonal sexual partners has not, to our knowledge, been formally reported for any isolate.

Population genetic analyses have provided additional support for the notion that *S. pombe* inbreeds. There are two major *S. pombe* lineages that diverged between 2,300 and 78,000 years ago (Tao et al. 2019; Tusso et al. 2019). Much of the sampled

variation within the species represents different admixed hybrids of those two ancestral lineages resulting from an estimated 20-60 outcrossing events (Tusso et al. 2019). This low outcrossing rate could result from limited opportunities to outcross, preferential same-clone mating, or a combination of the two. It is important to note, however, that the outcrossing rate estimates in *S. pombe* are likely low as pervasive meiotic drive and decreased recombination in heterozygotes suppress the genetic signatures that are used to infer outcrossing (Bravo Nunez et al. 2020b; Hu et al. 2017; Nuckolls et al. 2017; Zanders et al. 2014).

Accounting for all these factors to generate a more accurate *S. pombe* outcrossing rate from population genetic data is currently an insurmountable obstacle for several reasons. First, the landscape of meiotic drivers and their suppressors is complex in that more than four drivers and more than eight suppressors of drive could be present in a given heterozygote produced by outcrossing (Eickbush et al. 2019; Bravo Nunez et al. 2020a). Predicting how those factors will affect allele transmission is well beyond the capabilities of current population genetic models, even if the epistatic relationships within the drive systems were understood, which they currently are not. In addition to the drivers, *S. pombe* isolates contain an array of chromosomal rearrangements and other unknown barriers to recombination (Avelar et al. 2013; Brown et al. 2011; Jeffares et al. 2017; Tusso et al. 2019; Zanders et al. 2014). Importantly, recombination rates are also inextricably linked to the complex drive landscape because the presence of drivers also affects the recombination rate within viable offspring, independent of chromosomal rearrangements (Bravo Nunez et al. 2020b).

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134 Given those limitations, the current laboratory knowledge and outcrossing rate
135 estimates suggest that drivers would infrequently have the opportunity to act in *S.*
136 *pombe* (Egel 1977; Ekwall and Thon 2017; Forsburg and Rhind 2006; Gutz and Doe
137 1975; Miyata and Miyata 1981; Tusso et al. 2019). In fact, the notion that *S. pombe*
138 infrequently outcrosses has even been used to challenge the idea that *S. pombe*
139 meiotic drivers are truly selfish genes that persist due to meiotic drive (Sweigart et al.
140 2019). Nonetheless, the *S. pombe* genome houses numerous meiotic drive genes from
141 the *wtf* gene family (Bravo Nunez et al. 2018; Bravo Nunez et al. 2020a; Eickbush et al.
142 2019; Hu et al. 2017; Nuckolls et al. 2017).

143

144 The *wtf* drivers destroy the meiotic products (spores) that do not inherit the driver from a
145 heterozygote. Each *wtf* drive gene encodes both a Wtf^{poison} and an Wtf^{antidote} protein
146 that, together, execute targeted killing of the spores that do not inherit the *wtf* driver (Hu
147 et al. 2017; Nuckolls et al. 2017). In the characterized *wtf4* driver, the Wtf4^{poison} protein
148 assembles into toxic protein aggregates that are packaged into all developing spores.
149 The Wtf4^{antidote} protein co-assembles with Wtf4^{poison} only in the spores that inherit *wtf4*
150 and likely neutralizes the poison by promoting its trafficking to the vacuole (Nuckolls et
151 al. 2020). Spore killing by *wtf* drivers leads to the loss of about half of the spores and
152 almost exclusive transmission (>90%) of the *wtf* driver from a heterozygote (Hu et al.
153 2017; Nuckolls et al. 2017). Despite their heavy costs to the fitness of heterozygotes,
154 the drivers are successful in that all assayed *S. pombe* isolates contain multiple *wtf*
155 drivers (4-14 drivers; Bravo Nunez et al. 2020a; Eickbush et al. 2019; Hu et al. 2017). In

addition to those drivers, the *S. pombe* isolates also contain between 8-17 suppressors of drive that encode only a Wtf^{antidote} protein.

In this work, we exploited the tractability of *S. pombe* to better understand how mating phenotypes, particularly inbreeding propensity, could affect the spread of a meiotic driver in this species. Despite limited genetic diversity amongst isolates, we observed natural variation in inbreeding propensity and other mating phenotypes. Some natural isolates preferentially undergo same-clone mating in the presence of a potential outcrossing partner, whereas others mate more randomly. Additionally, we found that the level of same-clone mating can be altered by cell density and affected by the available sexual partners. This is important as it highlights that our measured values in the lab are not meant to indicate precise levels of outcrossing that would occur under unknown natural conditions.

To explore the effects that varying mating phenotypes could have on the spread of a *wtf* driver in a population, we used both mathematical modeling and an experimental evolution approach. We found that, while the spread of a *wtf* driver could be slowed by the observed levels of same-clone mating, the driver could still spread in the absence of linked deleterious traits. We incorporated our observations into a model in which rapid *wtf* gene evolution and occasional outcrossing facilitate the maintenance of *wtf* drivers. More broadly, this study illustrates how the success of drive systems are impacted by mating phenotypes.

Results

Same-clone mating propensity differs amongst *S. pombe* natural isolates

To quantify mating propensities of homothallic *S. pombe* strains in the presence of other potential homothallic sexual partners, we first generated fluorescently tagged strains to easily observe mating via microscopy (Figure 1A). We marked strains with either GFP or mCherry (both constitutively-expressed and integrated at the *ura4* locus). We then mixed equal proportions of GFP-expressing and mCherry-expressing haploid cells and plated them on a medium (SPA) that induces one round of mating and meiosis. We imaged the cells immediately after plating to measure the starting frequency of both parent types. We then imaged again 24-48 hours later when many cells in the population had mated to either form zygotes or fully developed spores. We inferred the genotypes (homozygous or heterozygous) of each zygote and ascus (spore sac) based on their fluorescence (Figure 1A). Homozygotes were produced by mating of two cells carrying the same fluorophore, while heterozygotes were produced by mating between a GFP-labeled and an mCherry-labeled cell (Figure 1B and C). Finally, we calculated the inbreeding coefficient (F) by comparing the observed frequency of heterozygotes to the frequency expected if mating was random ($F=1-\text{observed heterozygotes}/\text{expected heterozygotes}$; Figure 1A). Exclusive mating between cells with the same fluorophore, random mating, and exclusive mating between cells with different fluorophores would yield coefficients of 1, 0, and -1, respectively (Hartl and Clark 2007). This assay occurs in a specific laboratory context, so the conditions differ from those in the wild. Importantly, however, we assay all strains under the same conditions, so the assay is

sufficient to explore possible variation in mating phenotypes between different isolates of *S. pombe*.

In homothallic cells of the common lab isolate, *Sp*, we measured an average inbreeding coefficient of 0.57 using our microscopy assay (Figure 1B and 1D). As a control, we also assayed a mixed heterothallic population containing roughly equal amounts of GFP-expressing and mCherry-expressing cells of both mating types. We expected this control population to exhibit random mating between GFP and mCherry-expressing cells, as heterothallic cells cannot undergo same-clone mating. We did observe random mating of this mixed heterothallic population ($F=-0.05$), which helps validate our assay (Figure 1D). To further validate our microscopy results, we also assayed *Sp* cells using an orthogonal approach employing traditional genetic markers. For this analysis, we mixed haploid cells on supplemented SPA medium (SPAS) to induce them to mate and undergo meiosis. We then manually genotyped the progeny and used the fraction of recombinant progeny to calculate inbreeding coefficients (Figure 1-figure supplement 2A). The average inbreeding coefficients measured using the genetic assay were very similar to the values we measured using the microscopy assay (0.49 for homothallic *Sp* cells; Figure 1-figure supplement 2B). Together, our results confirm and quantify previous observations of non-random mating in homothallic *Sp* cells (Bendezu and Martin 2013; Egel 1977). In addition, we demonstrate that our fluorescence assay provides a powerful tool to quantify mating events.

223 We next extended our analyses to other *S. pombe* natural isolates collected from the
224 wild. We assayed six additional isolates, FY29043, FY29022, FY28981, FY28974,
225 FY29044 and *S. kambucha* (*Sk*), using our fluorescence microscopy assay. We chose
226 these isolates because they each contain different fractions of the two inferred ancestral
227 *S. pombe* lineages (Tusso et al. 2019). In addition, the strains we chose are
228 homothallic, sporulated well, were non-clumping, and we were able to transform them
229 with the GFP and mCherry markers described above (Figure 1-source data 1, Figure 1-
230 figure supplement 3) (Jeffares et al. 2015). We found that the measured inbreeding
231 coefficients varied significantly between the different natural isolates (Figure 1D). The
232 phenotype of FY29043 was similar to *Sp*, but other *S. pombe* isolates, including *Sk*,
233 mated more randomly (Figure 1D). We also observed variation in mating efficiency
234 ranging from 10% of cells mating in FY28981 to 50% of cells mating in *Sk* (Figure 1E).
235
236 Given that *S. pombe* cells are immobile, we thought that cell density could affect their
237 propensity to undergo same-clone mating. To test this, we compared the inbreeding
238 coefficients of both homothallic *Sp* and *Sk* isolates at three different starting cell
239 densities: our standard mating density (1X), high density (10X), and low density (0.1X).
240 Because crowding prevented us from assaying high-density cells using our microscopy
241 approach, we used the genetic assay for each condition. We found that inbreeding
242 coefficients were higher in both *Sp* and *Sk* isolates when cell densities were lower
243 (Figure 1-figure supplement 2C and 2D). This is likely because cells plated at low
244 density tended to be physically distant from potential sexual partners that were not part
245 of the same clonally growing patch of cells (Figure 1-figure supplement 4). However, a

control population consisting of mixed heterothallic *Sp* cells (*h*⁺ and *h*⁻ cells of two different genotypes mixed in equal proportions) that cannot mate within a clonal patch of cells showed near random mating between the two genotypes at all cell densities assayed (Figure 1-figure supplement 2C). Overall, these experiments demonstrate that inbreeding coefficients vary within *S. pombe* homothallic isolates and can be affected by cell density.

Additional phenotypes associated with reduced inbreeding coefficients in *Sk*

We next used time-lapse imaging to explore the origins of the different inbreeding coefficients, focusing on the *Sp* and *Sk* isolates. Previous work claimed that *Sk* cells have reduced mating-type switching efficiency, based on levels of the DNA break (DSB) that initiates switching (Singh and Klar 2002). A mutation at the *mat-M* imprint site was proposed to be responsible for the reduced level of DSBs (Singh and Klar 2003).

We reasoned that less mating-type switching could lead to less same-clone mating, as a small population of cells clonally derived from the same progenitor via mitosis would be less likely to contain cells of compatible mating types. This would promote cells mating outside their clonal lineage. One can see evidence consistent with this phenomenon in our experiments varying the cell density of homothallic *Sp* cells (Figure 1-figure supplement 2C and D). Specifically, we observed more random mating when cells were at higher density, which likely results from more opportunity for cells to mate outside their clonal lineage. We aspired to directly compare mating type switching rates between *Sp* and *Sk* cells, although such direct assays have not, to our knowledge, been

done in any strain background. We attempted to develop a direct assay using previously described cytological reporters of mating type. The reporters have been used to assay ratios of *h+* and *h-* cells in a population (Jakociunas et al. 2013), but we found they were not well-suited for following switching events live in time-lapse imaging in our hands (Figure 2-figure supplement 1A).

Because of this, we decided to further explore the possible differences in mating-type switching frequencies indirectly using time-lapse assays, similar to those used to originally determine the patterns of mating-type switching in *Sp* (Miyata and Miyata 1981). As in the classic study of Miyata and Miyata (1981), we used the first mating event as an imperfect proxy for when cells were capable of mating (i.e., they had a partner of the opposite mating type). For these assays, we tracked the fate of individual homothallic founder cells plated on SPA at low density (0.25X to our standard mating density used above) to quantify how many mitotic generations occurred prior to the first mating event. When the first mating event occurred, we recorded the proportion of the cells present that mated (prior to the appearance of cells from next mitotic generation). We also recorded the relationships between the cells that did mate (Figure 2A). Specifically, we scored whether the mated cells were the product of a single mitotic division. Historically, these have been called ‘sister cells’ in the *S. pombe* mating literature, although we use the term ‘sibling cells’ to be gender neutral (e.g., Miyata and Miyata 1981). We did not consider cells that were born in mitotic generations past the one in which mating first occurred (Figure 2A). For example, if cells within a given lineage first mated at generation 3, we scored the relationships between those mated

cells and recorded how many unmated cells remained at the end of generation 3 (even if descendants of the unmated cells eventually mated). We then did not consider that lineage any further, so lineages in which cells first mated at generation 2 are not represented in the generation 3 data.

Classic work characterizing mating-type switching patterns in *Sp* found that one in a group of four clonally derived cells will have the opposite mating type relative to the other three cells. A newly switched cell will be compatible to mate with close relatives, most typically its sibling cell (Figure 2A) (Miyata and Miyata 1981). Under the same switching model, a smaller portion of cells derived from a single division could be of opposite mating types and thus sexually compatible. For example, if one considers switchable cells (e.g., Ps in Figure 2A), they must divide only once to generate a pair of mating-competent cells. In *Sp* cells, we observed mating amongst the clonal descendants of some progenitor cells after a single mitotic division (i.e., at generation 2). By the third generation, we observed mating amongst the descendants of more than half of the progenitor cells. Almost all the observed mating events were between sibling cells (Figure 2A and 2B). These observations are consistent with published work assaying mating patterns within lineages of clonal *Sp* cells (Bendezu and Martin, 2013; Klar 1990; Miyata and Miyata 1981).

Sk cells plated at 0.25X density on SPA divided significantly more than *Sp* cells prior to the first mating (Wilcox rank sum test; $p < 0.005$ Figure 2B). *Sk* progenitor cells most frequently started mating at the fourth mitotic generation (Figure 2B). This phenotype is

consistent with less mating-type switching as more generations would be required on average to produce a cell with the opposite mating type (Singh and Klar 2002). In addition, many mating events were between non-sibling cells. This phenotype can also be explained indirectly by reduced mating-type switching. Specifically, after cells undergo several divisions, they generate non-linear cell clusters in which comparably more non-sibling cells are in close proximity (Figure 2-figure supplement 1A-B). This clustering could lead to more non-sibling mating than when cells are mating-competent after fewer divisions and the low number of cells are largely arranged linearly.

To better understand the differences between *Sp* and *Sk*, we compared the sequence of the mating-type locus in the two isolates as many distinct alleles have been identified (Nieuwenhuis et al. 2018). Consistent with previous work, we found that the mating-type regions of *Sp* and *Sk* are highly similar (Singh and Klar 2002). However, using a previously published mate-pair sequencing dataset, we discovered a ~5 kb insertion of nested *Tf* transposon sequences in the *Sk* mating type region (Eickbush et al. 2019). We confirmed the presence of the insertion using PCR (Figure 2-figure supplement 2A-B). We also found evidence consistent with the same insertion in FY28981, which also mates more randomly than *Sp* (Figure 2-figure supplement 2B, Figure 1D). We did not, however, formally test if the insertion affects mating phenotypes. Even if it does have an effect, it is insufficient to explain all the mating variation we observed as FY29044 mates randomly, yet it lacks the insertion (Figure 1D and Figure 2-figure supplement 2B).

To further explore the hypothesis that decreased mating-type switching efficiency in *Sk* could contribute to the mating differences we observed (Figure 2B), we carried out time-lapse analyses of cells, at our standard 1X cell mating density. We reasoned that at this density, any given cell is likely to have a cell of opposite mating type nearby, even if mating-type switching is infrequent. We again used mixed populations of GFP and mCherry-expressing cells to facilitate the scoring of mating patterns (Figure 2C, Figure 2-video supplement 1). We found that the *Sp* cells predominantly mated in the second and third mitotic generations and most mating events were between sibling cells (Figure 2C).

The mating behavior of *Sk* cells changed more dramatically between 0.25x density and the higher 1X density. Whereas *Sk* cells tended to first mate in the fourth generation at 0.25X density, at 1X density *Sk* cells, like *Sp*, generally mated in the second and third mitotic generations (Figure 2C, Figure 2-video supplement 2). Additionally, we observed significantly reduced levels of mating between *Sk* sibling cells at 1X density relative to 0.25X (10% and 56%, respectively; Figure 2E and 2B). These phenotypes are consistent with reduced mating-type switching in *Sk*. Specifically, our data suggest that *Sk* cells do not need to undergo more divisions before they are competent to mate. Rather, the additional divisions that occurred at 0.25X density in *Sk* could have been necessary to produce a pair of cells with opposite mating types. At 1X density, additional divisions are not expected to be required as additional non-sibling compatible partners are available.

It is important to note, however, that our experiments combined with the previous work showing fewer switching-initiating DSBs in *Sk* (Singh and Klar 2002), support, but do not conclusively demonstrate that mating-type switching occurs less frequently in *Sk*. In particular, our assays to understand switching frequencies use mating as a proxy for switching, which is not ideal. Therefore, reduced switching in *Sk* represents a promising model that remains to be tested. Still, our results conclusively demonstrate the key point that the mating phenotypes previously measured in *Sp* do not apply to all *S. pombe* isolates. Despite very little genetic diversity, *S. pombe* isolates maintain significant natural variation in key mating phenotypes (Jeffares et al. 2017).

Ascus variation

While assaying inbreeding cytologically, we noticed that the *Sk* natural isolate displayed tremendous diversity in ascus size and shape (Figure 1C, Figure 2-figure supplement 3A, Figure 2-video supplement 2). This was due to high variability in the size of the mating projections, known as shmoos. *Sk* produced long shmoos only in response to cells of the opposite mating type and not as a response to nitrogen starvation alone (Figure 2-figure supplement 3B). The long *Sk* shmoos motivated us to quantify asci length across all the natural isolates described above. We found that most isolates generated zygotes or asci that were ~10-15 μm , similar to *Sp*. The majority of *Sk* zygotes and asci also fell within this range, but ~25% of *Sk* zygotes and asci were longer than 15 μm , with some exceeding 30 μm (Figure 2-figure supplement 3C). We also assayed zygote/ascus length in an additional natural isolate in which we were

unable to quantify inbreeding due to a clumping phenotype (FY29033). This isolate also showed populations of long asci, like *Sk* (Figure 2-figure supplement 3C).

Additionally, we occasionally noticed a fused asci phenotype in *Sk* (Figure 2-figure supplement 3D). Time-lapse analyses of mating patterns, described above, revealed these fused asci can result from an occasional disconnect between mitotic cycles and the physical separation of cells (Figure 2-figure supplement 3E). This phenotype is reminiscent of *adg1*, *adg2*, *adg3* and *agn1* mutants in *Sp* that have defects in cell fission (Alonso-Nunez et al. 2005; Gould and Simanis 1997; Sipiczki 2007). Although we observed this phenotype in all time lapse experiments using *Sk* cells, the prevalence of this phenotype varied greatly between experiments. We rarely observed this phenotype in *Sp* cells. We did not analyze time-lapse images of the other natural isolates, where this phenotype is most easily observed, so it is unclear if this septation phenotype occurs in other natural isolates.

Mating phenotypes are affected by available mating partners

We next assayed if the mating preferences of *S. pombe* isolates *Sp* and *Sk* were invariable, or if they could be affected by the available mating partners due to mating incompatibilities (Seike et al. 2019b). To test this idea, we used both still and time-lapse imaging of cells mated at 1X density on SPA. For these experiments, we mixed fluorescently labeled *Sp* and *Sk* cells in equal frequencies.

We observed in experiments employing still images that the overall inbreeding coefficient of the mixed *Sk/Sp* population of cells was intermediate between single-isolate crosses and mixed crosses (Figure 2D). In time-lapse experiments, we observed that *Sk* cells maintained low levels of mating between sibling cells in the mixed *Sk/Sp* population (9.2% compared to 9.8% in a homogeneous population; Figure 2E). Amongst the *Sp* cells, mating between sibling cells decreased significantly from 56.8% to 29.7% in the mixed mating environment (Figure 2E; t-test, $p=0.04$). Together, these results suggest that *Sk* cells can interfere with the ability of *Sp* cells to undergo same-clone mating. Although sibling cell mating preference changed, we did not observe a significant decrease in the mating efficiency of *Sp* cells in a mixed *Sp/Sk* population relative to a pure *Sp* population (Figure 2-figure supplement 4A). Instead, the mating efficiency in the mixed *Sp/Sk* population was intermediate of those observed in pure *Sp* and *Sk* populations, indicating these isolates do not affect each other's ability to mate.

We were intrigued by the idea that long shmoos (mating projections) of *Sk* could contribute to its ability to disrupt *Sp* sibling mating. We were unable to address this idea directly. We did, however, find that *Sk/Sp* matings produce significantly longer zygotes/asci than either *Sk/Sk* or *Sp/Sp* matings (Figure 2-figure supplement 4B). This was true even when we compared *Sk/Sp* zygote/ascus length to the length of heterozygous *Sk/Sk* or heterozygous *Sp/Sp* zygotes/asci. While this result does not prove that long *Sk* shmoos disrupt *Sp* sibling mating, it does show that long shmoos tend to be used in these outcrossing events.

We next extended our analyses by assaying mating efficiency and inbreeding coefficients in all pairwise combinations of *Sp*, *Sk*, FY29043, and FY29044 using still images of mated cells. After adjusting for mating efficiencies and parental inbreeding coefficients (see Methods), the phenotypes we observed in these crosses were mostly additive, in that they were intermediate to the pure parental strain phenotypes (Figure 2-figure supplement 5). The two exceptions were in the crosses between *Sk* and the isolates FY20943 and FY20944. *Sk* formed more *Sk/Sk* homozygotes than expected in the two crosses (one tailed t-test, $p=0.043$ and $p=0.038$, respectively); suggesting that *Sk* cells may not be fully sexually compatible with FY20943 and FY20944 (Figure 2-figure supplement 5). However, the magnitude of the effect was small in both cases and the statistical significance is lost after Bonferroni correction for multiple testing. Overall, our observations indicate that mating phenotypes of a given isolate can be affected by different mating partners. Importantly, however, our results suggest mating incompatibilities are unlikely to have a major role in limiting outcrossing within *S. pombe*.

Population genetics modeling of the effect of inbreeding on a *wtf* meiotic driver

We next wanted to test how the observed range of inbreeding coefficients would affect the spread of a *wtf* driver in a population. To do this, we first used population genetic modeling. We used the meiotic drive model presented by J. Crow, but we also introduced an inbreeding coefficient (Hartl and Clark 2007; Crow 1991) (See Methods for a full description of the model). The model considers a population with two possible alleles at the queried locus and no genetic drift. We assumed a *wtf* driver would exhibit

450 98% drive (transmission to 98% of spores) in heterozygotes based on measured values
451 for the *Sk wtf4* driver (Nuckolls et al. 2017). We assumed that all genotypes have the
452 same fitness during haploid cell growth. For diploid cells induced to produce spores, we
453 assumed homozygotes have a fitness of 1 (e.g., maximal fitness), whereas *wtf* driver
454 heterozygotes have a fitness of 0.51, since meiotic drive destroys nearly half of the
455 spores (Nuckolls et al. 2017). The inbreeding coefficient dictates the frequency of
456 heterozygotes and thus the frequency at which the *wtf* driver can act. We varied the
457 inbreeding coefficient (F) from 1 (all matings generate homozygotes) to -1 (all matings
458 generate heterozygotes).

459

460 We used the model to calculate the predicted change in the frequency of a *wtf* driver
461 after only one sexual generation (Figure 3A). We also calculated the spread of a *wtf*
462 driver in a population from a 5% starting frequency (Figure 3B) and from lower starting
463 frequencies (Figure 3-figure supplement 1A) over generations of sexual reproduction. If
464 we used an inbreeding coefficient of 1, the frequency of the driver does not increase
465 after sexual reproduction or spread in a population over time (Figures 3A and 3B). No
466 change in driver frequency was expected because no heterozygotes are produced
467 under this condition, so no drive can occur. The *wtf* driver has the greatest advantage if
468 the inbreeding coefficient is -1, as all matings generate heterozygotes. Under all other
469 conditions, including the range of inbreeding coefficients we measured experimentally in
470 *S. pombe* natural isolates, some heterozygotes form. The *wtf* driver thus increases in
471 frequency over generations of sexual reproduction, even when the driver starts at very
472 low (anything greater than 0) frequencies (Figures 3A, 3B and Figure 3-figure

supplement 1A, see Methods) (Crow 1991). This model predicts that *wtf* drivers can spread under the modeled conditions if some non-same-clone mating occurs, even if it is infrequent. This observation is consistent with previous theoretical analyses demonstrating that inbreeding can slow the spread of meiotic drivers (Martinossi-Alilibert et al. 2021).

We next wanted to consider the spread of a *wtf* driver under conditions in which genetic drift could occur (Figure 3-figure supplement 1B and C). To do this, we simulated populations of different sizes (10-100,000 total individuals) that started with one driver. We followed the population for 1000 generations of mating and spore formation. We randomly selected surviving ‘haploids’ to populate the next generation to keep the population size fixed. We assumed that all genotypes have equal fitness during haploid cell growth. For mathematical simplicity, we assumed complete drive and a corresponding fitness of 0.5 in heterozygous diploids. We again assumed that both types of homozygous diploids had a fitness of 1. We also varied the inbreeding coefficient from 0 (random mating) to 1 (all matings generate homozygotes). Surprisingly, we found that the probability a driver’s success was related to populations size, similar to the recent results of Martinossi-Alilibert et al (2021). Specifically, the driver was most likely to be maintained in the smaller populations and in populations with more random mating due to the driver’s positive effect on its own allele transmission (Figure 3-figure supplement 1C). In larger populations in which the drivers started at a lower initial frequency, the drive allele generally took a long time to increase

its frequency compared to the alternate allele, or it was lost due to drift. This was especially true when inbreeding coefficients were high (Figure 3-figure supplement 1C).

Inbreeding and linked deleterious alleles can suppress the spread of *wtf* drivers

We next wanted to test if our predictions reflect the behavior of *wtf* drive alleles in a laboratory population of *Sp* cells over many generations. To do this, we constructed an experimental evolution system employing the GFP and mCherry fluorescent markers described above to measure changes in allele frequencies in a population over time using cytometry. To mark drive alleles, we linked the fluorescent markers with the *Sk wtf4* driver and integrated the whole construct at the *ura4* locus in *Sp* (Nuckolls et al. 2017). For nondriving alleles, we used GFP or mCherry integrated at the *ura4* locus without a linked *wtf* gene. We call the non-*wtf* alleles 'empty vector.' We started the experimental evolution populations with a defined ratio of GFP and mCherry-expressing cells. We then induced a subset of the population to mate and sporulate followed by collection and culturing of the progeny (spores). From these cells, we remeasured GFP and mCherry frequencies using flow cytometry, and we initiated the next round of mating and meiosis (Figure 4A).

Because our experiments rely on comparing the frequency of GFP and mCherry-expressing cells over time, we needed to test the relative fitness of the markers. We found that both fluorescent markers were lost from all our experimental populations over time (Figure 4-figure supplement 1A-B). This was likely because insertion of the markers disrupted the *ura4* gene and cells that excised the marker reverted the *ura4*

mutation and thereby gained a fitness benefit. We therefore only considered fluorescent cells for our analyses and stopped the experiments when more than 95% cells lacked a fluorescent marker. In addition, in one set of experiments we also sorted cells at defined timepoints to remove non-fluorescent cells from our populations (Figure 4D-E, Figure 4-figure supplement 1B and Figure 4-figure supplement 2C-D, described below).

To assay for potential differences in the fitness costs of GFP and mCherry markers, we carried out our analyses in two control populations without drive. One control population lacked the *Sk wtf4* driver while the other had *Sk wtf4* linked to both fluorescent markers. For both types of controls, we analyzed homothallic (inbreeding coefficient ~ 0.5) and mixed heterothallic (inbreeding coefficient ~ 0) cell populations (Figure 1D). We found in most cases that the number of mCherry-expressing cells increased at the expense of GFP-expressing cells over time (Figure 4-figure supplement 2 A-D). The notable exception was in heterothallic populations containing *Sk wtf4* linked to both fluorophore alleles, where we did not observe a different cost of the GFP allele compared to mCherry (Figure 4-figure supplement 2D). The origin of the differential fitness between mCherry and GFP alleles and why this cost was not observed in the one heterothallic population are both unclear. We did not determine the cause of the differences between them.

To allow us to predict expected allele frequencies more accurately, we wanted to obtain a gross estimate of the fitness cost of the GFP-marked alleles relative to the mCherry-marked alleles in our experiments. To do this, we used the first six generations of data

from our control crosses (Figure 4-figure supplement 2) to fit a maximum likelihood model in which all parameters were fixed except the fitness values of GFP/mCherry heterozygotes and GFP/GFP homozygotes. We found that the fitness cost of the GFP in homozygotes was 0.234 (see Methods). We found the dominance of the GFP cost was 0.083 (low fitness cost of GFP in GFP/mCherry heterozygotes). We then used these costs, calculated from our controls without meiotic drive, to calculate expected values in our experimental analyses in which drive can occur (Figure 4B-E).

For our experiments competing *Sk wtf4* with an empty vector allele, we first assayed populations in which the alleles both started at 50% frequency. In homothallic (inbreeding coefficient ~ 0.5) populations, we observed that *wtf4* alleles spread in the population over several generations of sexual reproduction. The driver spread faster when linked to mCherry than when linked to GFP, presumably due to the aforementioned fitness costs linked to GFP (Figure 4B-C). In both cases, the rate of spread of the allele was very close to our model's predictions if we assumed an inbreeding coefficient of 0.5 (black lines, Figure 4B-C) and differed considerably from the model's predictions assuming random mating (inbreeding coefficient=0; gray lines, Figure 4B-C).

We saw similar spread of *wtf4* in homothallic populations in a set of repeat experiments in which we sorted the cell populations twice to remove non fluorescent cells (Figure 4D and 4E). In these experiments, we also assayed mixed heterothallic populations with an equal mix of GFP- and mCherry-marked cells from both mating types. As described

above, these mixed heterothallic populations show random mating (inbreeding coefficient ~ 0) between GFP- and mCherry-labeled cells (Figure 1D). In the mixed heterothallic populations, the *wtf4* driver spread significantly faster than in homothallic cells. In generations 1-6, the spread of *wtf4* was very similar to that predicted by our model if we assumed random mating (inbreeding coefficient=0). In later generations, our observations did not fit the model well. We suspect extensive loss of fluorescent cells, especially those with mCherry, and the resulting decrease in population size could contribute to this effect (Figure 4D and 4E; Figure 4-figure supplement 1).

We compared our data to a model in which driver heterozygotes have a fitness of 0.51, but we also considered variants of the model in which heterozygotes have fitness greater than 0.5, as can occur with spore killers in *Podospora anserina* (Vogan et al 2021; Martinossi-Aliliberti et al. 2021). In *S. pombe*, an increase in driver heterozygote fitness could occur if the driving alleles benefit from drive beyond the benefits gained directly by killing spores bearing the alternate allele. For example, the surviving meiotic products could theoretically gain fitness during spore development by scavenging increased resources from the killed meiotic products (Nauta and Hoekstra 1993). We found that increasing heterozygote fitness beyond 0.51 decreased the fit of our data to the model (Figure 5-figure supplement 2), suggesting *wtf* drivers do not gain additional benefits beyond killing spores bearing the alternate allele.

Overall, our results demonstrate that our population genetics model with driver heterozygote fitness at 0.51 is good at describing the spread of *wtf4* in our experimental

population, particularly in the first few generations. Our results also confirm that inbreeding coefficients near 0.5 slow, but does not stop, the spread of drivers in a population.

Meiotic drivers tend to accumulate linked deleterious alleles in nature (Atlan et al. 2004; Dyer et al. 2007; Finnegan et al. 2019; Fishman and Saunders 2008; Higgins et al. 2018; Lyon 2003; Olds-Clarke 1997; Planchart et al. 2005; Unckless et al. 2015; Wilkinson and Fry 2001; Wu 1983). While the linkage of individual drivers to deleterious alleles in *S. pombe* has not been extensively investigated, driving alleles in the *Sk* isolate are linked to a chromosomal translocation that decreases fitness in heterozygotes (Zanders et al 2014). Similar chromosomal rearrangements involving chromosome 3, which houses most *wtf* genes, are common in *S. pombe*, so it is likely drivers are frequently linked to rearrangements (Bowen et al 2003; Brown et al 2011; Avelar et al 2013). We therefore used the population genetic model to calculate the ability of a driver to spread when tightly linked to alleles with fitness costs ranging from 0 to 0.8. We also varied the inbreeding coefficient from 0 (random mating) to 1 (no heterozygotes are produced).

We found that, in the absence of additional linked costs, *wtf* drivers are predicted to spread in a population at all initial frequencies greater than 0 (Figure 5A). As described above, this spread is slowed by inbreeding, but is not stopped until inbreeding coefficients reach 1 (no heterozygotes are produced). When the driver is burdened by additional fitness costs, it can still spread in a population. Importantly, however, as the

fitness costs of linked deleterious alleles increase, the driver must start at a higher initial frequency to spread. If the fitness costs are recessive or close to recessive (dominance coefficient h near 0), a driver can invade at low frequencies in a randomly mating population, but a driver would require a higher initial frequency to spread in an inbreeding population (inbreeding coefficients >0 ; Figure 5A, Figure 5-figure supplement 1A). If the fitness costs are partially dominant (50% dominance), the drivers require a higher initial frequency to spread, even with random mating (Figure 5-figure supplement 1B).

In finite populations in which drift can occur, we also observed that linked costs could limit the maintenance of a driver particularly in populations that inbreed (inbreeding coefficients >0 ; Figure 5-Supplement figure 1C-D). This is in line with previous modeling and can be explained because the cost of the deleterious allele is fixed, but the benefit a driver gains from drive is frequency dependent (Drury et al. 2017; Martinossi-Allibert et al. 2021; Nauta and Hoekstra 1993).

We next tested these predictions experimentally using the *Sk wtf4* allele linked to GFP in homothallic cells. As described above, the GFP allele is linked to an unknown deleterious trait (estimated 1.9% and 23.4% cost in heterozygotes and homozygotes, respectively). We varied the inbreeding coefficients of the populations by assaying cells mated at 1X and 0.1X density. As reported above, homothallic cells mated at 1X density exhibit an inbreeding coefficient of 0.5 to 0.57, but that is increased to 0.8 to 0.95 by mating the cells at low (0.1X) density (Figure 1D, Figure 1-figure supplement 2B and C).

Consistent with the predictions of our model, we observed in the experimental populations that the driver failed to spread when the initial frequency was less than 0.25 (Figure 5B). When the *wtf4::GFP* allele was found in roughly half of the population, it could spread when the inbreeding coefficient was low but decreased in frequency when the inbreeding coefficient was increased (Figure 5B). Similar, but less dramatic, effects were observed at higher initial frequencies of the *wtf4::GFP* allele. Altogether, our experimental analyses are consistent with the predictions of our model and show that both inbreeding and linked deleterious alleles can impede the spread of a *wtf* meiotic driver.

Discussion

Natural variation in mating phenotypes in *S. pombe*

Mating phenotypes, particularly the outcrossing rate, are key parameters that affect the evolution of species (Muller 1932; Otto and Lenormand 2002). We sought to explore mating phenotypes in *S. pombe* to better understand the evolution of the *wtf* gene family found in this species. Although genetic variation within *S. pombe* is limited, past studies found variation in mating efficiency and uncovered genetic diversity of the mating-type locus (Jeffares et al. 2015; Nieuwenhuis et al. 2018; Rhind et al. 2011; Singh and Klar 2002). In this work, we assayed mating in an array of homothallic *S. pombe* natural isolates under a variety of laboratory conditions. Similar to previous work, we found *Sp* mating efficiency close to 40% and observed variable mating efficiencies for natural isolates (Merlini et al. 2016; Seike et al. 2019a). In addition, we quantified the propensity of natural isolates undergo same-clone mating when given the opportunity to mate with

656 non-clonally related cells. We found this trait, as measured using an inbreeding
657 coefficient, was variable between natural isolates and could be affected by cell density
658 or available sexual partners.

659

660 All of the *S. pombe* natural isolates we studied are homothallic and thus capable of
661 same-clone mating. Despite this, we found that all isolates underwent some non-same-
662 clone mating. In addition, some isolates, like *Sk*, showed considerable non-same-clone
663 mating (inbreeding coefficient near 0) under standard mating conditions. This provides
664 additional data supporting homothallism is compatible with outcrossing between non-
665 clonally related isolates (Attanayake et al 2014). In addition, *S. pombe* asci undergo a
666 programmed degeneration process (endolysis) shortly after spore formation (Encinar
667 del Dedo et al. 2009). This presumably frees spores to potentially distribute (in wind,
668 water, or associated with animals) separate from the other spores produced in the same
669 meiosis. The physical independence of *S. pombe* spores could also facilitate
670 outcrossing in this homothallic fungus (Billiard et al 2012).

671

672 We did not definitively identify the molecular mechanisms underlying the variation in
673 mating phenotypes we observed. Our data is, however, consistent with a model in
674 which less frequent mating-type switching in the *Sk* isolate contributes to more random
675 mating in *Sk* than in the common lab isolate, *Sp*. Specifically, if switching is less
676 frequent in *Sk*, sibling cells are less likely to be compatible to mate. Incompatibility of
677 sibling cells opens the possibility for mating with other, perhaps non-clonally derived,
678 cells in the population. Singh and Klar were the first to propose that *Sk* switched less

frequently than *Sp* when they noticed less of the DNA break that initiates switching (Singh and Klar 2002). We discovered a large, nested insertion of transposon sequences in the mating-type locus of *Sk*, and we posit that this insertion could contribute to reduced DNA break formation and, potentially, decreased mating-type switching. It is important to note, however, that not all strains that mate randomly share this transposon insertion. We also stress that, as in previous work (e.g., Miyata and Miyata 1981), we did not directly assay switching rates and instead used mating events to infer information about switching. The long shmooos we observed in *Sk* may also contribute to more random mating in this isolate, as the long shmoo may increase the available number of partners within range.

Additional previously described natural variation that we did not functionally explore may also contribute to differences in inbreeding propensity in *S. pombe*. For example, heterothallic natural isolates are predicted to exclusively undergo non-same-clone mating with cells of the opposite mating type (Jeffares et al. 2015; Nieuwenhuis et al. 2018). In addition, homothallic isolates with atypical mating type loci with extra copies of the *mat* cassettes could grow into populations that are biased towards one mating type (Nieuwenhuis and Immler 2016). Indeed, we analysed the presumably expressed mating type locus (*mat1*) in several isolates for which we had nanopore sequencing data and found an approximate 3:1 excess of the *h+* allele in FY29033 (Figure 2-figure supplement 2C). The excess of one mating type is predicted to also facilitate non-same-clone mating.

It is important to note that our study does not address the actual frequency of outcrossing in *S. pombe* populations in the wild. Very little is known about the ecology of fission yeast, including how frequently genetically distinct isolates are found in close enough proximity to mate (e.g., closer than ~40 microns apart) (Jeffares 2018). Outcrossing rates have been estimated using genomic data, but those estimates generally assume both that heterozygous recombination rates will match those observed in pure *Sp* and that allele transmission is Mendelian (Farlow et al. 2015; Tusso et al. 2019). Although these genomic estimates are reasonable, neither of these assumptions is consistent with empirical analyses (Bravo Nunez et al. 2020b; Hu et al. 2017; Zanders et al. 2014). These assumptions have, therefore, likely led to an underestimation of the true outcrossing rate.

The effect of mating-type phenotypes on the spread of *wtf* meiotic drivers

To understand the evolution of the *wtf* drive genes, it is not necessarily essential to understand how frequently significantly diverged natural isolates, like those assayed in this work, mate. Instead, it is important to understand how often a driver is found in a heterozygous state. We suspect that heterozygosity for *wtf* drivers does not absolutely require outcrossing between more distantly related strains. This is because the *wtf* gene family, particularly the genes involved in meiotic drive, exhibit extremely rapid evolution. Even though genetic diversity within *S. pombe* is low (<1% average DNA sequence divergence in non-repetitive regions), the *wtf* genes present in different isolates tend to be largely distinct (Eickbush et al. 2019; Hu et al. 2017; Jeffares et al. 2015; Rhind et al. 2011). The number of *wtf* genes per isolate varies from 25-38 *wtf* genes (including

pseudogenes), and even genes found at the same locus can be dramatically different (e.g., <61% coding sequence identity between alleles of *wtf24*) (Eickbush et al. 2019). For example, *wtf22* is a predicted pseudogene in one strain, a predicted antidote in another strain, and is predicted to encode distinct drivers (i.e., mutually killing) in two more strains. There is only one *wtf* locus where all four strains that were surveyed each contain a meiotic driver, *wtf4* (Eickbush et al. 2019). Still, we do not consider this a fixed driver because the sequence of *wtf4* is different in each strain, which, in all cases tested, leads to a distinct drive phenotype (Bravo Nunez et al. 2018; Bravo Nunez et al. 2020a; Bravo Nunez et al. 2020b; Eickbush et al. 2019; Hu et al. 2017). For example, because *Sk wtf4* and *Sp wtf4* have different sequences, the antidote of *Sk wtf4* does not neutralize *Sp wtf4* and vice versa (Bravo Nunez et al. 2020a). The rapid evolution of *wtf* genes is driven largely by non-allelic gene conversion within the family and expansion or contraction of repetitive sequences within the coding sequences of the genes (Eickbush et al. 2019).

Because *wtf* genes generally provide no protection against *wtf* drivers with distinct sequences, the variation in *wtf* gene sequences has profound consequences (Bravo Nunez et al. 2018; Bravo Nunez et al. 2020a; Hu et al. 2017). Even small sequence changes in *wtf* drivers can cause the birth and death of drivers. When a cell bearing a novel *wtf* driver mutation mates, with a cell without the mutation, the driver is heterozygous, and thus, drive can occur and the novel allele can potentially spread through the otherwise largely homogeneous population. Given fission yeast cells have no inherent mobility, the novel drive alleles could arise within small isolated

subpopulations (demes) in which drivers have an increased possibility of establishing, as was formally described by Martinossi-Alilibert and colleagues (2021).

Previous work assayed the strength of drive and the associated fitness reduction due to heterozygous *wtf* drivers (Bravo Nunez et al. 2018; Bravo Nunez et al. 2020a; Hu et al. 2017; Nuckolls et al. 2017). Those data, along with the inbreeding coefficients measured in this study, allowed us to mathematically model the spread of a *wtf* meiotic driver in an *S. pombe* population. Our modeling showed that the inbreeding coefficients we observed in *S. pombe* could slow the spread of a *wtf* driver. In the absence of drift, however, even the highest inbreeding coefficients we observed in *S. pombe* do not halt the spread of a driver, except in cases where the driver is found in low frequencies and linked to a deleterious allele. Given the tractability of *S. pombe*, we were also able to test the predictions of the model experimentally. Overall, our experimental results were quite similar to the model's predictions discussed above. This suggests that our model encompasses all critical parameters. In addition, our experiments show how the *wtf* drivers can persist and spread in *S. pombe*, even if outcrossing is infrequent. The variation of mating phenotypes also indicates that the rate of spread of a *wtf* driver is expected to vary between different populations of *S. pombe*.

Overall, our results are consistent with previous empirical and modeling studies of meiotic driver dynamics in populations. For example, like our fortuitously deleterious GFP allele, meiotic drivers are often linked to deleterious alleles that can hitchhike with the driver (Atlan et al. 2004; Dyer et al. 2007; Finnegan et al. 2019; Fishman and

Saunders 2008; Higgins et al. 2018; Lyon 2003; Olds-Clarke 1997; Planchart et al. 2005; Unckless et al. 2015; Wilkinson and Fry 2001; Wu 1983). The added costs reduce the spread of drivers, which can lead a population to harbor a driver at stable intermediate frequency (Dyer and Hall 2019; Finnegan et al. 2019; Fishman and Kelly 2015; Hall and Dawe 2018; Manser et al. 2011). Additionally, inbreeding can be selected as it increases fitness in a population when a driver is recessive lethal (cost ~ 1 , $h=0$), such as in synthetic drive systems (Bull 2016).

***S. pombe* as a tool to experimentally model complex drive dynamics**

To conclude, we would like to highlight the potential usefulness of the *S. pombe* experimental evolution approach developed for this study. With this system, we were able to observe the effects of altering allele frequencies, inbreeding rate, and fitness of a driving haplotype. In the future, this system could be used to experimentally explore additional questions about drive systems. For example, one could experimentally model meiotic drivers that bias sex ratios by linking the driver to the mating type locus in a heterothallic population. In addition, one could explore the evolution of complex multi-locus drive systems employing combinations of multiple *wtf* meiotic drivers or drivers and suppressors. This tool could lead to novel insights about natural drivers, but it may also be particularly useful for exploring potential evolutionary trajectories of artificial gene drive systems (Burt and Crisanti 2018; Drury et al. 2017; Price et al. 2020; Wedell et al. 2019).

Materials and methods

Generation of *ura4*-integrating vectors and fluorescent strains

We introduced the fluorescent genetic markers into the genome using plasmids that integrated at the *ura4* locus. To generate the integrating plasmids, we first ordered gBlocks from IDT (Coralville, IA) that contained mCherry or GFP under the control of a *TEF* promoter and *ADH1* terminator (Hailey et al. 2002; Sheff and Thorn 2004). We digested the gBlocks with *SpeI* and ligated the GFP cassette into the *SpeI* site of pSZB331 and the mCherry cassette into the *SpeI* site of pSEZB332 (alternate clone of pSZB331; (Bravo Nunez et al. 2020a; Bravo Nunez et al. 2020b)) to generate pSZB437 and pSZB882, respectively. We then linearized the plasmids with *KpnI* and transformed them into *S. pombe* using the standard lithium acetate protocol (Schiestl and Gietz 1989). We independently transformed the isolates GP50 (*S. pombe*), *S. kambucha*, FY28974, FY28981, FY29022, FY29033, FY29043, and FY29044. We were unsuccessful in transforming FY28969, FY29048, and FY29068. FY29033 was not included in the inbreeding analyses due to its proclivity to clump. The homothallic and heterothallic strains carrying mCherry or GFP were transformed using the same method.

To add *Sk wtf4* to the *Sp* genome, we again used a *ura4*-integrating plasmid. To generate this plasmid, we amplified *Sk wtf4* from SZY13 using the oligos 688 and 686. We digested the amplicon with *SacI* and ligated into the *SacI* site of pSZB332 to generate pSZB716 (Bravo Nunez et al. 2020a; Bravo Nunez et al. 2020b). We then

separately introduced the GFP and mCherry gBlocks into the SpeI site of pSZB716 to generate pSZB904 and pSZB909, respectively. We introduced the resulting plasmids into yeast as described above.

Crosses

We performed crosses using standard approaches (Smith 2009). We cultured each haploid parent to saturation in 3 mL YEL (0.5% yeast extract, 3% dextrose, and 250 mg/L adenine, histidine, leucine, lysine, and uracil) for 24 hours at 32°C. We then mixed an equal volume of each parent (700 μ L each for individual homothallic strain, 350 μ L for heterothallic parents), pelleted and resuspended in an equal volume of ddH₂O (1.4 mL total), then plated 200 μ L on SPA (1% glucose, 7.3 mM KH₂PO₄, vitamins and agar) for microscopy experiments or SPAS (SPA + 45 mg/L adenine, histidine, leucine, lysine and uracil) for genetics experiments. We incubated the plates at 25°C for 1-4 days, depending on the experiment (see figure legends for exact timing). When we genotyped spore progeny, we scraped cells off of the plates and isolated spores after treatment with B-Glucuronidase (Sigma) and ethanol as described in (Smith 2009).

Iodine staining

We grew haploid isolates to saturation in 3 mL YEL overnight at 32°C. We washed the cells once with ddH₂O then resuspended them in an equal volume ddH₂O. We then spotted 10 μ L of each strain onto an SPAS plate, which we then incubated at 25°C for 4 days prior to staining with iodine (VWR iodine crystals) vapor (Forsburg and Rhind 2006).

839

840 **Mating-type locus assembly and PCR**

841 We used mate-pair Illumina sequencing reads to assemble the mating-type locus of *S.*
842 *kambucha* with previously published data (Eickbush et al. 2019). We assembled the
843 mating-type locus using Geneious Prime software, (<https://www.geneious.com>; last
844 accessed March 18, 2019) using an analogous approach to that described to assemble
845 *wtf* loci (Eickbush et al. 2019).

846

847 **DNA extraction for Nanopore sequencing**

848 To extract DNA for Nanopore sequencing we used a modified version of a previously
849 developed protocol (Jain et al. 2018). We pelleted 50 ml of a saturated culture and
850 proceeded as described, with the addition of 0.5 mg/mL zymolyase to the TLB buffer
851 immediately prior to use.

852

853 **Nanopore sequencing and assembly**

854 We used a MinION instrument and R9 MinION flow cells for sequencing. For library
855 preparation, we used the standard ligation sequencing prep (SQK-LSK109), including
856 end repair using the NEB End Prep Enzyme, FFPE prep using the NEB FFPE DNA
857 repair mix, and ligation using NEB Quick Ligase. We did not barcode samples and thus
858 used each flow cell for a single genome. We used guppy version 2.1.3 for base calling.
859 We removed sequencing adapters from the reads using porechop version 0.2.2 and
860 then filtered the reads using filtlong v0.2.0 to keep the 100x longest reads. We then
861 error corrected those reads, trimmed the reads and de novo assembled them using

canu v 1.8 and the ovl overlapper with a predicted genome size of 13 mb and a corrected error rate of 0.12 (Koren et al. 2017). Base called reads are available as fastq files at the SRA under project accession number PRJNA732453.

In order to count allele frequency within the active *mat* locus, we mapped raw reads back to the corresponding de novo assembly using graphmap v0.5.2 and processed using samtools v1.12 (Li et al. 2009; Sović et al. 2016). We then visually observed the reference-based assemblies using IGV v2.3.97 to count the number of *h+* and *h-* alleles present at the active mating type locus with anchors to unique sequence outside the *mat* locus (Robinson et al. 2011).

Measuring inbreeding coefficients by microscopy

We mixed haploid parents (a GFP and an mCherry-expressing strain) in equal proportions on SPA as described above. We then left the plate to dry for 30 minutes and then took a punch of agar from the plate using a 1271E Arch Punch (General Tools, Amazon). We then inverted the punch of agar into a 35 mm glass bottomed dish (No. 1.5 MatTek Corporation). We used this sample to count the initial frequency of the two parental types. We then imaged a second punch of agar taken from the same SPA plate after 24 hours incubation at 25°C for homothallic cells and 48 hours for heterothallic cells. Each biological replicate was constituted by a separate cross.

To image the cells, we used an AXIO Observer.Z1 (Zeiss) wide-field microscope with a 40x C-Apochromat (1.2 NA) water-immersion objective. We excited mCherry using a

885 530–585 nm bandpass filter which was reflected off an FT 600 dichroic filter into the
886 objective and collected emission using a long-pass 615 nm filter. To excite GFP, we
887 used a 440-470 nm bandpass filter, reflected the beam off an FT 495 nm dichroic filter
888 into the objective and collected emission using a 525-550 nm bandpass filter. We
889 collected emission onto a Hamamatsu ORCA Flash 4.0 using μ Manager software. We
890 imaged at least three different fields for each sample as each technical replicate.

891

892 We used cell shape to identify mated cells (zygotes and asci) and used fluorescence to
893 identify the genotype of each haploid parent. To measure both fluorescence and the
894 length of asci, we used Fiji (<https://imagej.net/Fiji>) software to hand draw 5 pixel-width
895 lines through the length of each zygote or ascus. After subtracting background using a
896 rolling ball background subtraction with width 50 pixels, we then measured the average
897 intensity for the GFP and mCherry channels. When measuring the log₁₀ ratio of GFP
898 over mCherry, the mCherry homozygotes have the lowest ratio, homozygotes for GFP
899 the highest ratio, and heterozygotes intermediate.

900

901 To calculate the inbreeding coefficient, we used the formula $F = 1 - (\text{observed}$
902 $\text{heterozygotes} / \text{expected heterozygotes})$. We used Hardy-Weinberg expectations to
903 calculate the expected frequency of heterozygotes ($2p(1-p)$) for each sample, where ' p '
904 is the fraction of mCherry+ cells and $(1-p)$ is the fraction of GFP+ cells measured prior
905 to mating (Hartl and Clark 2007).

906

907 **Visualizing mating and meiosis using time-lapse microscopy**

For time-lapse imaging of cells mated at 1X density (Figure 2C), we prepared cells using the agar punch method described above. For cells at 0.25X density (Figure 2B), we used the same approach, except we cultured cells in 3 mL EMM (14.7 mM $C_8H_5KO_4$, 15.5 mM Na_2HPO_4 , 93.5 mM NH_4Cl , 2% w/v glucose, salts, vitamins, minerals) then washed three times with PM-N (8mM Na_2HPO_4 , 1% glucose, EMM2 salts, vitamins and minerals) before plating cells to SPA. While imaging the cells, we added a moistened kimwipe to the MatTek dish to maintain humidity. We sealed the dish lids on with high-vacuum grease (Corning). We imaged cells using either a Ti Eclipse (Nikon) coupled to a CSU W1 Spinning Disk (Yokagawa), or a Ti2 (Nikon) widefield using the 60x oil immersion objective (NA 1.45), acquiring images every ten minutes for 24-48 hours, using a 5x5 grid pattern with 10% overlap between fields. The Ti Eclipse was used for one replicate each of the 1x crosses and the Ti2 was used for all remaining experiments. We used an Okolab stage top incubator to maintain the temperature at 25°C. For the Ti2 (widefield) data we excited GFP through a 470/24 nm excitation filter and collected through an ET515/30m emission filter. For mCherry on this system, we excited through a 550/15 nm excitation filter and collected through an ET595/40m emission filter. For the Ti Eclipse (confocal) data, we excited GFP with a 488 nm laser and collected its emission through an ET525/36m emission filter. For mCherry on this system, we excited with a 561 nm laser and collected through an ET605/70m emission filter.

To monitor mating in 1X crosses (Figure 2C-2E), we recorded the number of divisions and mating choice of the progeny of 286 cells until an expected mating efficiency for the

population being filmed was attained. The expected mating efficiency was calculated from still images of the same crosses. We recorded two videos of each cross.

To monitor the number of divisions required before mating could occur in 0.25X cultures (Figure 2B), around 200 individual cells were monitored through the duration of the generation in which the first mating event occurred. If cells failed to mate, they were monitored throughout the duration of the movie. If a cell or its mitotic offspring interacted with a neighboring cell cluster, it was not included in the analysis. We recorded two videos for each isolate.

Calculation of mating efficiency

We calculated mating efficiency from microscopic images using the following formula:

$$Mating\ Efficiency(\%) = \frac{2Z + 2A + S/2}{V + 2Z + 2A + S/2} * 100$$

Where Z represents the number of zygotes, A represents the number of asci, S represents the number of free spores and V represents the number of vegetative cells (Seike and Niki 2017).

Switching assay

In an attempt to directly observe mating-type switching we used the strain TP220 (Jakociunas, 2013). This strain contains a dual reporter system with YFP under control of the h^- cell specific *mfm3* promoter and CFP under control of the h^+ cell-specific *map2* promoter. Cells were cultured for 24 hours in PM-N+ade+leu and then plated to

952 SPA+ade+leu (at 0.5x density) or SPA-S (at 1x density) (see figure legends). Plates
953 were incubated at 25 °C for 1 or 12 hours (SPA+ade+leu and SPA-S, respectively)
954 before we prepared cells for microscopy using the agar punch method described above.
955 Two microscopes were used for this analysis. In one case, a Nikon Ti microscope
956 coupled to a Yokogawa CSU W1 spinning disc was used. CFP was excited with 445 nm
957 and YFP was excited with 515 nm laser light. Cells were imaged with a 60x Plan
958 Apochromat Objective (NA 1.4). CFP fluorescence was filtered through a 480/30
959 bandpass filter and YFP was filtered through an ET535/36m bandpass filter.
960 Fluorescence was recorded with an iXon DU897 EMCCD (Andor). In a separate
961 experiment, a Nikon Ti2-E widefield microscope was used. Here, a bulb was filtered to
962 excite CFP with a 440/20 nm bandpass filter while YFP was excited with a 510/25 nm
963 bandpass filter. The cells were imaged with a 60x Plan Apochromat phase 3 objective
964 (NA 1.4). The fluorescence of CFP was filtered through an ET472/24 nm bandpass filter
965 and YFP was filtered through a 542/21 nm bandpass filter. Here, images were acquired
966 with a Prime 95 sCMOS camera (Photometrics). In both data sets, images were
967 acquired every 20 minutes for 24 hours. Images from these datasets were gaussian
968 blurred 1 pixel, then rolling ball background subtracted with a radius of 50 pixels.
969 Sample movement was eliminated by registering the images using the plugin
970 “StackRegJ_” in Fiji. Subsections of the image were created by duplicating out 500 x
971 500 pixel regions from this large image. These were then gaussian blurred 1 pixel again
972 and brightness and contrast adjusted as needed to illustrate the fluorescence. Finally,
973 these small fields of view, were cropped again to yield the images in Figure 2-figure
974 supplement 1.

975

976 **Measuring inbreeding coefficients using genetics**

977 We used a high-throughput system to genotype the progeny from each cross. First, we
978 crossed two parental populations to generate spore progeny as described above. In
979 addition to placing the mixed haploid cells on SPAS, we also diluted a subset of the mix
980 and plated it onto YEAS (YEA + 45 mg/L adenine, histidine, leucine, lysine and uracil).
981 We genotyped the colonies that grew on that YEAS plate to measure the starting
982 frequency of each parental strain in the cross. We plated the spores produced by the
983 cross on YEAS and grew them at 32°C for 4 days. We picked the colonies using a Qpix
984 420 Colony Picking System and cultured them in YEL for 24 hours at 30°C in 96 well
985 round-bottom plates (Axygen). We then used a Singer RoTor robot to spot the cultures
986 to YNP dropout and YEAS drug plates and incubated them at 32°C for three days. We
987 then imaged the plates using an S&P robotics SPImager with a Canon EOS Rebel T3i
988 camera. We analyzed each picture using the *subtract background* feature in Fiji and
989 assigned regions of interest (ROIs) to the 384 spots where cells were pinned. We then
990 measured the average intensity of each spot and classified cells as grown or not by a
991 heuristic threshold. We genotyped some cross progeny manually using standard
992 techniques due to robot unavailability, with indistinguishable results. A single cross was
993 considered as one biological replicate.

994

995 We then inferred the frequency of mating between cells of distinct genotypes based on
996 the frequency of recombinant progeny using a combination of either two or three
997 unlinked genetic markers. If mating was random, we expect the progeny to reflect

998 Hardy-Weinberg expectations ($p^2 + 2p(1 - p) + (1 - p)^2 = 1$), where $p^2 + (1 - p)^2$
 999 reflect the expected frequency of homozygotes and $2p(1 - p)$ reflects the expected
 1000 frequency of outcrossing. Homozygotes of either parental genotype can only produce
 1001 offspring with the parental genotypes. If the two parental strains outcross to generate
 1002 heterozygotes, they will make the parental genotypes and recombinant genotypes all in
 1003 equal frequencies (2^n total genotypes where n is the number of segregating markers).
 1004 For our crosses with three markers, we therefore expected the true 'observed'
 1005 frequency of progeny produced by outcrossing to be equal to the number of observed
 1006 recombinants divided by 6/8. For our crosses with two unlinked markers, we divided by
 1007 2/4. We then calculated the inbreeding coefficient using the formula,

$$F = 1 - \frac{(the\ true\ observed\ fraction\ of\ progeny\ produced\ by\ outcrossing)}{2p(1 - p)}.$$

1008

1009 **Zygote frequency expectation under inbreeding and different mating efficiencies**

1010 We calculated the expected zygote frequencies when isolates were mated with different
 1011 isolates on SPA (see Measuring inbreeding coefficients by microscopy) using an
 1012 additive model that incorporated mating efficiencies and inbreeding coefficients
 1013 measured from the isogenic crosses. The model assumed that each strain contributes
 1014 equally to the cross, and that they do not change their own mating in response to the
 1015 mating partner.

1016

1017 We calculated the expected frequency of homozygotes for parental strain 1 as:

$$= [p^2 + p(1 - p)F_{I1}](1 - u_{s1}),$$

1018 where the inbreeding coefficient is F_{I1} and the mating efficiency is $(1 - u_{s1})$ for parental
 1019 strain 1 ($s1$), considering its initial frequency p . The expected heterozygote frequency is:

$$= 2p(1 - p) \left(1 - \frac{F_{I1} + F_{I2}}{2} \right) \left(1 - \frac{u_{s1} + u_{s2}}{2} \right).$$

1020 The expected fraction of homozygotes for parental strain 2 is:

$$= 1 - (\text{Homozygote frequency strain 1} + \text{Heterozygote frequency})$$

1021

1022 **Calculating expected allele frequencies after sexual reproduction**

1023 To model the expected changes in allele frequencies in a randomly mating population
 1024 over time, we used the equations described by Crow (Crow 1991). For nonrandomly
 1025 mating populations, we included the ' F ' inbreeding coefficient in the equations, similar to
 1026 (Hartl and Clark 2007). We assumed that *wtf4* had a drive strength (' k ') of 0.98 based
 1027 on experimental observations (Nuckolls et al. 2017). We assumed the control allele
 1028 exhibited Mendelian transmission ($k=0.5$). Simulations for the spread of a driver in
 1029 Figure 3 only considered drive and inbreeding coefficients. To simulate drive in
 1030 fluorescent populations, the starting frequencies of each allele (i.e., ' p ') were determined
 1031 empirically for each experiment using either traditional genetic approaches or cytometry.
 1032 Predicted values were calculated from each biological replicate (independent cross),
 1033 composed of three technical replicates measured via cytometry per cross. We assumed
 1034 all genotypes had equal fitness as haploids. For relative fitness of diploids, we assigned
 1035 mCherry/mCherry homozygotes a fitness of $w_{11} = 1$, regardless of whether they were
 1036 EV/EV or *wtf4/wtf4* homozygotes. In all but one cross (see below), we observed a
 1037 fitness cost linked to the GFP alleles relative to mCherry alleles during sexual
 1038 reproduction, regardless of *wtf4*. We therefore used our data (see below) to calculate

0.234 as the fitness cost of the GFP-linked variant. Because of that, we assigned a fitness value of 0.766 to GFP homozygotes, w_{22} . We found the fitness cost linked to GFP had low dominance (see below) and thus assigned a fitness value of 0.98 for GFP/mCherry homozygous for *wtf4* or empty vector, w_{12} . For GFP/mCherry heterozygotes that were also heterozygous for *wtf4*, we assigned a fitness of (0.5) that accounts for the death of spores killed by drive (0.51) and the GFP cost (0.234) and dominance (0.083).

The calculation for allele frequency for a *wtf* meiotic driver in consecutive sexual cycles from haploid populations is,

$$p_{g+1} = \frac{[p_g^2 + Fp_g(1-p)_g]w_{11} + 2p_g(1-p)_gk(1-F)w_{12}}{\bar{W}_g}.$$

Where the mean fitness of the population at each generation (g),

$$\bar{W}_g = p_g^2 w_{11} + 2p_g(1-p)_g w_{12} + (1-p)_g^2 w_{22} + Fp_g(1-p)_g(w_{11} + w_{22} - 2w_{12}).$$

When the fitness reduction in heterozygotes is only due to dead cells by drive with no additional costs, $w_{12} = \frac{1}{2k}$, the drivers spread under any initial frequency greater than 0 (Crow 1991).

To determine the fitness cost of the GFP linked variant that was present in most of our experiments, we used the L-BFGS-B algorithm to find a fitness that maximized the likelihood that our observed allele frequencies varied only due to the linked cost of GFP (c) and dominance (h) in the first six generations of the control experiments shown in Figure 4-figure supplement 2 (Byrd et al. 1995). To do this, we used the *mle* function

from the R *stats4* package (Team 2019). We assumed the fitness cost alters the relative fitness of both homozygotes $w_{22} = 1 - c$ and heterozygotes $w_{12} = 1 - ch$. The fitting was done using only the initial six generations due to a rapid loss of fluorescent cells from seven to ten generations (Figure 4-figure supplement 1).

To calculate the minimum initial frequency of driver required to spread in a population when linked to alleles with varying additional fitness costs, we use the additional linked cost (c) and their dominance (h) associated to each genotype and solved using WolframAlpha (Wolfram Research 2021). For simplicity we also assumed complete transmission bias, $k = 1$. A *wtf* meiotic driver linked to a deleterious allele can spread under the condition (critical value) that

$$F_{critical} < \frac{ch(p - 1) - cp + p}{c(h - 1)(p - 1) + p}.$$

Where p is the initial frequency of a *wtf* driver, c is the cost linked to the driver and h is the dominance coefficient. For this case the assigned relative fitness for homozygote carrying the *wtf* driver is, $w_{11} = 1 - c$.

Modeling driver success in finite populations

To determine the possibility of a meiotic driver to spread and be fixed in a finite population, we ran stochastic simulations using a Wright-Fisher model considering multiple population sizes. We assumed no fitness differences between genotypes during haploid cell growth, complete drive ($k=1$), gamete killing proportional to drive in heterozygotes, no linked deleterious alleles, and non-overlapping generations. We used characters of “0” for non-driving cells and “1” for driving cells (Figure 3-figure

supplement 1B). Initially the population starts with population size (N). We next sampled a fixed population size (n) with replacement, assuming infinite gamete supply used for mating (e.g., as if mitotic expansion of the population occurred prior to mating). In our simulations $N=n$. We sampled mating groups without replacement for an inbreeding population (S_{pop}) with a fraction F and the rest of cells ($n - S_{pop}$) mate randomly. Populations that mate randomly are proportional to Hardy-Weinberg genotype frequencies with approximated integer values. Homozygotes ("0,0" and "1,1") produce four progeny ("0,0,0,0" and "1,1,1,1", respectively), while heterozygotes produce two progeny that inherit the drive allele ("1,1"). When a fitness cost is associated with a genotype, we decimated the gametic products in proportion to the cost. The collection of 0's and 1's after this process constituted the next generation. We repeated 1000 generations for 1000 iterations for each tested condition.

Measuring allele frequencies for experimental evolution analyses

We performed the crosses and collected spores as described above. We then started the next generation by culturing 60 μ L of spores from each cross in each of three different wells with 600 μ L fresh YEL media in 96 deep-well-round-bottom plates (Axygen) and cultured for 24 hours at 1200 rpm at 32°C. We then transferred 60 μ L from each culture to a new plate with 600 μ L YEL and cultured for 12-14 hours at 1200 rpm at 32°C. We then pooled the culture replicates in an Eppendorf tube, spun down, and resuspended them in an equal volume of ddH₂O. We then took 100 μ L of this sample to assay via cytometry (described below). We also plated 200 μ L of each sample on SPAS plates and incubated at 25°C for 5 days to allow the cells to mate and sporulate.

1105
1106 To detect and quantify fluorescent cells via flow cytometry, we used the ZE5 Cell
1107 Analyzer (Bio-Rad). We spun down 100 μ L of each culture, washed the cell pellet with
1108 water, spun down again and resuspended the cells in 200 μ L of 1X PBS (phosphate
1109 buffered saline) with 1.5 μ L 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, 100
1110 ng/ml). DAPI stains dead cells, so we considered DAPI-negative cells as live cells. To
1111 image DAPI, we used 355 nm laser excitation and a 447/60 nm detector. To image
1112 GFP, we excited using a 488 nm laser and detected emission with a 525/35 nm filter. To
1113 image mCherry, we excited using a 561 nm laser and detected emission with a 615/24
1114 nm filter. We used 405 nm and 488 nm (FSC 405 and FSC 488) lasers for forward
1115 scatter and 488 nm laser for side scatter (SSC 488).

1116
1117 To quantify the frequency of GFP or mCherry positive cells, we analyzed the Flow
1118 Cytometry Standard files in R/Bioconductor using the packages FlowTrans and
1119 FlowClust (Lo et al. 2009). We first separated the cells that had round and uniform
1120 shape and similar granularity. This step allowed us to detect a more uniform population
1121 of single cells. We then discarded DAPI positive cells. To determine the GFP positive
1122 and mCherry positive cells, we used limits for each channel. The limits varied
1123 throughout the experiment due to reconfiguration in the flow cytometer. For every
1124 measurement, we corrected with standard samples of cells that only expressed either
1125 GFP or mCherry. Fluorescent GFP positive and mCherry positive cells always showed
1126 non-overlapping cell populations. We quantified cells that were not classified as non-
1127 fluorescent cells.

1128

1129 **Fluorescent marker loss in experimental evolution**

1130 In the experimental evolution experiments (Figure 4, Figure 5B, and Figure 4-figure
1131 supplement 2), a large proportion of cells lost their fluorescent marker over time (Figure
1132 4-figure supplement 1 A-B). We assume this is because we introduced the markers
1133 using an integrating plasmid that enters the genome following a single crossover event.
1134 Because of this, a single crossover can then pop the marker and the associated vector
1135 out of the genome. We repeatedly observed that the loss occurred faster with the
1136 mCherry markers. Consistent with this model, cells that lost fluorescence generally also
1137 lost the associated drug resistance marker present on the integrating vector.

1138

1139 We only considered fluorescent cells in our analyses. In the experiments (Figure 4B-C,
1140 Figure 4-figure supplement 1A-B, and Figure 5B) we extended the evolution up to ten
1141 generations. In other experiments (Figure 4D-E and Figure 4-figure supplement 2C-D),
1142 we removed non-fluorescent cells by cell sorting after generations two and five. To sort
1143 cells, we first collected and cultured spores as described above with the first culture
1144 done for 12 hours. We then transferred 60 μ L of germinated cells into 600 μ L fresh YEL
1145 media. We used three cultures for each experimental line and pooled all in equal
1146 amounts to have 1.4 mL of each line. We spun each sample down and resuspended the
1147 cells in 5 ml ddH₂O prior to sorting. We removed non-fluorescent cells and retained GFP
1148 positive (488 nm laser for excitation and a filter 507 nm) and mCherry positive (561 nm
1149 laser for excitation and a 582 nm filter) cells using the laser the BD FACSMelody cell
1150 sorter software. We collected 1.2 million cells for each line into 1X PBS. We then spun

down the cells and resuspended the pellets in 200 μ L YEL. We then took 60 μ L from each sample and diluted the cells into 600 μ L YEL and continued with the time course as described above. This restored fluorescently labelled cells populations as expected for homothallic and heterothallic lines (Figure 4-figure supplement 1B). Cell sorting did not affect our results as we observed the same patterns in replicate experiments in which we did not remove the non-fluorescent cells (Figure 4).

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1172 **Competing interests**

1173 SEZ is an Inventor on patent application 834 serial 62/491,107 based on *wtf* killers. We

1174 confirm that other authors have no competing interests.

1175

1176 **Data availability**

1177

1178 **Figure 1-source data 1. *S. pombe* natural isolates.**

1179 List of *S. pombe* natural isolates reported by (Tusso et al. 2019). Those selected to
1180 measure inbreeding coefficients using microscopy are highlighted and observations for
1181 these from other studies are reported (Jeffares et al. 2015; Nieuwenhuis et al. 2018).

1182

1183 **Figure 1-figure supplement 2-source data 1. Raw data of allele transmission**
1184 **values reported in Figure 1-figure supplement 2.**

1185 Crosses shown in Figure 1-figure supplement 2 were genotyped before and after
1186 meiosis. The absolute and relative frequencies of each genotype were quantified.
1187 Segregation for each genetic marker is reported. From each cross, the list of
1188 recombinant genotypes are described. In tables 1-3 on spreadsheet 1 (columns B-K) we
1189 show the genotypes, number of colonies counted and initial frequencies of the two
1190 parents used in each cross represented in Figure 1-figure supplement 2B. In columns L,
1191 O and R we show the expected frequency of Parent1, Parent 2 and recombinants,
1192 respectively, predicted using Hardy-Weinberg. Columns M-Y show the results obtained
1193 after meiosis. In columns M-N, P-Q and S-T we report the observed frequency and total
1194 number of colonies counted for Parent 1, Parent 2, and recombinants, respectively. In
1195 columns U-W (U-X for table 3) we report the transmission frequency of each individual
1196 marker in the recombinants. The inbreeding coefficient for each cross is reported in
1197 Column Y. In tables 4,5 and 6 we show the numbers of each genotype obtained after
1198 meiosis for each replicate experiment. Parental genotypes are shown in white.

1199 Discordant genotypes are highlighted in blue at the bottom of each table. We also
1200 specify which experiments were performed manually and which were performed using
1201 robotics. Spreadsheet 2 shows similar data for the multiple density experiments shown
1202 in Figure 1-figure supplement 2C-D. Column Q also shows the G of fit G-test p-value.

1203

1204 **Figure 2-figure supplement 1- source data 1**

1205 Original file of the full raw unedited gel shown in Figure 2-figure supplement 1B.

1206

1207 **Figure 2-figure supplement 1- source data 2**

1208 Original file of the full raw unedited gel shown in Figure 2-figure supplement 1B with
1209 relevant band labelled.

1210

1211 **Supplemental file 1. Yeast strains used in this study.**

1212 Strains used and created in this study are listed.

1213

1214 **Supplemental file 2. Plasmids used in this study.**

1215 Plasmids used in this study.

1216

1217 **Supplemental file 3. Oligo table.**

1218 Oligos used in this study.

1219

1220 **Figure 2-video supplement 1. Homothallic *Sp* mating video.**

1221 Homothallic *Sp* cells plated on mating inducing media (SPA) were recorded for 24
1222 hours. Cells labelled with constitutively-expressing fluorophores mCherry (magenta) or
1223 (GFP) were mixed in equal proportion 1X standard cell density.

1224

1225 **Figure 2-video supplement 2. Homothallic *Sk* mating video.**

1226 Homothallic *Sk* cells plated on mating inducing media (SPA) were recorded for 24
1227 hours. Cells labelled with constitutively-expressing fluorophores mCherry (magenta) or
1228 (GFP) were mixed in equal proportion at 1X standard cell density.

1229

1230 **Software. Flow cytometry data analysis and modeling scripts.**

1231 Analysis performed to filter, measure, and compare flow-cytometry with simulations are
1232 located in the Zanders Lab Github repository, [https://github.com/Zanders-](https://github.com/Zanders-Lab/Diverse_mating_phenotypes_impact_the_spread_of_wtf_meiotic_drivers_in_S.-pombe)
1233 [Lab/Diverse_mating_phenotypes_impact_the_spread_of_wtf_meiotic_drivers_in_S.-](https://github.com/Zanders-Lab/Diverse_mating_phenotypes_impact_the_spread_of_wtf_meiotic_drivers_in_S.-pombe)
1234 [pombe](https://github.com/Zanders-Lab/Diverse_mating_phenotypes_impact_the_spread_of_wtf_meiotic_drivers_in_S.-pombe)

1235

References

- Alonso-Nunez ML, An H, Martin-Cuadrado AB, Mehta S, Petit C, Sipiczki M, del Rey F, Gould KL, de Aldana CR. 2005. Ace2p controls the expression of genes required for cell separation in *Schizosaccharomyces pombe*. *Mol Biol Cell* **16**:2003-2017. doi: 10.1091/mbc.e04-06-0442
- Attanayake RN, Tennekoon V, Johnson DA, Porter LD, del Rio-Mendoza L, Jiang D, Chen W. 2014. Inferring outcrossing in the homothallic fungus *sclerotinia sclerotiorum* using linkage disequilibrium decay. *Heredity (Edinb)* **113**:353-363. doi: 10.1038/hdy.2014.37
- Atlan A, Joly D, Capillon C, Montchamp-Moreau C. 2004. Sex-ratio distorter of *drosophila simulans* reduces male productivity and sperm competition ability. *J Evol Biol* **17**:744-751. doi: 10.1111/j.1420-9101.2004.00737.x
- Avelar AT, Perfeito L, Gordo I, Ferreira MG. 2013. Genome architecture is a selectable trait that can be maintained by antagonistic pleiotropy. *Nat Commun* **4**:2235. doi: 10.1038/ncomms3235
- Bendezu FO, Martin SG. 2013. Cdc42 explores the cell periphery for mate selection in fission yeast. *Curr Biol* **23**:42-47. doi: 10.1016/j.cub.2012.10.042
- Billiard S, Lopez-Villavicencio M, Hood ME, Giraud T. 2012. Sex, outcrossing and mating types: Unsolved questions in fungi and beyond. Review of *J Evol Biol* **25**:1020-1038. doi: 10.1111/j.1420-9101.2012.02495.x
- Bravo Nunez MA, Lange JJ, Zanders SE. 2018. A suppressor of a wtf poison-antidote meiotic driver acts via mimicry of the driver's antidote. *PLoS Genet* **14**:e1007836. doi: 10.1371/journal.pgen.1007836

1259 Bravo Nunez MA, Sabbarini IM, Eickbush MT, Liang Y, Lange JJ, Kent AM, Zanders
 1260 SE. 2020a. Dramatically diverse *Schizosaccharomyces pombe* wtf meiotic
 1261 drivers all display high gamete-killing efficiency. *PLoS Genet* **16**:e1008350. doi:
 1262 10.1371/journal.pgen.1008350
 1263 Bravo Nunez MA, Sabbarini IM, Eide LE, Unckless RL, Zanders SE. 2020. Atypical
 1264 meiosis can be adaptive in outcrossed *schizosaccharomyces pombe* due to wtf
 1265 meiotic drivers. *Elife* **9**. doi: 10.7554/eLife.57936
 1266 Brown WR, Liti G, Rosa C, James S, Roberts I, Robert V, Jolly N, Tang W, Baumann P,
 1267 Green C, Schlegel K, Young J, Hirschaud F, Leek S, Thomas G, Blomberg A,
 1268 Warringer J. 2011. A geographically diverse collection of *schizosaccharomyces*
 1269 *pombe* isolates shows limited phenotypic variation but extensive karyotypic
 1270 diversity. *G3 (Bethesda)* **1**:615-626. doi: 10.1534/g3.111.001123
 1271 Bull JJ. 2016. Lethal gene drive selects inbreeding. *Evol Med Public Health* **2017**:1-16.
 1272 doi: 10.1093/emph/eow030
 1273 Burt A, Crisanti A. 2018. Gene drive: Evolved and synthetic. *ACS Chem Biol* **13**:343-
 1274 346. doi: 10.1021/acschembio.7b01031
 1275 Burt, A. and Trivers R. 1998. Selfish DNA and breeding system in flowering plants.
 1276 *Proceeding of the The Royal Society* **265**: 141-146. doi: 10.1098/rspb.1998.0275
 1277 Burt A, Trivers R. 2006. Genes in conflict : The biology of selfish genetic elements.
 1278 Cambridge, MA: Belknap Press of Harvard University Press. **Book**
 1279 Byrd R, Lu P, Nocedal J, Zhu C. 1995. A limited memory algorithm for bound
 1280 constrained optimization. *SIAM Journal on Scientific Computing*:1190-1208.
 1281 Crow JF. 1988. The ultraselfish gene. *Genetics* **118**:389-391.

1282 Crow JF. 1991. Why is Mendelian segregation so exact? *Bioessays*:305-312. doi:
 1283 10.1002/bies.950130609
 1284 Drury DW, Dapper AL, Siniard DJ, Zentner GE, Wade MJ. 2017. Crispr/cas9 gene
 1285 drives in genetically variable and nonrandomly mating wild populations. *Sci Adv*
 1286 **3**:e1601910. doi: 10.1126/sciadv.1601910
 1287 Dyer KA, Charlesworth B, Jaenike J. 2007. Chromosome-wide linkage disequilibrium as
 1288 a consequence of meiotic drive. *Proc Natl Acad Sci U S A* **104**:1587-1592. doi:
 1289 10.1073/pnas.0605578104
 1290 Dyer KA, Hall DW. 2019. Fitness consequences of a non-recombining sex-ratio drive
 1291 chromosome can explain its prevalence in the wild. *Proc Biol Sci* **286**:20192529.
 1292 doi: 10.1098/rspb.2019.2529
 1293 Egel R. 1977. Frequency of mating-type switching in homothallic fission yeast. *Nature*
 1294 **266**:172-174. doi: 10.1038/266172a0
 1295 Eickbush MT, Young JM, Zanders SE. 2019. Killer meiotic drive and dynamic evolution
 1296 of the *wtf* gene family. *Mol Biol Evol* **36**:1201-1214. doi: 10.1093/molbev/msz052
 1297 Egel R, Eie B. 1987. Cell lineage asymmetry in *Schizosaccharomyces pombe*:
 1298 Unilateral transmission of a high-frequency state for mating-type switching in
 1299 diploid pedigrees. *Current Genetics* **12**:429–433
 1300 Ekwall K, Thon G. 2017. Setting up *Schizosaccharomyces pombe* crosses/matings.
 1301 *Cold Spring Harb Protoc* **2017**:pdb prot091694. doi: 10.1101/pdb.prot091694
 1302 Encinar del Dedo J, Duenas E, Arnaiz Y, del Rey F, Vazquez de Aldana CR. 2009.
 1303 Beta-glucanase eng2 is required for ascus wall endolysis after sporulation in the

1304 fission yeast *Schizosaccharomyces pombe*. *Eukaryot Cell* **8**:1278-1286. doi:
 1305 10.1128/EC.00148-09

1306 Farlow A, Long H, Arnoux S, Sung W, Doak TG, Nordborg M, Lynch M. 2015. The
 1307 spontaneous mutation rate in the fission yeast *Schizosaccharomyces pombe*.
 1308 *Genetics* **201**:737-744. doi: 10.1534/genetics.115.177329

1309 Finnegan SR, White NJ, Koh D, Camus MF, Fowler K, Pomiankowski A. 2019. Meiotic
 1310 drive reduces egg-to-adult viability in stalk-eyed flies. *Proc Biol Sci*
 1311 **286**:20191414. doi: 10.1098/rspb.2019.1414

1312 Fishman L, Kelly JK. 2015. Centromere-associated meiotic drive and female fitness
 1313 variation in *Mimulus*. *Evolution* **69**:1208-1218. doi: 10.1111/evo.12661

1314 Fishman L, Saunders A. 2008. Centromere-associated female meiotic drive entails male
 1315 fitness costs in monkeyflowers. *Science* **322**:1559-1562. doi:
 1316 10.1126/science.1161406

1317 Forsburg SL, Rhind N. 2006. Basic methods for fission yeast. *Yeast* **23**:173-183. doi:
 1318 10.1002/yea.1347

1319 Giraud T, Yockteng R, Lopez-Villavicencio M, Refregier G, Hood ME. 2008. Mating
 1320 system of the anther smut fungus *Microbotryum violaceum*: Selfing under
 1321 heterothallism. *Eukaryot Cell* **7**:765-775. doi: 10.1128/EC.00440-07

1322 Glemin S, Francois CM, Galtier N. 2019. Genome evolution in outcrossing vs. Selfing
 1323 vs. Asexual species. *Methods Mol Biol* **1910**:331-369. doi: 10.1007/978-1-4939-
 1324 9074-0_11

1325 Grognet P, Lalucque H, Malagnac F, Silar P. 2014. Genes that bias mendelian
 1326 segregation. *PLoS Genet* **10**:e1004387. doi: 10.1371/journal.pgen.1004387

1327 Gould KL, Simanis V. 1997. The control of septum formation in fission yeast. *Genes*
1328 *Dev* **11**:2939-2951. doi: 10.1101/gad.11.22.2939

1329 Gutz H, Doe FJ. 1975. On homo- and heterothallism in *Schizosaccharomyces pombe*.
1330 *Mycologia* **67**:748-759.

1331 Hailey DW, Davis TN, Muller EG. 2002. Fluorescence resonance energy transfer using
1332 color variants of green fluorescent protein. *Methods Enzymol* **351**:34-49. doi:
1333 10.1016/s0076-6879(02)51840-1

1334 Hammond TM, Rehard DG, Xiao H, Shiu PK. 2012. Molecular dissection of neurospora
1335 spore killer meiotic drive elements. *Proc Natl Acad Sci U S A* **109**:12093-12098.
1336 doi: 10.1073/pnas.1203267109

1337 Hall DW, Dawe RK. 2018. Modeling the evolution of female meiotic drive in maize. *G3*
1338 *(Bethesda)* **8**:123-130. doi: 10.1534/g3.117.300073

1339 Hartl DL, Clark, AG. 2007. Principles of population genetics. *Oxford University Press*.
1340 **Book**

1341 Higgins DM, Lowry EG, Kanizay LB, Becraft PW, Hall DW, Dawe RK. 2018. Fitness
1342 costs and variation in transmission distortion associated with the abnormal
1343 chromosome 10 meiotic drive system in maize. *Genetics* **208**:297-305. doi:
1344 10.1534/genetics.117.300060

1345 Hu W, Jiang ZD, Suo F, Zheng JX, He WZ, Du LL. 2017. A large gene family in fission
1346 yeast encodes spore killers that subvert Mendel's law. *Elife* **6**. doi:
1347 10.7554/eLife.26057

1348 Hurst GD, Werren JH. 2001. The role of selfish genetic elements in eukaryotic
1349 evolution. *Nat Rev Genet* **2**:597-606. doi: 10.1038/35084545

1350 Jain M, Koren S, Miga KH, Quick J, Rand AC, Sasani TA, Tyson JR, Beggs AD, Diltthey
 1351 AT, Fiddes IT, Malla S, Marriott H, Nieto T, O'Grady J, Olsen HE, Pedersen BS,
 1352 Rhie A, Richardson H, Quinlan AR, Snutch TP, Tee L, Paten B, Phillippy AM,
 1353 Simpson JT, Loman NJ, Loose M. 2018. Nanopore sequencing and assembly of
 1354 a human genome with ultra-long reads. *Nat Biotechnol* **36**:338-345. doi:
 1355 10.1038/nbt.4060

1356 Klein J, Sipos P, Figueroa F. 1984. Polymorphism of t-complex genes in european wild
 1357 mice. *Genetics Research* **44**:39-46. doi:
 1358 <https://doi.org/10.1017/S0016672300026239>

1359 Jakociunas T, Holm LR, Verhein-Hansen J, Trusina A, Thon G. 2013. Two portable
 1360 recombination enhancers direct donor choice in fission yeast heterochromatin.
 1361 *PLoS Genet* **9**:e1003762. doi: 10.1371/journal.pgen.1003762

1362 Jeffares DC. 2018. The natural diversity and ecology of fission yeast. *Yeast* **35**:253-260.
 1363 doi: 10.1002/yea.3293

1364 Jeffares DC, Jolly C, Hoti M, Speed D, Shaw L, Rallis C, Balloux F, Dessimoz C, Bahler
 1365 J, Sedlazeck FJ. 2017. Transient structural variations have strong effects on
 1366 quantitative traits and reproductive isolation in fission yeast. *Nat Commun*
 1367 **8**:14061. doi: 10.1038/ncomms14061

1368 Jeffares DC, Rallis C, Rieux A, Speed D, Prevorsevsky M, Mourier T, Marsellach FX,
 1369 Iqbal Z, Lau W, Cheng TM, Pracana R, Mulleder M, Lawson JL, Chessel A, Bala
 1370 S, Hellenthal G, O'Fallon B, Keane T, Simpson JT, Bischof L, Tomiczek B, Bitton
 1371 DA, Sideri T, Codlin S, Hellberg JE, van Trigt L, Jeffery L, Li JJ, Atkinson S,
 1372 Thodberg M, Febrer M, McLay K, Drou N, Brown W, Hayles J, Carazo Salas RE,

1373 Ralser M, Maniatis N, Balding DJ, Balloux F, Durbin R, Bahler J. 2015. The
 1374 genomic and phenotypic diversity of *Schizosaccharomyces pombe*. *Nat Genet*
 1375 **47**:235-241. doi: 10.1038/ng.3215
 1376 Klar AJ. 1990. The developmental fate of fission yeast cells is determined by the pattern
 1377 of inheritance of parental and grandparental DNA strands. *EMBO J* **9**:1407-1415.
 1378 Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu:
 1379 Scalable and accurate long-read assembly via adaptive. *Genome Res* **27**:722-
 1380 736. doi: 10.1101/gr.215087.116
 1381 Leupold U. 1949. Die vererbung von homothallie und heterothallie bei
 1382 *Schizosaccharomyces pombe*. *CR Trav Lab Carlsberg Ser Physiol*:381–480.
 1383 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G,
 1384 Durbin R, Subgroup GPDP. 2009. The sequence alignment/map format and
 1385 samtools. *Bioinformatics* **25**:2078-2079. doi: 10.1093/bioinformatics/btp352
 1386 Lindholm AK, Dyer KA, Firman RC, Fishman L, Forstmeier W, Holman L, Johannesson
 1387 H, Knief U, Kokko H, Larracuenta AM, Manser A, Montchamp-Moreau C,
 1388 Petrosyan VG, Pomiankowski A, Presgraves DC, Safronova LD, Sutter A,
 1389 Unckless RL, Verspoor RL, Wedell N, Wilkinson GS, Price TAR. 2016. The
 1390 ecology and evolutionary dynamics of meiotic drive. *Trends Ecol Evol* **31**:315-
 1391 326. doi: 10.1016/j.tree.2016.02.001
 1392 Lo K, Hahne F, Brinkman RR, Gottardo R. 2009. Flowclust: A bioconductor package for
 1393 automated gating of flow cytometry data. *BMC Bioinformatics* **10**:145. doi:
 1394 10.1186/1471-2105-10-145

1395 Lyon MF. 2003. Transmission ratio distortion in mice. *Annu Rev Genet* **37**:393-408. doi:
1396 10.1146/annurev.genet.37.110801.143030

1397 Maki T, Ogura N, Haber JE, Iwasaki H, Thon G. 2018. New insights into donor
1398 directionality of mating-type switching in *Schizosaccharomyces pombe*. *PLoS*
1399 *Genet* **14**:e1007424. doi: 10.1371/journal.pgen.1007424

1400 Manser A, Lindholm AK, Konig B, Bagheri HC. 2011. Polyandry and the decrease of a
1401 selfish genetic element in a wild house mouse population. *Evolution* **65**:2435-
1402 2447. doi: 10.1111/j.1558-5646.2011.01336.x

1403 Martinossi-Aliliberti I, Veller C, Ament-Velasquez SL, Vogan AA, Rueffler C,
1404 Johannesson H. 2021. Invasion and maintenance of meiotic drivers in
1405 populations of ascomycete fungi. *Evolution* **75**:1150-1169. doi:
1406 10.1111/evo.14214

1407 McDonald MJ, Rice DP, Desai MM. 2016. Sex speeds adaptation by altering the
1408 dynamics of molecular evolution. *Nature* **531**:233-236. doi: 10.1038/nature17143

1409 Merlini L, Dudin O, Martin SG. 2013. Mate and fuse: How yeast cells do it. *Open Biol*
1410 **3**:130008. doi: 10.1098/rsob.130008

1411 Merlini L, Khalili B, Bendezu FO, Hurwitz D, Vincenzetti V, Vavylonis D, Martin SG.
1412 2016. Local pheromone release from dynamic polarity sites underlies cell-cell
1413 pairing during yeast mating. *Curr Biol* **26**:1117-1125. doi:
1414 10.1016/j.cub.2016.02.064

1415 Miyata H, Miyata M. 1981. Mode of conjugation in homothallic cells of
1416 *Schizosaccharomyces pombe*. *Journal of General and Applied Microbiology*.

1417 Muller HJ. 1932. Some genetic aspects of sex. *The American Naturalist* **66**:118-138.

1418 Nauta MJ, Hoekstra RF. 1993. Evolutionary dynamics of spore killers. *Genetics*
1419 **135**:923-930.

1420 Nieuwenhuis BP, Immler S. 2016. The evolution of mating-type switching for
1421 reproductive assurance. *Bioessays* **38**:1141-1149. doi: 10.1002/bies.201600139

1422 Nieuwenhuis BPS, Tusso S, Bjerling P, Stangberg J, Wolf JBW, Immler S. 2018.
1423 Repeated evolution of self-compatibility for reproductive assurance. *Nat Commun*
1424 **9**:1639. doi: 10.1038/s41467-018-04054-6

1425 Novitski LSaE. 1957. Meiotic drive as an evolutionary force. *American Society of*
1426 *Naturalists* **91**:105-110.

1427 Nuckolls NL, Bravo Nunez MA, Eickbush MT, Young JM, Lange JJ, Yu JS, Smith GR,
1428 Jaspersen SL, Malik HS, Zanders SE. 2017. Wtf genes are prolific dual poison-
1429 antidote meiotic drivers. *Elife* **6**. doi: 10.7554/eLife.26033

1430 Nuckolls NL, Mok AC, Lange JJ, Yi K, Kandola TS, Hunn AM, McCroskey S, Snyder JL,
1431 Bravo Nunez MA, McClain M, McKinney SA, Wood C, Halfmann R, Zanders SE.
1432 2020. The wtf4 meiotic driver utilizes controlled protein aggregation to generate
1433 selective cell death. *Elife* **9**. doi: 10.7554/eLife.55694

1434 Olds-Clarke P. 1997. Models for male infertility: The t haplotypes. *Rev Reprod* **2**:157-
1435 164. doi: 10.1530/ror.0.0020157

1436 Osterwalder A. 1924. *Schizosaccharomyces liquefaciens* n. Sp., eine gegen freie
1437 schweflige säure widerstandsfähige gärhefe. *Mitt Gebiete Lebensmittelunters*
1438 *Hyg* **15**:5-28.

1439 Otto SP, Lenormand T. 2002. Resolving the paradox of sex and recombination. *Nat Rev*
1440 *Genet* **3**:252-261. doi: 10.1038/nrg761

1441 Perrin N. 2011. What uses are mating types? The “developmental switch” model.
 1442 Review of. *Evolution* **66**:947-956. doi: [https://doi.org/10.1111/j.1558-](https://doi.org/10.1111/j.1558-5646.2011.01562.x)
 1443 [5646.2011.01562.x](https://doi.org/10.1111/j.1558-5646.2011.01562.x)
 1444 Price TAR, Windbichler N, Unckless RL, Sutter A, Runge JN, Ross PA, Pomiankowski
 1445 A, Nuckolls NL, Montchamp-Moreau C, Mideo N, Martin OY, Manser A, Legros
 1446 M, Larracuente AM, Holman L, Godwin J, Gemmell N, Courret C, Buchman A,
 1447 Barrett LG, Lindholm AK. 2020. Resistance to natural and synthetic gene drive
 1448 systems. *J Evol Biol* **33**:1345-1360. doi: 10.1111/jeb.13693
 1449 Rhind N, Chen Z, Yassour M, Thompson DA, Haas BJ, Habib N, Wapinski I, Roy S, Lin
 1450 MF, Heiman DI, Young SK, Furuya K, Guo Y, Pidoux A, Chen HM, Robbertse B,
 1451 Goldberg JM, Aoki K, Bayne EH, Berlin AM, Desjardins CA, Dobbs E, Dukaj L,
 1452 Fan L, FitzGerald MG, French C, Gujja S, Hansen K, Keifenheim D, Levin JZ,
 1453 Mosher RA, Muller CA, Pfiffner J, Priest M, Russ C, Smialowska A, Swoboda P,
 1454 Sykes SM, Vaughn M, Vengrova S, Yoder R, Zeng Q, Allshire R, Baulcombe D,
 1455 Birren BW, Brown W, Ekwall K, Kellis M, Leatherwood J, Levin H, Margalit H,
 1456 Martienssen R, Nieduszynski CA, Spatafora JW, Friedman N, Dalgaard JZ,
 1457 Baumann P, Niki H, Regev A, Nusbaum C. 2011. Comparative functional
 1458 genomics of the fission yeasts. *Science* **332**:930-936. doi:
 1459 [10.1126/science.1203357](https://doi.org/10.1126/science.1203357)
 1460 Rick CM. 1966. Abortion of male and female gametes in the tomato determined by
 1461 allelic interaction. *Genetics* **53**:85-96.

1462 Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov
 1463 JP. 2011. Integrative genomics viewer. *Nat Biotechnol* **29**:24-26. doi:
 1464 10.1038/nbt.1754
 1465 Schiestl RH, Gietz RD. 1989. High efficiency transformation of intact yeast cells using
 1466 single stranded nucleic acids as a carrier. *Curr Genet* **16**:339-346. doi:
 1467 10.1007/BF00340712
 1468 Planchart A, Reynolds JL, Schimenti JC. 2005. Mutations in Serac1 or Synj2 cause
 1469 proximal t haplotype-mediated male mouse sterility but not transmission ratio
 1470 distortion. *Proc Natl Acad Sci U S A* **102**:3342-3347. doi:
 1471 10.1073/pnas.0407970102
 1472 Schlake T, Gutz H. 1993. Mating configurations in *Schizosaccharomyces pombe* strains
 1473 of different geographical origins. *Current Genetics*:108-114.
 1474 Seike T, Maekawa H, Nakamura T, Shimoda C. 2019a. The asymmetric chemical
 1475 structures of two mating pheromones reflect their differential roles in mating of
 1476 fission yeast. *J Cell Sci* **132**. doi: 10.1242/jcs.230722
 1477 Seike T, Niki H. 2017. Mating response and construction of heterothallic strains of the
 1478 fission yeast *Schizosaccharomyces octosporus*. *FEMS Yeast Res* **17**. doi:
 1479 10.1093/femsyr/fox045
 1480 Seike T, Shimoda C, Niki H. 2019b. Asymmetric diversification of mating pheromones in
 1481 fission yeast. *PLoS Biol* **17**:e3000101. doi: 10.1371/journal.pbio.3000101
 1482 Sheff MA, Thorn KS. 2004. Optimized cassettes for fluorescent protein tagging in
 1483 *Saccharomyces cerevisiae*. *Yeast* **21**:661-670. doi: 10.1002/yea.1130

1484 Singh G, Klar AJ. 2003. DNA sequence of the mat2,3 region of *Schizosaccharomyces*
1485 *kambucha* shares high homology with the corresponding sequence from sz.
1486 *Pombe*. *Yeast* **20**:1273-1278. doi: 10.1002/yea.1042

1487 Singh G, Klar AJS. 2002. The 2.1-kb inverted repeat DNA sequences flank the mat2,3
1488 silent region in two species of *Schizosaccharomyces* and are involved in
1489 epigenetic silencing in *Schizosaccharomyces pombe*. *Genetics*:**162**:591-602.

1490 Sipiczki M. 2007. Splitting of the fission yeast septum. *FEMS Yeast Res* **7**:761-770. doi:
1491 10.1111/j.1567-1364.2007.00266.x

1492 Smith GR. 2009. Genetic analysis of meiotic recombination in *Schizosaccharomyces*
1493 *pombe*. *Methods Mol Biol* **557**:65-76. doi: 10.1007/978-1-59745-527-5_6

1494 Sović I, Šikić M, Wilm A, Fenlon SN, Chen S, Nagarajan N. 2016. Fast and sensitive
1495 mapping of nanopore sequencing reads with graphmap. *Nat Commun* **7**:11307.
1496 doi: 10.1038/ncomms11307

1497 Sun S, Klebaner F, Tian T. 2017. Mathematical modelling for variations of inbreeding
1498 populations fitness with single and polygenic traits. *BMC Genomics* **18**:196. doi:
1499 10.1186/s12864-017-3492-1

1500 Svedberg J, Vogan AA, Rhoades NA, Sarmarajeewa D, Jacobson DJ, Lascoux M,
1501 Hammond TM, Johannesson H. 2021. An introgressed gene causes meiotic
1502 drive in *Neurospora sitophila*. *Proc Natl Acad Sci U S A* **118**. doi:
1503 10.1073/pnas.2026605118

1504 Sweigart AL, Brandvain Y, Fishman L. 2019. Making a murderer: The evolutionary
1505 framing of hybrid gamete-killers. Review of. *Trends Genet* **35**:245-252. doi:
1506 10.1016/j.tig.2019.01.004

1507 Tao YT, Suo F, Tusso S, Wang YK, Huang S, Wolf JBW, Du LL. 2019. Intraspecific
 1508 diversity of fission yeast mitochondrial genomes. *Genome Biol Evol* **11**:2312-
 1509 2329. doi: 10.1093/gbe/evz165
 1510 Taylor DR, Saur MJ, Adams E. 1999. Pollen performance and sex-ratio evolution
 1511 in a dioecious plant. *Evolution* **53**:1028-1036. doi: 10.1111/j.1558-
 1512 5646.1999.tb04518.x
 1513 Team RC. 2019. R: A language and environment for statistical computing.
 1514 Tusso S, Nieuwenhuis BPS, Sedlazeck FJ, Davey JW, Jeffares DC, Wolf JBW. 2019.
 1515 Ancestral admixture is the main determinant of global biodiversity in fission yeast.
 1516 *Mol Biol Evol* **36**:1975-1989. doi: 10.1093/molbev/msz126
 1517 Unckless RL, Larracuenta AM, Clark AG. 2015. Sex-ratio meiotic drive and y-linked
 1518 resistance in drosophila affinis. *Genetics* **199**:831-840. doi:
 1519 10.1534/genetics.114.173948
 1520 van der Gaag M, Debets AJ, Oosterhof J, Slakhorst M, Thijssen JA, Hoekstra RF. 2000.
 1521 Spore-killing meiotic drive factors in a natural population of the fungus *podospora*
 1522 *anserina*. *Genetics* **156**:593-605.
 1523 Vogan AA, Ament-Velasquez SL, Bastiaans E, Wallerman O, Saupe SJ, Suh A,
 1524 Johannesson H. 2021. The enterprise, a massive transposon carrying spok
 1525 meiotic drive genes. *Genome Res* **31**:789-798. doi: 10.1101/gr.267609.120
 1526 Wedell N, Price TAR, Lindholm AK. 2019. Gene drive: Progress and prospects. *Proc*
 1527 *Biol Sci* **286**:20192709. doi: 10.1098/rspb.2019.2709
 1528 Wilkinson GS, Fry CL. 2001. Meiotic drive alters sperm competitive ability in stalk-eyed
 1529 flies. *Proc Biol Sci* **268**:2559-2564. doi: 10.1098/rspb.2001.1831

1530 Wolfram Research I. Mathematica online. Wolfram Research, Inc.
1531 <https://www.wolfram.com/mathematica>.
1532 Wu CI. 1983. Virility deficiency and the sex-ratio trait in *Drosophila pseudoobscura*. I.
1533 Sperm displacement and sexual selection. *Genetics* **105**:651-662.
1534 Zanders SE, Eickbush MT, Yu JS, Kang JW, Fowler KR, Smith GR, Malik HS. 2014.
1535 Genome rearrangements and pervasive meiotic drive cause hybrid infertility in
1536 fission yeast. *Elife* **3**:e02630. doi: 10.7554/eLife.02630
1537 Zeyl C, Bell G, Green DM. 1996. Sex and the spread of retrotransposon Ty3 in
1538 experimental populations of *Saccharomyces cerevisiae*. *Genetics* **143**:1567-
1539 1577.
1540

Figure 1. Inbreeding coefficients vary between homothallic isolates of *S. pombe*.

A) Experimental strategy to quantify mating patterns between mixed homothallic isolates. GFP (cyan)- and mCherry (magenta)-expressing cells were mixed and placed on SPA medium that induces mating and meiosis. An agar punch from this plate was imaged to assess the initial frequencies of each haploid strain. After incubation at 25°C for at least 24 hours, another punch was imaged to determine the number of homozygous and heterozygous zygotes/asci based on their fluorescence. The inbreeding coefficient (F) was calculated using the formula shown. **B and C)** Representative images of the mating in *Sp* (**B**) and *Sk* (**C**) isolates after 24 hours. Filled arrowheads highlight examples of homozygous asci whereas open arrowheads highlight heterozygous asci. A few additional zygotes are also outlined with dotted lines in the images. Scale bars represent 10 μ m. **D)** Inbreeding coefficient of homothallic natural isolates and complementary heterothallic (h^+, h^- mCherry and h^+, h^- GFP) *Sp* lab strains. At least three biological replicates per isolate are shown (open shapes). **E)** Mating efficiency of the isolates shown in **D** (%) $\pm$ standard error from three biological replicates of each natural isolate.

Figure 1-figure supplement 1. Sexual cycle of *S. pombe*. **A)** *S. pombe* haploid cells of compatible mating types h^+ (P) and h^- (M) can be induced to mate upon nitrogen starvation to form a diploid zygote. If starvation continues, the zygote undergoes meiosis to form haploid spores. **B)** Patterns of mating-type switching in clonally expanding *Sp* cells. An unswitchable cell (u) divides to produce a switchable cell (s) and an unswitchable cell. Switchable cells divide to produce another switchable cell of the same mating type and an unswitchable cell of the opposite mating type. Cells that

switch mating type (P to M or M to P) during mitotic growth are compatible within the clonal populations. Mating between cells derived from the same haploid progenitor are considered same-clone mating in this study. Mating events between descendants from different founder haploid cells are considered non-same-clone mating.

Figure 1-figure supplement 2. Inbreeding coefficients can be affected by cell density.

A) Experimental strategy to infer inbreeding coefficient by genotyping the segregation of unlinked genetic markers. The composition of the starting population was measured by genotyping cells placed on rich medium (YEAS). Cells were also plated on SPAS, where they mate and undergo meiosis. After meiosis, spores were genotyped and we compared the expected number of recombinant progeny to the number observed to calculate the inbreeding coefficient (F). **B)** Inbreeding coefficients calculated as described in **A** using the indicated genetic markers at standard 1X mating density. Each color indicates a different cross. *** indicates p-value<0.005, One tailed t-test on at least three biological replicates (open shapes). **C)** Inbreeding coefficients calculated as described in **A** for *Sp* crosses plated at low (0.1X), standard (1X), and high (10X) density. *** indicates p-value <0.005, G-test. Colonies were randomly sampled for each cross, 320 for left panel and 144 colonies for the second and third cross sets. **D)** Inbreeding coefficients calculated as described in **A** for *Sk* crosses plated at low (0.1X), standard (1X), and high (10X) density. *** indicates p-value <0.005, *indicates p-value < 0.05, G-test. 325 and 340 colonies were sampled from the crosses in the left and right panels, respectively.

Figure 1-figure supplement 3. Homothallism of natural isolates. Saturated cultures were spotted on SPAS to induce mating and meiosis. After five days at 25°C, cells were exposed to iodine vapor, which stains spores brown. The top panel shows natural isolates of homothallic (*Hom*) strains and *h-* *Sp* as a negative control. The bottom panel shows individual heterothallic strains, mixed complimentary heterothallic strains and a homothallic isolate of *Sp*.

Figure 1-figure supplement 4. Density variation in plated cells. Homothallic GFP (cyan)- and mCherry (magenta)-expressing cells were mixed, plated on SPAS, incubated at 25°C and then imaged. On this medium, cells tend to divide until confluent prior to mating. When plated at low density (0.1X; **A**), cells form large clusters of clonal cells after ~72 hours. Cells mixed and plated at high density (10X; **B**) reach confluency after just 24 hours and form clusters with more contact zones between cells of different genotypes. Scale bars represents 10 μ m.

Figure 2. Variation between mating behaviors in *Sp* and *Sk* cells. **A)** Schematic showing cell divisions, switching and mate choice in *Sp*. Mitotic generations are shown on the left. Cells are either *h+* (P) or *h-* (M) and their status is switchable (s) or unswitchable (u). **B)** Division and mating phenotypes of wildtype *Sp* and *Sk* cells plated at low cell density (0.25X) on SPA plates. Single founder cells and their descendants were monitored through time-lapse imaging. Mating patterns were categorized until the end of the generation in which the first mating event occurred. The data presented were

pooled from two independent time-lapses in which over 400 founder cells were tracked.

C) Division and mating phenotypes of fluorescently labeled *Sp* and *Sk* cells plated at standard (1X) density on SPA plates. Individual cells were monitored until the population reached a typical mating efficiency for the specific cross and the mate choice of their progeny and the mitotic generation of those events was classified. The cells labeled with GFP are indicated with a cyan line around the cell whereas the mCherry-labeled cells are outlined in magenta. Pooled data from two independent experiments are presented, with 286 cells scored from each experiment. In the cross of *Sp* (GFP) and *Sk* (mCherry), we present an additional plot (far right) in which the fate of each founder is separated by isolate. **D)** Inbreeding coefficient calculated from still images of cells plated at 1X density on SPA. *** indicates p-value < 0.005, Multiple t-test, Bonferroni corrected. At least three biological replicates per isolate are shown (open shapes). **E)** Breakdown of sibling cell mating by fluorophore in the indicated crosses from C. * indicates p-value=0.04, one-tailed t-test comparing isogenic and mixed *Sp* cells from two videos.

Figure 2-figure supplement 1. Mating-type switching dual reporter assay using mating type-specific promoters in *Sp*. A-C) Cells expressing both YFP under control of the *h*- cell specific *mfm3* promoter and CFP under control of the *h*+ cell-specific *map2* promoter (from Jakociunas et al 2013) were visualized using time-lapse microscopy. Image contrast was enhanced due to signal bleaching at later time-points and to better distinguish switching events from background. **A-B)** Switching events (CFP to YFP and YFP to CFP) were generally only visible after progenitor cells

produced eight clonal descendants. Progenitors and clonal descendants are highlighted with dotted lines. The observed pattern of fluorophore switching is different than the expected mating-type switching pattern where one out of four cells derived from the same progenitor is expected to have the opposite mating type as the other three cells (Figure 1-figure supplement 1B). **A)** Cells were grown for 24 hours in PM-N+ade+leu, washed x3 in PM-N, then plated at 0.5x to SPA+ade+leu. One-hour post-plating a time-lapse was recorded using a Nikon Ti spinning disc microscope at 25°C. Arrows indicate cells that switched fluorescent protein expression. **B)** Cells were grown for 24 hours in PM-N+ade+leu, washed x3 in ddH₂O, then plated at 1x to SPAS. 12 hours post-plating a time-lapse was recorded using a Nikon Ti2-E widefield microscope at 25°C. In three of the four cell divisions that led to the eight cell stage, a fluorophore switching event occurred. The fourth cell may have switched prior to the division as both sister cells appear to have switched. These observations also violate the current model of mating type switching (Figure 1-figure supplement 1B). Arrows indicate cells involved in mating after switching fluorescent protein expression. **C)** Sibling cells expressing CFP mated (dashed line) without an accompanying fluorophore shift. Cells were treated and time-lapses recorded as in B). The two mating partners are highlighted with dotted lines. Scale bars represent 10 µm.

Figure 2-figure supplement 2. Mating-type locus variation in *S. pombe* isolates. A)

Schematic representation of the mating-type locus. We found a 5 kb insertion of transposon sequences in the *Sk* isolate using published Illumina mate-pair sequencing data (Eickbush et al. 2019). The insert is within SPNCRNA.388, near the double-strand

break site (DSB) where switching initiates. **B)** PCR amplification of the 5 kb insertion using the oligos 3178 and 3179 shown in **A** suggests the insertion is also in the FY28981 isolate. **C)** We aligned long Nanopore sequencing from the indicated strains to the *Sp* reference genome *mat* locus, which is *h*-. We then quantified the frequency of *h*+ alleles from the aligned reads. We used Fisher's exact tests to compare our observations to the indicated ratios.

Figure 2-figure supplement 3. Variation in cell division and asci phenotypes in natural isolates. A) Examples of *Sk* long asci formed by GFP- and mCherry-expressing cells mated on SPA. **B)** *Sk* cells of each mating type were plated to SPA plates individually or in combination and imaged after 48 hours at 25°C. Cells only form long projections (shmoos) in response to cells of the opposite mating type. **C)** Tip-to-tip length (tracing through the center of the cells) of zygotes and asci of distinct natural isolates. Cells were plated on SPA and incubated for 24 hours at 25°C. *FY29033 cells showed a clumpy phenotype upon nitrogen starvation. 336 sampled cells from three independent biological replicates. **D)** Examples of *Sk* asci fused at uncleaved septa. White arrows indicate uncleaved septa. **E)** Time-lapse showing *Sk* cells with a septum (black arrow) that remained uncleaved after two rounds of fission. Additional septa (white arrows) are also shown that cleaved prior to mating. Scale bar represents 10 µm.

Figure 2-figure supplement 4. Mating efficiency and asci length of *Sp*/*Sk* heterozygotes. A) Mating efficiency calculated from still images taken after 24 hours on SPA at 25°C from isogenic and mixed isolate crosses. The open shapes represent

values from three biological replicates per cross. ** indicates p-value <0.01, t-test. **B)** Length of zygotes/asci from isogenic and mixed isolate crosses of *Sp* and *Sk*. Cells were plated on SPA at 1X density and incubated for 24 hours at 25°C before being imaged. For each category (e.g., homozygote) of zygote, 100 cells were sampled from three independent biological replicates. Multiple Wilcoxon Rank Sum Tests, Bonferroni corrected; ***, p-value<0.005.

Figure 2-figure supplement 5. Mostly additive phenotypes in crosses between natural isolates. Four natural isolates with distinct mating efficiencies and inbreeding coefficients were mixed and mated in all pair-wise combinations. After 24 hours on SPA at 25°C, cells were imaged and the mating efficiencies and inbreeding coefficients were calculated using fluorescent markers scored from still images, obtained as in Figure 1A. On the outer diagonal are isogenic crosses color-coded with the respective natural isolate. For the non-isogenic crosses, the observed homozygotes (hom) are color-coded by the respective parent isolate. The observed frequencies of hybrid (hyb) zygotes and asci are plotted in black. The expected values (gray bars) account for the inbreeding coefficient and mating efficiency from each isolate assuming the parental phenotypes are additive. Inbreeding coefficients (*F* values) and standard error (+/-) for each cross are indicated above each plot. The open shapes represent experimental replicates. * indicates p-value<0.05, one-tailed t-test.

Figure 3. Inbreeding is predicted to slow the spread of a *wtf* driver. We assumed a 0.98 transmission bias favoring the *wtf* driver and a fitness of 0.51 in *wtf*+/*wtf*-

heterozygotes. We assumed that homozygote fitness is 1. We simulated the spread of a *wtf* driver for 30 generations. **A)** Change of *wtf* meiotic driver frequency after a single meiosis assuming varying levels of inbreeding. **B)** The spread of a *wtf* driver in a population over time assuming varying levels of inbreeding.

Figure 3-figure supplement 1. Spread of *wtf* under varying initial frequencies and

populations sizes. A) Distinct initial frequencies (line type) were simulated under varying inbreeding coefficients (colors) to evolve throughout multiple sexual generations. Populations with complete inbreeding remained constant, while all populations with an initial driver frequency greater than 0 reached fixation. **B)** Evolution of a single cell carrying a *wtf* meiotic driver in different population sizes under a Wright-Fisher model (see Methods). Driving cells are represented as “1”s and non-driving as “0”s. We introduced only one driver cell among the distinct population sizes. We repeated the process over 1000 sexual generations by 1000 iterations. **C)** Proportion of iterations that did not show extinction of the driver (i.e., either fixed or still evolving).

Figure 4. Inbreeding slows the spread of the *wtf4* meiotic driver in homothallic

strains. A) Experimental strategy to monitor allele frequency through multiple generations of sexual reproduction. GFP (green) and mCherry (magenta) markers are used to follow empty vector (EV) alleles or the *wtf4* meiotic driver. Starting allele frequencies and allele frequencies after each round of sexual reproduction were monitored using cytometry. **B)** Homothallic population with mCherry marking *wtf4* and GFP marking an EV allele. Allele dynamics were predicted using drive and fitness

parameters described in the text assuming an inbreeding coefficient of 0.5 (black lines) and random mating (inbreeding coefficient of 0; gray lines). The individual spots represent biological replicates and the shaded areas around the lines represent 95% confidence intervals. **C)** Same experimental setup as in **B**, but with mCherry marking the EV allele and GFP marking *wtf4*. **D-E)** Repeats of the experiments shown in **B** and **C** with two alterations. First, these experiments tracked heterothallic populations (dotted lines) in addition to homothallic cells (solid lines). Second, the populations were sorted by cytometry at generations 3 and 5 (vertical long-dashed lines) to remove non-fluorescent cells.

Figure 4-figure supplement 1. Loss of fluorescent markers in experimental evolution analyses. A) Fluorescent marker loss from cell populations carrying only one type of fluorescent marker (GFP or mCherry) linked to an empty vector (EV). Cells were grown as in Figure 4A in parallel to the cell populations depicted in Figure 4 B-C. **B)** Similar to A, but both heterothallic and homothallic cells were analyzed in parallel to the cell populations depicted in Figure 4 D-E. The cells were sorted after generation 5 to remove non-fluorescent cells (vertical gray dotted line).

Figure 4-figure supplement 2. Allele transmission in homozygous control homothallic and heterothallic populations. A) Homothallic population with both mCherry and GFP marking EV alleles. The individual spots represent biological replicates and the shaded areas around the lines represent 95% confidence intervals. **B)** Same experimental setup as in **A**, but with both markers linked to a *wtf4* allele. **C-D)**

Repeats of the experiments shown in **A** and **B** with two alterations. First, these experiments tracked heterothallic populations (dotted lines) in addition to homothallic cells (solid lines). Second, the populations were sorted by cytometry at generations 3 and 5 (vertical long-dashed lines) to remove non-fluorescent cells.

Figure 5. Inbreeding purges *wtf* drivers with linked deleterious alleles. A) Modeling the predicted impacts of inbreeding, the fitness of the driving haplotype, and the initial frequency of the driver on the spread of a *wtf* driver linked to a deleterious allele with low dominance ($h=0.083$) in a population. The circle indicates the predicted initial frequency necessary for the GFP::*wtf4+* allele to spread in a population when mated at standard (1X) density where $F=0.5$. The diamond indicates the predicted initial frequency necessary for the GFP::*wtf4+* allele to spread in a population when mated at low (0.1X) density where $F=0.8-0.95$ measured in Figure 1-figure supplement 2C. **B)** Experimental analyses of the impacts of inbreeding and the initial driver frequency on the spread of the GFP::*wtf4+* allele in a population. A single biological replicate was used for each initial frequency and inbreeding condition.

Figure 5-figure supplement 1. Evolution of *wtf* meiotic drivers in populations for recessive and codominant costs. A-B) Modeling the predicted impacts of inbreeding, the fitness of the driving haplotype, and the initial frequency of the driver on the spread of a *wtf* driver linked to a completely recessive (**A**; $h=0$) or codominant (**B**; $h=0.5$) deleterious allele. The oval indicates the predicted initial frequency necessary for the GFP::*wtf4+* allele to spread in a population when mated at standard (1X) density where

1771 $F=0.5$. The diamond indicates the predicted initial frequency necessary for the
 1772 GFP::*wtf4+* allele to spread in a population when mated at low (0.1X) density where
 1773 $F=0.8-0.95$ measured in Figure 1-figure supplement 2C. **C-D)** Evolution of a population
 1774 in which a single cell carrying a *wtf* driver is introduced under a Wright-Fisher model
 1775 with different fixed population sizes. Simulations were performed under three inbreeding
 1776 coefficients (F). We simulated for 1000 generations and 1000 iterations for each
 1777 condition. The proportion of iterations that maintained the meiotic driver are shown for
 1778 simulations in which the linked costs were recessive ($h=0$) in **B)** or codominant ($h=0.5$)
 1779 in **D)**.

1780

1781 **Figure 5-figure supplement 2. Assessing if *wtf+* spores gain fitness as a result of**
 1782 **spore killing A)** Simulation of the evolution of a *wtf* meiotic driver starting at a
 1783 frequency of 0.05 in the population. This analysis is similar to that presented in Figure
 1784 3B, except the inbreeding coefficient (F) was held constant at 0.5 and fitness values of
 1785 *wtf* driver heterozygotes greater than 1 were considered. A driver heterozygote with
 1786 fitness of 0.51 (as in Figure 3, Figure 4, Figure 5A, and Figure 5-figure supplement 1A-
 1787 B) is shown with an orange line. Increasing the fitness of the driver heterozygote
 1788 $w_{12}>0.51$ (gray lines) results in a faster spread of the meiotic driver. **B-E)** Comparison of
 1789 the *wtf4* predicted meiotic driver spread assuming different driver heterozygote fitness
 1790 values (black lines) to the experimentally observed values (colored lines). **B)** Observed
 1791 values from Figure 4B compared to multiple relative heterozygote fitness. **C)** Observed
 1792 values from Figure 4C compared to multiple relative heterozygote fitness. **D)** Observed
 1793 values from Figure 4D compared to multiple relative heterozygote fitness. Cell sorting

1794 events are indicated with vertical gray lines. **E)** Observed values form Figure 4E
1795 compared to multiple relative heterozygote fitness. Cell-sorting events are indicated with
1796 vertical gray lines.

Figure 1

A

B

S. pombe (Sp, Lab strain)

C

S. kambucha (Sk)

D

E

Mating efficiency %

FY29043	33±2
Sp	33±1
FY29022	25±3
FY28981	10±4
FY28974	23±7
FY29044	14±2
Sk	50±2

Figure 1-figure supplement 1

A

B

Figure 1-figure supplement 2

A

B

C

D

Figure 1-figure supplement 3

Figure 1-figure supplement 4

A

Lab *Sp* 0.1X 72 h post plating on SPAS

B

Lab *Sp* 10X 24 h post plating on SPAS

Figure 2

Figure 2-figure supplement 1

A

B

C

Figure 2-figure supplement 2

C

Isolate	Frequency h^+	Reads	P-value, 50:50	P-value, 75:25
<i>S. pombe</i> ref (h^-)	0	363	<0.0001	--
<i>Sk</i>	0.44	182	0.294	--
FY29033	0.76	227	<0.0001	0.913

Figure 2-figure supplement 3

Figure 2-figure supplement 4

Figure 2-figure supplement 5

Figure 3

A **B**

Figure 3- figure supplement 1

A

B

C

Figure 4

Figure 4-figure supplement 1

A

B

Figure 4-figure supplement 2

A

B

C

D

Figure 5

A **B**

Figure 5-Figure supplement 1

Figure 5-figure supplement 2