Self-compatibility in yeast is selected for reproductive assurance not population level compatibility

Bart P.S. Nieuwenhuis* [1] - nieuwenhuis@bio.lmu.de, ORCID: 0000-0001-8159-4784
Rasha Shraim [1, 2, 3] - rshraim@tcd.ie, ORCID: 0000-0002-3351-1179
Hamed Al Ghaithi [1] - h.ghaithi@gmail.com

[1] Division of Evolutionary Biology, Faculty of Biology, Ludwig-Maximilians-Universität München, Germany; **[2]** The SFI Centre for Research Training in Genomics Data Sciences, National University of Ireland Galway; **[3]** Department of Public Health and Primary Care, School of Medicine, Trinity College Dublin, Republic of Ireland; * Corresponding author

Published in Evolution
Nieuwenhuis, B. P. S., R. Shraim, and H. Al Ghaithi. 2023. Self-compatibility in yeast is selected for reproductive assurance not population-level compatibility. Evolution qpad076.
https://doi.org/10.1093/evolut/qpad076

Keywords: Mating-type switching, homothallism, heterothallism, outcrossing, evolution of self-compatibility

Abstract

In haploid species, sexual reproduction by selfing lacks the common benefits from recombination and is indistinguishable from asexual reproduction at the genetic level. Nevertheless, the evolution of self-compatibility, known as homothallism in organisms with mating types, has occurred hundreds of times in fungi. Two main hypotheses have been proposed for the evolution of homothallism. First, that homothallism offers reproductive assurance, which is especially important when species have an obligatory sexual phase in their lifecycle. Second, that homothallism is associated with population level compatibility, increasing the chance of outbreeding. Here, we test these hypotheses using the fission yeast *Schizosaccharomyces pombe*, which is homothallic by mating-type switching, leveraging natural variation for switching efficiency in this species. Combining empirical tests with cellular automaton simulations, we show that homothallism by switching increases mating success of switching genotypes, but does not affect population level compatibility. Experiments show that outcrossing is actually reduced under homothallism. Our simulations explain these findings, because due to local mating, gametes that mated through intra-clonal selfing are no longer available for outcrossing. Our results suggest that the recurrent evolution of haploid self-compatibility is likely driven by selection for mating assurance, not to increase the potential for outcrossing.

Introduction

Sexual reproduction requires the fusion of gametes, and in most organisms, these gametes differ from each other: the gametes are of opposite sexes, or of different mating types. Mating between non-like types enforces outcrossing, but reduces the chance of finding a compatible partner, especially when the number of compatible types is low (Fisher 1930; Lande and Schemske 1985). When exactly two different types exist, the chance to meet a compatible partner is limited to 50%, assuming equal frequencies of each type in the population (Fisher 1930). If mates become limiting due to lack of compatible types, it is not surprising that self-compatibility evolves, which indeed has occurred repeatedly in different clades, including plants, animals, green and brown algae, and fungi (Whitehouse 1949; Baker 1955; Gioti et al. 2013; McDaniel et al. 2013; Wright et al. 2013; Beukeboom and Perrin 2014; Wilson et al. 2015; Hanschen et al. 2018). In fungi, transitions can occur regularly within a group, for example in *Neurospora* at least seven times (Nygren et al. 2011) and within the Saccharomycetaceae at least 31 times (Krassowski et al. 2019).

The consequences of self-compatibility differ greatly between diploid and haploid species (Holsinger 1987; Billiard et al. 2011; Szövényi et al. 2014). In diploid species, selfing occurs between gametes produced by the same individual, resulting in increased homozygosity with associated fitness reduction due to recessive deleterious alleles (i.e. inbreeding depression), nonetheless, existing heterozygosity in the parent will generate some variation among the offspring (Charlesworth and Willis 2009). In haploid species, selfing occurs between identical gametes (this is also known as intrahaploid mating, sameclone mating, or gametophytic selfing; Giraud et al. 2008; Epinat and Lenormand 2009; Billiard et al. 2012), hence the zygote will be completely homozygous. Consequently, recombination will not yield variation in the offspring and the sexual cycle is effectively indistinguishable from the asexual cycle with regards to the genetic outcomes (Xu 2023). Taking into account the high costs of sexual reproduction relative to asexual reproduction (Otto 2009), it is remarkable that sexual reproduction has been lost completely in only a few species (Lee et al. 2010; Nieuwenhuis and James 2016). A hypothesis for the maintenance of sex in selfing haploid species states that sexual reproduction might have become entwined with essential life-history traits, such as virulence or production of resting spores, which are mechanistically dependent on meiosis (Aanen and Hoekstra 2007; Heitman et al. 2014; Nieuwenhuis and James 2016; Ohtsuka et al. 2022). Additionally, even though sex can occur through haploid selfing, outcrossing is not precluded and, even when outcrossing is rare, sexual lineages might be favored over asexual ones (Hadany and Otto 2009).

In this study, we focus on mating in fungi. Most fungi are self-incompatible and require a partner of a different mating type, known as heterothallism (Blakeslee 1904; Lee et al. 2010). This is the ancestral state of the Dikarya, which contains the Ascomycota and Basidiomycota clades, and might even be older (Dyer 2008; Ropars et al. 2016; Coelho et al. 2017). Self-compatibility in most fungi is thus a derived state. The breeding system in which mating can occur between clonal gametes is known as homothallism (Whitehouse 1949; Wilson et al. 2015). Homothallism has two main consequences, which have each been proposed as the evolutionary driver of the loss of

Fig. 1

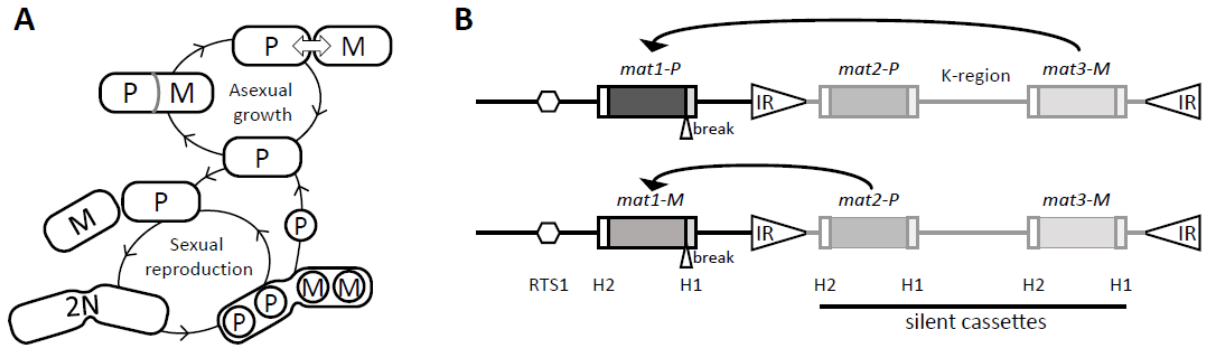

Fig 1. The general lifecycle of fission yeast with a schematic of the mating-type switching process. **A)** During mitotic reproduction the haploid cell divides during which mating type switching occurs on average every second cell division, resulting in daughter cells of opposite mating type. Induced by nitrogen starvation, cells of opposite mating types fuse, resulting in a diploid zygote that goes into meiosis, producing four haploid asco-spores. **B)** The three mating-type loci (*mat1*, *mat2* and *mat3*) indicated with the grey box. The heterochromatic region between the two inverted repeats (IR triangles) contains the two silent cassettes *mat2-P* and *mat3-M* interspersed with the K-region. A break is initiated in *H1* at *mat1* to initiate switching, which is facilitated by blocked centromere proximate DNA replication at replication termination site *RTS1*, to facilitate mating type switching. *H1* and *H2* are homology boxes involved in strand invasion during gene conversion.

heterothallism (Giraud et al. 2008; Billiard et al. 2012). First, homothallism allows for mating within a single haploid individual or between genetically identical clonally derived individuals (Billiard et al. 2011). Second, the loss of types realises population level compatibility (universal compatibility): each individual in the population is compatible with all others (Billiard et al. 2011, 2012). However, these two forces are not mutually exclusive, and which selective force is most prevalent has not been thoroughly empirically evaluated. While the benefit of reproductive assurance, especially in immotile species, has been shown (e.g. Hanson et al. 2014; Nieuwenhuis et al. 2018), the role of homothallism on outcrossing has not been extensively analyzed. Note that the two-fold advantage of self-fertilization as it occurs in hermaphroditic plants is unlikely to play a role in haploid species (Fisher 1941; Nagylaki 1976).

In fungi, homothallism has evolved through various mechanisms (Wilson et al. 2015; Krassowski et al. 2019). The most common form of homothallism is the incorporation of both mating-type alleles into the haploid genome, resulting in universal compatibility, as wells as self-compatibility (Yun et al. 1999; Dyer 2008). Another, less common form is same-mating-type mating (‘unisexual mating’) in which mutations of genes involved in the initiation of sexual reproduction allow cells of one genetic type to complete the sexual cycle (Lin et al. 2005; Wilson et al. 2021). A third form

of homothallism, the form central in this study, is mating-type switching, where each cell is one of two mating types, but where the mating type changes during mitosis, resulting in genetic compatibility between the daughter cells (Oshima and Takano 1971; Beach 1983; Perkins 1987; Krassowski et al. 2019). When no other individuals are present and cannot be encountered due to the predominantly sessile nature of fungi, switching assures sexual reproduction through selfing (Hanson et al. 2014). However, even when compatible genotypes are abundant, switching is beneficial because it provides the immotile yeast cells with a clonally derived partner that is of the opposite mating type (Nieuwenhuis and Immler 2016; Nieuwenhuis 2017). This is not possible for a heterothallic (i.e. non-switching) strain, because it forms a clonal patch within which mating is not possible; a compatible partner can only be found at the edge of the patch. Though beneficial for selfing, homothallism through mating-type switching should not affect population level compatibility, as each cell remains compatible with only half of the population. Nevertheless, switching might affect interactions at the edge.

In this study, we present data from a collection of natural isolates of the fission yeast *Schizosaccharomyces pombe*, which is haploid throughout its life cycle (**Fig. 1A**) and homothallic by mating-type switching (Beach 1983; Klar et al. 2014; Arcangioli and Gangloff 2023). Mating-type switching in fission yeasts consists of a three-cassette system (**Fig. 1B**), similar to, though mechanistically different from the independently evolved switching system in the budding yeast *Saccharomyces cerevisiae* (Hanson and Wolfe 2017). In *S. pombe*, the haploid cell can be one of two possible mating types, Plus or Minus (denoted as P and M, respectively), depending on the genes encoded in the 1kb mating-type cassette at the mating-type locus *mat1*. Next to the mating-type cassette at *mat1*, there are two additional cassettes located approximately 17kb centromere distal, containing a silent copy of the P and M cassettes, at *mat2* and *mat3* respectively. During mitotic division, a break at *mat1* is initiated, which is then repaired with the genetic information from one of the two silent cassettes as a template that is introduced at *mat1*. The choice of cassette used depends on the information that was present at *mat1*, with a preference given for the cassette encoding the opposite mating type, i.e. a P cell will preferentially use the M cassette and an M cell will preferentially use the P cassette (Jakočiūnas et al. 2013). In the standard homothallic laboratory strain (Leupold's h^{90} strain), switching is highly efficient occurring about 90% of the time in the right direction, either from P to M or from M to P, and this directional switching occurs in equal frequencies (i.e. switching is 'symmetrical'; (Leupold 1949; Jakočiūnas et al. 2013; Maki et al. 2018; Thon et al. 2019). In the other 10%, switching occurs using the cassette that was already present at *mat1* or using the sister chromatid, resulting in no switching. Previous research suggests that mating-type switching among natural isolates may vary in efficiency and symmetry (P→M ≠ M→P) (Singh and Klar 2002; Jeffares et al. 2015; Maki et al. 2018; Nieuwenhuis et al. 2018; López Hernández et al. 2021).

Here, we reanalyse whole-genome sequencing data showing asymmetries in the ratios of the mating-type cassettes at *mat1*. Our results show that the mating-type ratios indicate variation in switching directionality and – as we will show – can be used as a proxy for mating efficiency.

Based on this quantitative result, we analyse how homothallism – especially under spatial structure – affects its two proposed drivers: mating efficiency and outcrossing rate. We combine empirical results with simulation data to assess why homothallism evolved in yeast. Our results suggest it is to improve mating efficiency not to increase outcrossing rate.

Material and methods

Strains and media used

We analysed the 57 'core' natural isolates from a set of 161 isolates as described in (Jeffares et al. 2015) that were gifted by the Bähler lab. To test for outcrossing, we used strains EBC662 and EBC663, *h+* and *h-* respectively. These contain a marker on each of the chromosomes: mCherry fluorescent protein with a strong constitutive promoter on Chr I, a *hphMX* resistance marker at the mating type locus on Chr II, the color marker *ade6-M216* on Chr. Additionally, we used Leupold 690 derived h^{90} and an h^{+S} and h^{-S} strain as controls for an efficiently switching symmetrical homothallic and two stable heterothallic strains, respectively (Heim 1990). All strains with their genotypes are documented in Supplementary Table 1. The strains were grown on YES or synthetic medium PMG (Forsburg 2001; EMM with NH_4Cl replaced by 5g/L glutamic acid) at 32°C and mated on solid synthetic medium with the amount of glutamic acid reduced to 1g/L and 20g/L agar, referred to as PMG-N at 25°C.

Mating-type switching symmetry

To assess the symmetry in mating-type switching, a proxy for homothallism, we analysed two previously published data sets for the frequency of P and M cassettes at the active mating-type locus: 1) A set of 161 natural isolates for which the genomes were sequenced at mean coverage of 76X for 150bp paired-end reads using Illumina Hiseq (Jeffares et al. 2015) and 2) subset of 20 of these isolates that had been sequenced at mean coverage of 240X for paired-end 150bp reads using Illumina HiSeq and with long-read SMRT sequencing technology (Pacific Biosiences) (Tusso et al. 2019). The long-read data from the subset was used to validate the methodology applied to the full set of samples. Note that in the subset data, approximately 5% of the Illumina reads may be miss-assigned to samples, due to pooled sequencing and index hopping (Tusso et al. 2019).

Because the mating type cassettes can be present in up to five copies in the genome (Heim 1990; Nieuwenhuis et al. 2018) and here we are only interested in the allele at the *mat1* locus, we developed a simple pipeline to isolate reads from that locus. In short, i) we mapped all reads to a reference genome, ii) retrieved all the reads whose pair mapped to the region directly 1000bp centromere-proximal adjacent to the *mat1* locus containing the *RTS1* locus (referred to as *RTS1* region; (Dalgaard and Klar 2001)), and iii) counted how many of these paired reads mapped to the Plus or the Minus mating type locus. The annotated scripts are available in Supplementary Material. The copies at loci other than *mat1* are silenced and are considered not to affect compatibility (Heim 1990).

Fastq and *fasta* files were obtained for the short-read and long-read raw data, respectively, from the European Nucleotide Archive under accessions PRJEB2733, PRJEB6284, and PRJNA527756. The short read data was mapped to the reference genome using *bwa mem* v0.7.15 (Li 2013). Mapping was performed on an artificially generated h^{90} reference genome in which the silent *mat2:3* region was replaced by the full *mat2P-mat3M* region (MTR-contig in reference genome; Wood et al. 2002). To avoid biasing the mapping, we applied the full pipeline using a P and an M reference, in which the *mat1* sequence was set to either the P or the M mating-type cassette (Beach 1983). Bam files were generated, sorted, and indexed using *samtools* v1.4.1 (Danecek et al. 2021). We then calculated coverage for the *RTS1* region (coordinates in reference genome II:2113449-2114449) and of a 100kb region to normalize. The IDs of the reads mapping to the *RTS1* region were extracted using 'samtools view –f 0x1 file.bam "II:2113093-2114093"'. The IDs were used to extract pair reads and converted to fastq using bedtools v2.26.0 (Quinlan and Hall 2010). The obtained reads were filtered for *mat1* flanking or mating-type genes and finally assigned as either P or M, in two consecutive rounds using *bbsplit* from BBMap v37.28 (Bushnell 2014) and counted. For strains with multiple Run Accessions, the read counts were summed before downstream analyses. Deviation from the expected 50:50 ratio was assessed using a Chi^2 test with false discovery rate (FDR) correction for multiple testing.

For the long read data, we first extracted the reads that mapped to a 100kb region around the mating type using *bwa mem* with '-x pacbio' flag. We then used blast (blast/2.6.0+ NCBI) to find the *RTS1* region and the genes from the P (*mat2-Pi* and *mat2-Pc*) and M (*mat2-Mi* and *mat2-Mc*) cassettes within each read. Reads with a P or an M in a region up to 3kb centromere distal from the *RTS1* region were counted. Read counts from the long read and short read (outlined above) analyses were compared. Additionally, we counted the number of copies of the genes from the M and P cassettes in all reads. Due to discrepancies in some of the sample identities in Jeffares et al. (2015) and Tusso et al. (2019) (as described in (Tusso et al. 2022)) comparisons were only made between samples from the latter project. Additionally, ratios were compared with the assumed copy number of the *P* cassette, *M* cassette, and *K* region (region within silent P and M cassettes) in the genome based on normalized coverage, as counted in Nieuwenhuis et al. (2018).

To test if mating-type switching asymmetries are driven by a genetic component, we calculated the narrow-sense heritability and performed a genome-wide association study (GWAS) using REML in *ldak* (Speed et al. 2012), following the variants and methods described in Jeffares et al. (2015). Briefly, we evaluated the association between the fraction of *P* reads at *mat1* with the SNP variant data from Jeffares et al. (2015). The SNP data was formatted using *plink* (v1.07) with *--make-bed* flag and the quantitative association analysis was performed with *ldak --reml*.

Sporulation efficiency

Sporulation efficiency was measured by counting the number of mated and unmated cells in microscopy observations for the 57 natural isolates. Strains were grown in 300µl PMG in a 96 deep-well plate for 24h on a plate shaker at 800rpm, after which cells were diluted 1:100 in 300µl PMG in a 96 deep-well plate and incubated for 12 hours. The cells were spun down, supernatant

removed and all cells were resuspended in 10µl PBS and a droplet was placed on a PMG-N plate. The high cell densities obtained reduces the number of cell divisions possible (counts of cells before and after show ~10-30 times more cells after incubation, i.e. ~4-5 cell divisions). After 5 days incubation at 25°C, all cells were harvested, well mixed, mounted on a slide, and brightfield imaged at 40X. For the images we counted the number of loose spores, asci, zygotes, and cells. Sporulation efficiency was calculated as $(2Z + 2A + S)/(V + 2Z + 2A + S)$, with Z, A, S and V describing the number of zygotes, asci, spores and vegetative cells, respectively (Seike & Niki (2017)).

Outcrossing measurements

As with sporulation efficiency, cells were grown twice in PMG then mixed in equal volume with either a P (EBC662) or M (EBC663) strain with markers at each chromosome. These mixes were spun down, resuspended in 10 µl and this high-density droplet placed on PMG-N. The high density reduces the number of asexual growth cycles to approximately five cell divisions. After 5 days incubation the colony was harvested, digested in 100 mg/ml Lallzyme MMX suspension overnight (Flor-Parra et al. 2014) followed by a 30 min incubation in 30% ethanol, which kills all cells but the spores (Nieuwenhuis et al. 2018). Spores were diluted and plated out on YES+2% agar medium and incubated for 5 days and replica plating on YES+hygromycin for the *hphMX* marker. Due to the large number of *wtf* spore killer loci on chromosome III, which might skew allele frequencies linked to these loci, the marker at *ade6* was not scored (Zanders et al. 2014; Hu et al. 2017). The markers at chromosomes I and II are likely less affected by this due to the large distance (>50cM) of these markers to the two known locations of the *wtf* loci (one for chromosome I and II each) on these chromosomes (Hu et al. 2017). Because a heterothallic tester strain cannot reproduce by itself, offspring produced always need to be derived from the natural isolate, which either selfed or outcrossed. Any colony that carries a marker that originates from the tester strain, must therefore represent offspring from an outcrossing event. For each marker, we counted the fraction of colonies with markers, using the highest fraction to calculate the outcrossing rate. This method cannot account for epistatic incompatibilities between the lab strain and the natural isolates; the result should be interpreted as the minimal level of outcrossing.

Simulations

Cellular automaton simulations were performed similar to (Nieuwenhuis et al. 2018). Briefly, on a square lattice, coordinates are occupied by an individual after which asexual growth occurs repeatedly. When all coordinates are occupied or after a certain number of growth cycles all cells are allowed to mate. Mating efficiency can then be calculated. Every growth cycle, an empty coordinate of which at least one of the eight neighbouring cell is occupied has a chance Nx that will become occupied, with N a growth rate constant and x the number of neighboring cells. If growth occurs, the empty coordinate becomes occupied with the genotype of a neighbouring cell and is considered a clone of that neighbour. During growth the mating type can switch to P or M, with a chance P or M, respectively and either the mother or the daughter cell can migrate. Within

Fig. 2

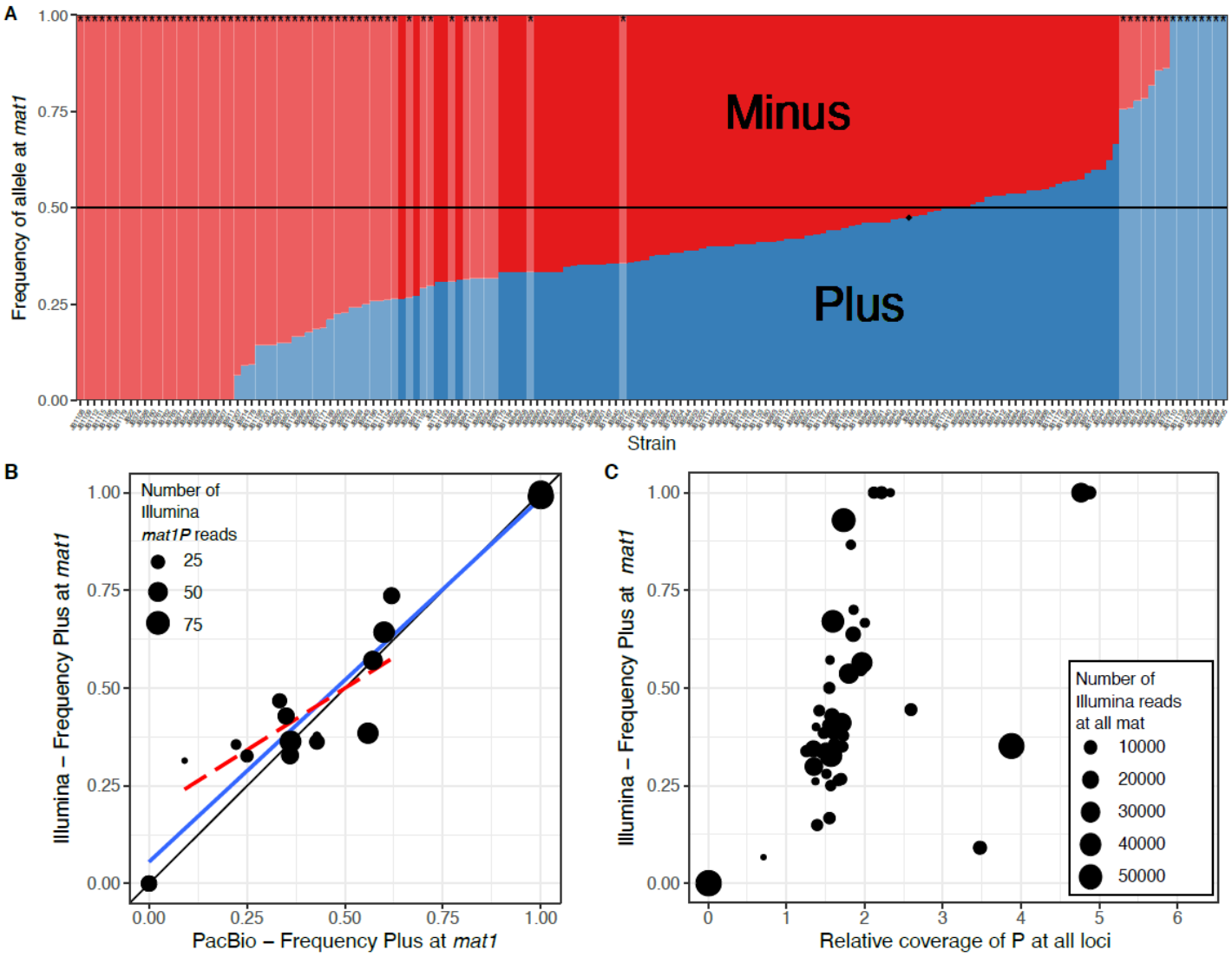

Fig 2. A) Frequency of the P and M mating-type cassettes at *mat1* per strain, calculated from the number of paired reads located adjacent to *RTS1*. The black line indicates the expected ratio of 1:1. The diamond gives the frequency of the h^{90} lab strain. Light columns indicated with an asterisk are significantly deviation from 1:1 at the significance level of $p < 0.05$ using a Chi-square comparison on the numbers of raw reads, followed by a False Discovery Rate correction for multiple comparisons. **B)** Correlation of allele frequencies for twenty strains for which both Pacific Bioscience SMART long-read sequencing data, as well as Illumina short-read data was available. The correlation is significant both for all strains (solid blue), and remains so when excluding apparent heterothallic strains (dashed red; GLM binomial, $z = 14.46$, $p < 10^{-15}$ and $z = 4.353$, $p < 10^{-5}$). **C)** Correlation between the coverage of the P cassette normalized to mean coverage and those counted at *mat1* shows that most of the variation in mating type frequency observed is within strains for the h^{90}-like genotype with three cassettes. Size of the point indicates total number of reads mapping to the P cassette.

each simulation, the switching frequencies are fixed. When the whole lattice is occupied, mating is induced: each cell assesses if one of the direct neighbours is of the opposite mating type and not mated, picks one of these cells at random and mates with that cell. When all cells that can mate have mated, the number of cells mated and the amount of inter-clonal and intra-clonal mating (outcrossing and haploid-selfing, respectively) is calculated. To test the importance of local interactions, additionally mating with more distant neighbours (up to 10 cells away) or full random mating was performed, for varying levels of switching. These simulations were also performed for single colonies for 8 or 16 asexual cycles to assess within colony dynamics, similar to (Hanson et al. 2014). Finally, also primary homothallism was tested, in which all cells are compatible to all others. In these simulations, grids direct comparisons were made for grids of cells that were allowed to mate as heterothallics, or as homothallics. Scripts are available in the supplementary material.

Results

Mating type switching is often not symmetrical

We assessed the variation in mating-type switching symmetry, a quantitative proxy for homothallism. By assigning the pairs of the reads that map to the *RTS1* region (a single copy region in all strains; **Fig. S1**) as M or P, we were able to obtain a measure for the number of *mat1P* or *mat1M* cells in each sample. Our results showed that of the 161 samples tested, 30 were heterothallic (8 for only *P* and 22 only *M* reads at *mat1*). Of the remaining 131 homothallic strains, 41 strains showed a significant deviation from the expected 1:1 ratio for *P* and *M* reads at *mat1* (**Fig. 2a**; χ^2-test with FDR correction $p < 0.05$). Comparing SMART long-read data with Illumina short read data for the subset of 20 strains showed a strong correlation between the skew in reads at *mat1* (**Fig 2b**, GLM binomial, $z = 14.46$, $p < 10^{-15}$) that is also maintained when excluding assumed heterothallic strains (GLM binomial, $z = 4.353$, $p < 10^{-5}$), validating our method. As expected Leupold's h^{90} reference strain 968 (JB50 in Jeffares et al. 2015) does not deviate significantly from a 1:1 ratio (frequency *mat1P* is 0.474 indicated by the diamond in **Fig. 2a**). Interestingly, a majority of the strains skewed towards the *M* mating-type cassette (34 skewed towards *M* compared to 7 towards *P*). In contrast, the ratio of reads at all mating-type loci was skewed towards the *P* mating type locus (**Fig. S2a**). Comparing the relative coverage of reads mapping to the *P* cassette for the entire genome shows a strong correlation with the relative coverage of the *K*-region, located between *mat2* and *mat3* (**Fig. S2b**). Repeats of the *K*-region are well documented resulting for example in the heterothallic h^{+N} genotype (Heim 1990; Schlake and Gutz 1993; Roseaulin et al. 2008), which in consecutive rounds can be extended to multiple copies. The frequency of *P* reads at the *mat1* locus within the 1.5X coverage group, probably h^{90}-like genotypes, spans almost the full spectrum suggesting that factors other than *P*-cassette copy numbers drive this variation (**Fig. 2c-d**). The correlation observed between the fraction of *P* reads at *mat1* and *P* reads for all loci (significant for all strains and when excluding presumed h^{-S} strains; **Fig. S2c**) is probably driven by the skew at *mat1* and not by copy number of other loci.

Fig. 3

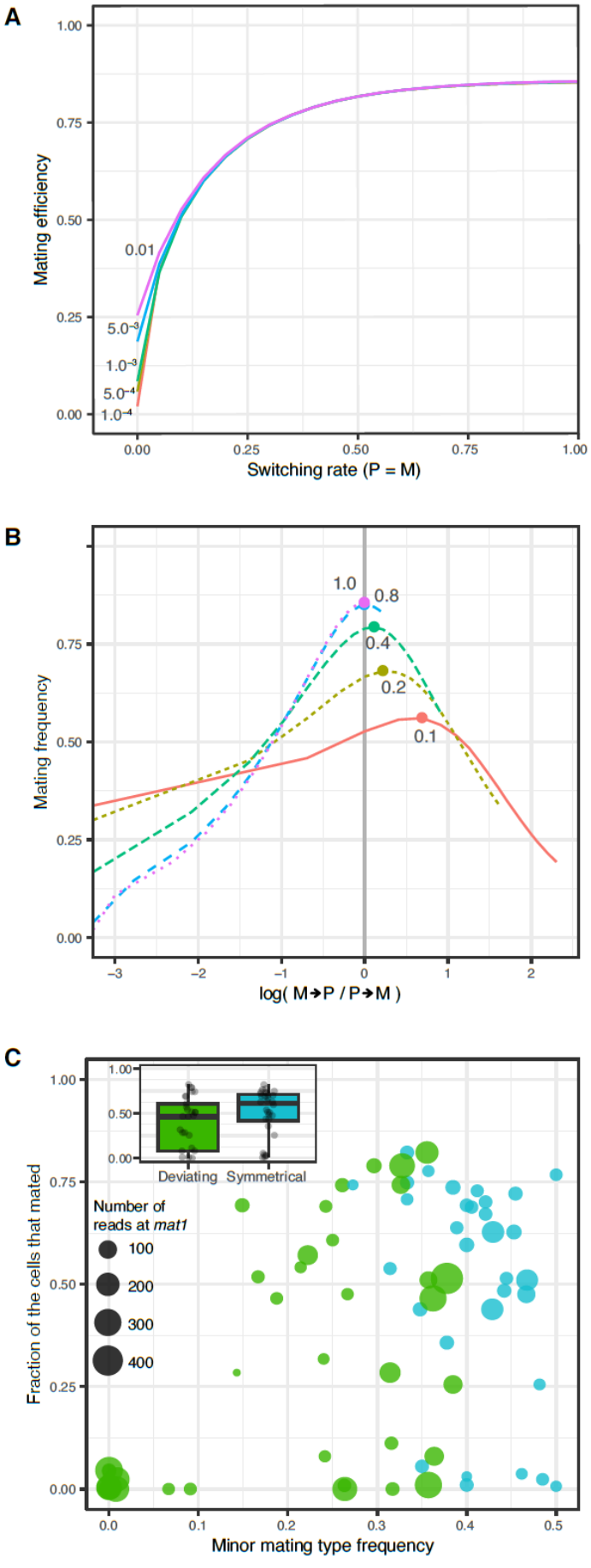

Fig 3. Mating efficiencies from cellular automaton simulations (A-B) and measurements (C). **A)** Mating efficiency (fraction of cells mated) under symmetrical switching (i.e. M→P = P→M) plotted against the rate of switching under different densities (indicated by value next to line) shows increase mating for with higher switching rates. **B)** Mating efficiency given for the switching rate M→P for a fixed value of switching P→M (values next to line). The x-axis gives the log of the rate M→P over P→M, which value is 0 when the two rates are equal and is positive when the rate of M→P is larger than P→M. A mutation that increases the switching rate away from symmetrical switching (value of 0) can increase mating efficiency, but only if the rate does not increase too much. The points indicate the maximum mating efficiency for each value P→M rate. **C)** Mating efficiency measured as asci, zygotes and spores per total number of cells, plotted against the minor mating-type frequency obtained from Illumina data. Color indicates if the strain showed significant deviation from 1:1 in *mat1* reads and size of the point indicates the reads on which the value is based. Inset shows the overall mating efficiency for deviating (left) and symmetrical (right) strains for strains in which the minor allele has a frequency of at least 0.05. These groups differ significantly (GLM, $z = 7.102$, $p < 1.23e\text{-}12$).

Heritability estimates using genome-wide genetic variants of all strains and the allele frequency at *mat1* as a quantitative trait analysed with *ldak* showed a significant narrow-sense heritability (0.33, SD = 0.137, p = 1.51e-03). However, we did not identify any variants significantly associated with this trait. This fits with previous research identifying at least 50 genes related to mating-type switching (Maki et al. 2018). In the following analyses, we used the observed variation in mating-type ratios to assess how the intensity of switching affects mating efficiency and outcrossing.

Switching rate and symmetry improve mating efficiency

To obtain a better understanding of how mating-type switching relates to sporulation and to outcrossing, we performed cellular automaton simulations under varying densities and varying amounts of switching. When the frequency of switching from P to M is the same as from M to P, our simulations show that higher mating-type switching rate increases spore production (**Fig. 3a**). This increase in mating is initially large, but levels off near full switching. Initial cell densities had little effect, except for at the lowest switching rates, indicating that most mating occurs through selfing (Nieuwenhuis et al., 2018). This also explains that density affects mating efficiency only at the lowest switching rates, when selfing is rare. The dynamics within single colonies when mating occurs after a few asexual cycles are similar to those in (Hanson et al. 2014) and are quantitatively similar to the runs using many genotypes and the full grid. This ag ain shows that the dynamics of the entire simulated area are mostly driven by the intra-clonal dynamics (**Fig. S3**).

Next, we introduced asymmetry in the rate of switching from P to M and *vice versa* in our simulations. Mating type ratios are directly proportional to switching symmetry, as the equilibrium ratio P/M is equal to the rate M-switches-to-P over the rate P-switches-to-M. Mating efficiency in our cellular automaton is proportional to the ratio of the mating types, however, not directly proportional, due to the spatial structure, which generally shows lower mating than would be expected under random mating. Adding asymmetries shows that a higher switching rate for one of the mating types can increase mating efficiency (it reduces the patch size of cells of the same mating type), but only when this rate is close to the switching rate of the other mating type (**Fig. 3b & Fig. S4**). When asymmetry in switching rate between the two mating types becomes too large, it strongly reduces mating efficiency due to the skew in mating-type ratios arising from asymmetrical switching (Hadjivasiliou et al. 2016). Evolution for increased switching efficiency is thus expected to occur with small incremental steps, with a potential for larger steps early in evolution when switching is likely inefficient.

Measurements of mating efficiency in natural yeast isolates (fraction of zygotes, asci and spores from all cells) vary widely across strains (**Fig. 3c**). While mating overall is higher when no deviation from equal ratios is observed (GLM binomial, z = 7.102, p < 1.23E-12, excluding heterothallic strains, **Fig. 3c** inset), high efficiencies are obtained for strains with large deviations in mating-type ratios. Low mating efficiencies are strongest for highly skewed ratios and

heterothallic strains, but are observed across all strains. This suggests that mechanisms other than mating type ratio affect the mating efficiency.

Homothallism reduces outcrossing

In our simulations, we were able to measure inter-clonal mating (outcrossing) by tracing the origin of the cells mating. Even though mating-type switching increases the chance that cells from different clonal decent are compatible, this did not result in an increased number of cells that outcross under any density (**Fig. 4A** solid lines). On the contrary, homothallism reduced the total number of cells that mate with another individual, because fewer cells were available, as these had already participated in intra-clonal mating. Indeed, when haploid selfing was not allowed, outcrossing did increase with increased switching (**Fig. 4A** dotted lines). Due to the large increase in offspring from haploid selfing, the relative number of outcrossed offspring decreased even more drastically than the absolute reduction (**Fig. 4B**).

We expected reduced outcrossing to be driven by the spatial structure in our cellular automaton. To test the importance of the spatial structure during mating, we varied the distance over which mating can take place beyond direct neighbours. Increasing this distance does affect the amount of outcrossing, however, the relationship of reduced outcrossing with increasing homothallism is maintained (**Fig. S5A**). Next, we tested if this observation is maintained under true homothallism (or 'primary homothallism') when all cells are compatible with all others (Wilson et al. 2015). Under all densities and mating distances, fewer outcrossing occurs in homothallic strains than in heterothallic strains, which shows that in structured environments with local mating, homothallism reduces outcrossing (**Fig. S5B**). Under the highest density and largest distance, the ratio becomes equal. For these parameters, the average patch size is equal in size to the mating distance; hence, between- and within-patch interactions are equally likely. Finally, we analysed the effect of local interactions on the amount of outcrossing. When mating occurs without any structure (random mating), the amount of outcrossing depends on the 'population level compatibility', which remains 50% and is not affected by the switching rate (**Fig. S5C**). When cells that did not meet a compatible mate at first are given opportunities to mate again, the number of successful mating increases, and so does the absolute number of outcrossing events. The dashed lines show selfing still diminishes outcrossing, but that homothallism overall improves outcrossing relative to heterothallism (**Fig. S5C**).

Finally, we empirically tested outcrossing rate for the 57 natural isolates, crossing each with a labelled tester strain of either the P or M mating type and scored the number of colonies from sexual offspring with marker (**Fig. 4C**). The heterothallic strains, which are obligate outcrossing, showed as expected that all offspring were obtained through outcrossing (median 1.04 and 0.959 for Minus and Plus strains respectively; **Fig. 4D**). Most homothallic strains do produce some outcrossed offspring, but less than the heterothallic stains, however there is quite some variation among the strains and also whether crossed to Plus or Minus (medians 0.124 and 0.267 respectively; **Fig. 4D**). Some strains mate almost exclusively through selfing, while others mate

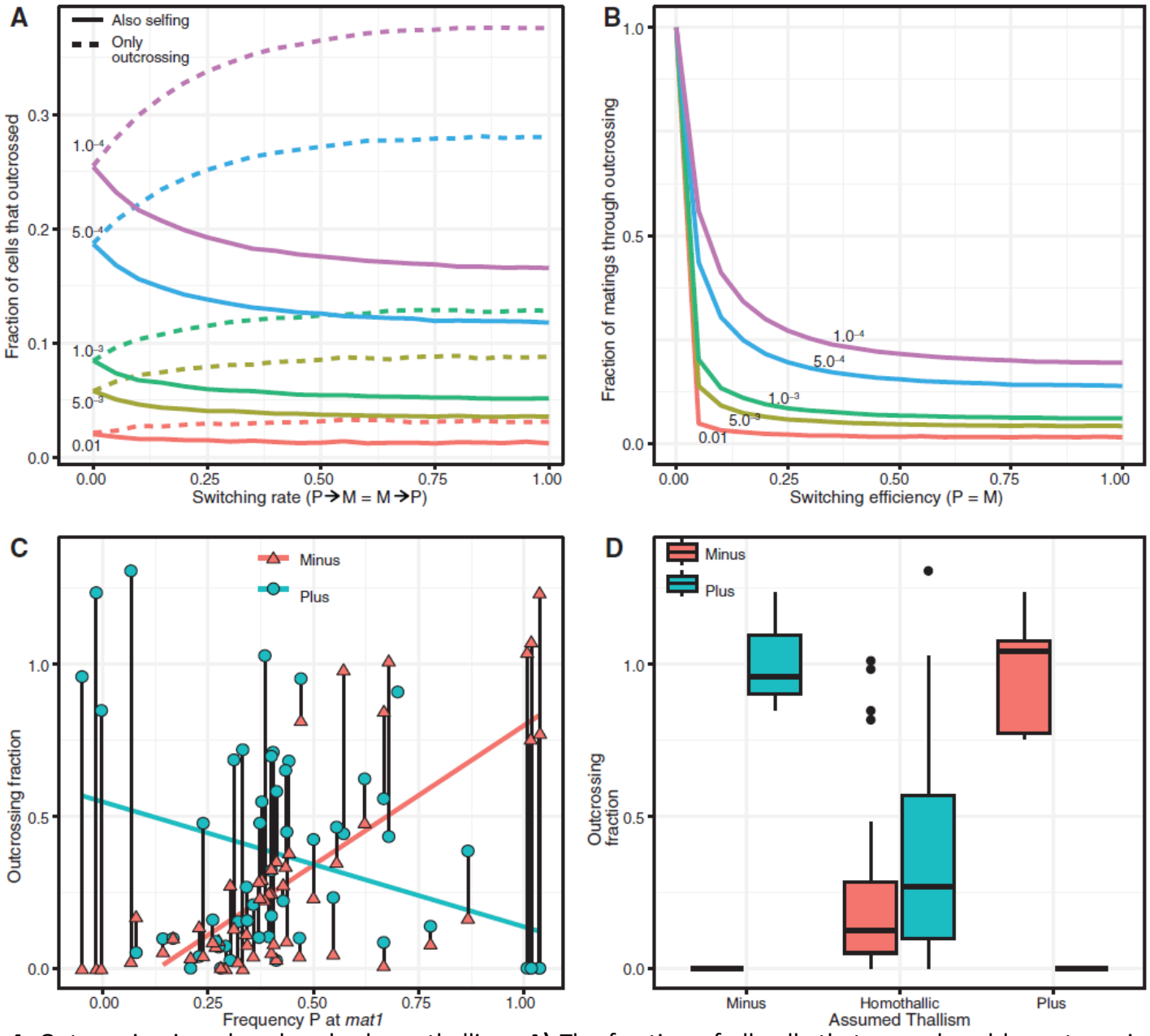

Fig 4. Outcrossing is reduced under homothallism. **A)** The fraction of all cells that reproduced by outcrossing in cellular automaton simulation shows that with higher switching rates the total number of cells that outcross decreases when haploid selfing is allowed (solid lines) under different densities indicated by values next to lines. The dashed lines show the same, but when outcrossing is blocked. **B)** Similar to A, but showing the outcrossed cell as a fraction of the cells that mated. Because the number of cells mating increases more rapidly, the relative decrease is much stronger. **C)** Fraction of outcrossing for 57 natural isolates when mixed in equal proportions with a heterothallic M or P strain (red or blue dot) against the frequency of Plus at *mat1*. As expected, heterothallic strains show high outcrossing rates. To avoid overlap of data points, the heterothallic strains are plotted next to each other (strains with $freq_P < 0$ or $freq_P > 1$ are $freq_P = 0$ or $freq_P = 1$ respectively). **D)** Summary of data from C) showing outcrossing rate per group.

almost exclusively through outcrossing, This suggests that other mechanisms beyond asymmetry in allele frequencies at *mat1* play a role here, as was also suggested to occur for some strains studied in (López Hernández et al. 2021).

Discussion

The loss of self-incompatibility in haploid species gives the potential for haploid selfing, but does not necessarily increase the chance of outcrossing. In this study, we measured the ratio of P and M cassettes at *mat1* in *S. pombe*, which deviate from a 1:1 ratio in many strains, and used this asymmetry as a quantitative proxy for homothallism. We find that symmetry in switching rate is associated with mating efficiency and that balanced ratios increase mating efficiency within a strain. Our results show that outcrossing is not common and not associated with switching symmetry and that high outcrossing frequencies are observed most strongly under heterothallism. We explored these finding for a larger parameters space using cellular automaton simulations, which further support our conclusions, showing that i) switching frequency increases mating efficiency, ii) mating-type switching asymmetries negatively affect mating efficiency, and iii) outcrossing under homothallism is reduced relative to heterothallism.

Variation in switching affects mating efficiency

The reanalyses of sequencing data showed variation in mating-type frequencies among yeast strains. The results are consistent across long- and short-read data and across samples sequenced in different labs, indicating that our method to assess mating-type frequencies is robust. The deviation in observed ratios from expected ratios suggests variation in the symmetry from P to M and from M to P, leading to a skew in mating type ratios within a clonal population (Jakočiūnas et al. 2013; Maki et al. 2018). The variation in mating types should thus affect the number of compatible cells within a patch and additionally reduce population-level compatibility (Fisher 1930; Hoekstra 1987; Hadjivasiliou et al. 2016). The ratios indicate skews in switching directionality but cannot directly inform about switching efficiency, for which direct observations of switching are required. Unfortunately, attempts to observe mating types in cell pedigrees using the fluorescent strain from Jakočiūnas et al. (2013) did not yield results in our lab. Nonetheless, variation in mating-type switching in natural isolates has been deduced by counting mating in pedigrees and has been shown to be associated with mating efficiency (López Hernández et al. 2021).

We observed a reduction in mating efficiency for natural isolates with stronger asymmetric ratios, however, this is mostly driven by strains in which asymmetry is very high. When both mating types can be found at *mat1*, a wide variety of mating efficiencies is observed, suggesting that other factors might be associated with this trait besides the mating-type region. This association has been observed in mutant screens before. Leupold isolated eight spontaneous mutants from the reference strain, in which sporulation was reduced to between 20% and 64% (Leupold 1949, 1955). Further variation has been shown to exist between strains, where mutations in the mating-type region reduced mating (Schlake and Gutz 1993). Reorganizations of the mating-type region are associated

with the transition from homothallism to heterothallism (Heim 1990). However, the variation we observe is probably not caused by such a reorganization, as most of the variation occurs within the group that has a standard configuration with a P or M cassette at *mat2* and *mat3*, respectively. Asymmetries at *mat1* showed no association with copy numbers of mating-type cassettes. On the contrary, while the active mating-type locus *mat1* skews towards M, the reads for all loci skew towards the P cassette. Interestingly, in a mutant screen for mating-type switching asymmetries, Maki et al. found almost exclusively mutants that shift the ratio towards M (Maki et al. 2018). Even though switching shows a narrow sense heritability of 0.33, no strong associations with genetic variants were observed in our GWAS, suggesting that the variation in asymmetries is a polygenic trait, which is supported by the known suite of genes that affect mating-type switching (Jakočiūnas et al. 2013; Maki et al. 2018).

Evolution of switching efficiency

Improved switching will improve mating efficiency, but only when symmetry in switching does not become unbalanced. Our results are in line with the model and the observations from Hanson et al. (2014), which showed that switching under symmetry can lead to a mating efficiency of 90%. Our results level-off at about 85% of cells mating, which is due to the lattice used in our simulations, in which only eight neighbouring cells exist; mating over ten neighbouring cells increases mating efficiency to ~98%.

The main hypothesis for the evolution of the three cassette system observed in fission yeasts and in the Saccharomycetaceae (Dalgaard and Klar 2001; Krassowski et al. 2019) states that this system evolved from a flip-flop system in which a part of the chromosome repeatedly inverts. With the inversion, the active cassette is exchanged to a centromere or sub-telomere adjacent region containing the silent cassette (Maekawa and Kaneko 2014; Wolfe and Butler 2022), which comes at the cost of potential meiotic disruption when crossover occurs between the two different mating-type orientations (Krassowski et al. 2019; Wolfe and Butler 2022). The three-cassette system overcomes this cost, but introduces selection pressure to enhance switching directionality. Where in a flip-flop system a switching is always in the correct direction, when two templates are available this will be correct only half of the time. Mutations for increased efficiency will likely be towards one or the other silent cassette (Maki et al. 2018), which will increase asymmetry. Our simulations show that small incremental changes can be selected, even as these cause some asymmetry, as these improve the overall mating efficiency within the colony. When switching is very rare, most mutations - even those increasing asymmetry - will increase mating. However, when switching becomes more frequent, improved efficiency can only occur incrementally by successive mutations that maintain a certain amount of symmetry. The large number of genes involved in switching in *S. pombe* and *Saccharomyces cerevisiae* support this finding (Thon et al. 2019).

Homothallism reduces outcrossing

Symmetrical mating-type switching resulting in balanced mating-type ratios increases within patch mating efficiency (Nieuwenhuis et al. 2018) as well as population level compatibility by balancing mating-type ratios (Hadjivasiliou et al. 2016). We empirically tested how homothallism affects outcrossing, and showed outcrossing in homothallic strains to be low relative to heterothallic strains. The simulations confirm a low amount of outcrossing compared to heterothallism: the absolute number of outcrossed zygotes decreased, and because the total number of zygotes increased due to haploid selfing, the relative contribution of outcrossings was reduced even more strongly. In a scenario where switching still occurs but haploid selfing is restricted through introduction of a second locus that discriminates self from non-self, the absolute reduction in outcrossing is no longer observed, and switching increases mating success. From this we can conclude that switching does increase compatibility, but due to mating within the same clone, unmated cells at the periphery of the local patch become less common, in turn reducing achieved outcrossings. Also under full homothallism, where within-clone compatibility is equal to between-clone compatibility, local structure reduces outcrossing. This reduction of available cells is similar to the process of seed discounting in plants where selfing reduces the opportunity for outcrossing through the female role (Uyenoyama and Waller 1991). From our data we thus conclude that homothallism in yeast is most strongly selected to increase intra-haploid selfing and not to increase universal compatibility.

Our models explicitly incorporate environmental structure. This is appropriate for most sessile species that reproduce through fusion *in situ*, such as yeasts, non-conidiating fungi, and fungi that have limited dispersal of fertilizing propagules. Strains that reproduce with higher dispersal will have higher mixing, which likely reduces discounting and homothallism in these species could thus be especially beneficial for universal compatibility. Nevertheless, in some filamentous homothallic species with conspecifics that have high dispersal, structures that improve outcrossing are lost (Billiard et al. 2012; Hadjivasiliou et al. 2015). If homothallism evolved to favour selfing and not outcrossing, loss of dispersal mechanisms could be driven by resource allocation from the male role (fertilizing propagule production for outcrossing) towards the female role (spore production), similar to the selfing syndrome in plants that lost self-incompatibility (Sicard and Lenhard 2011). For example, many homothallic *Neurospora* have lost the ability to form ‘male’ conidia and ‘female’ trichogynes (Glass et al. 1990; Nygren et al. 2011). Note that the causation could be in the other direction; loss of these structures could have selected for homothallism. The analysis of the effect of homothallism on filamentous conidiating species requires further empirical and theoretical studies.

Homothallic species have reduced effective population sizes, which is associated with a reduction in outcrossing (Taylor et al. 2015; Nieuwenhuis and James 2016). Homothallic species are predominantly located at the tips of phylogenetic trees with heterothallic sister clades suggesting a recent transition to homothallism (Takebayashi and Morrell 2001; Gioti et al. 2013; Krassowski et al. 2019). A main argument is that due to limited outcrossing, these lineages have low effective

populations sizes and eventually go extinct, while self-incompatibility is maintained through species level selection (Stebbins 1957; Goldberg et al. 2010). Nevertheless, long-lived homothallic taxa do exist, e.g. *Schizosaccharomyces* are probably homothallic for >100 My (Rhind et al. 2011) and Saccharomyceae for ~100 My (Krassowski et al. 2019). Possibly, the haplontic life-cycle with regular sexual reproduction acts as a selection arena (Aanen and Hoekstra 2007), and together with large census population sizes sporadic reproduction by outcrossing can protect the lineage from extinction. This hypothesis could be tested by assessing the age of self-compatibility and life history traits in a broader range of taxa, including non-yeast fungi, green and brown algae, and bryophytes. An alternative, do not mutually exclusive hypothesis is that homothallic species might maintain diversity through a mechanism that allows them to distinguish self from non-self through other means than the mating type. The evolution of such mechanisms can overcome the negative effects of discounting and increase outcrossing. For example, homothallic species that mate by dispersal of fertilizing propagules over longer distances might be able to improve outcrossing by temporal shifts of reproduction though the male and female roles. Propagules can perform fertilizations without the chance of self-fertilizing the female structures. In fission yeast, López Hernández et al. (2021) observed cells with elongated shmoos (yeast mating protrusions) specifically under outcrossing conditions, but it remains unclear if these will increase non-self mating. Similarly, variation in secreted pheromones or other cell-surface bound signals might be used as cues to distinguish among genotypes (Seike et al. 2019). Unfortunately, little is known about the natural occurrence of fission yeasts and thus how likely strains are to encounter other genotypes (Jeffares 2018; Brysch-Herzberg et al. 2022; Shraim and Nieuwenhuis 2022). Population genetic analyses of *S. pombe* shows persistent, but sporadic outcrossing, with large haplotype blocks and slow linkage disequilibrium decay over distance (Nieuwenhuis and James 2016; Tusso et al. 2019), which suggests outcrossing is very rare relative to asexual growth or haploid selfing.

Conclusions

The repeated loss of self-incompatibility in haploid species was long considered to have evolved to increase haploid selfing. However, this omitted the alternative hypothesis that self-compatibility might be driven by selection for population level compatibility. Here we show that in species where mating occurs with limited dispersal, homothallism is unlikely to have evolved for increased outcrossing, because outcrossing is reduced when intra-haploid mating is possible, due to gamete discounting. Our results support the hypothesis that homothallism evolved for reproductive assurance through haploid selfing. Analyses of more haploid species, including non-fungal taxa, is required to assess the generality of this principle.

References

Aanen, D. K., and R. F. Hoekstra. 2007. Why sex is good: on fungi and beyond. Pp. 527–534 *in* J. Heitman, J. W. Kronstad, J. W. Taylor, and L. A. Casselton, eds. Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, D.C.

Arcangioli, B., and S. Gangloff. 2023. The Fission Yeast Mating-Type Switching Motto: "One-for-Two" and "Two-for-One." Microbiol. Mol. Biol. Rev. 0:e00008-21. American Society for Microbiology.

Baker, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9:347–349.

Beach, D. H. 1983. Cell type switching by DNA transposition in fission yeast. Nature 305:682–688.

Beukeboom, L., and N. Perrin. 2014. The Evolution of Sex Determination. Oxford University Press, Oxford.

Billiard, S., M. López-Villavicencio, B. Devier, M. E. Hood, C. Fairhead, and T. Giraud. 2011. Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. Biol. Rev. 86:421–442.

Billiard, S., M. López-Villavicencio, M. E. Hood, and T. Giraud. 2012. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J. Evol. Biol. 25:1020–1038.

Blakeslee, A. F. 1904. Sexual reproduction in the Mucorineae. Proc. Am. Acad. Arts Sci. 40:205–319.

Brysch-Herzberg, M., G.-S. Jia, M. Seidel, I. Assali, and L.-L. Du. 2022. Insights into the ecology of *Schizosaccharomyces* species in natural and artificial habitats. Antonie Van Leeuwenhoek 115:661–695.

Bushnell, B. 2014. BBMap: A Fast, Accurate, Splice-Aware Aligner.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10:783–796. Nature Publishing Group.

Coelho, M. A., G. Bakkeren, S. Sun, M. E. Hood, and T. Giraud. 2017. Fungal Sex: The Basidiomycota. Microbiol. Spectr. 5.

Dalgaard, J. Z., and A. J. S. Klar. 2001. A DNA replication-arrest site *RTS1* regulates imprinting by determining the direction of replication at *mat1* in *S. pombe*. Genes Dev. 15:2060–2068.

Danecek, P., J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan, M. O. Pollard, A. Whitwham, T. Keane, S. A. McCarthy, R. M. Davies, and H. Li. 2021. Twelve years of SAMtools and BCFtools. GigaScience 10.

Dyer, P. S. 2008. Evolutionary biology: Genomic clues to original sex in fungi. Curr. Biol. 18:R207–R209.

Epinat, G., and T. Lenormand. 2009. The Evolution of Assortative Mating and Selfing with in- and Outbreeding Depression. Evolution 63:2047–2060.

Fisher, R. A. 1941. Average Excess and Average Effect of a Gene Substitution. Ann. Eugen. 11:53–63.

Fisher, R. A. 1930. Sexual reproduction and sexual selection. Pp. 121–145 *in* The genetical theory of natural selection. Oxford University Press, Oxford.

Flor-Parra, I., J. Zhurinsky, M. Bernal, P. Gallardo, and R. R. Daga. 2014. A Lallzyme MMX-based rapid method for fission yeast protoplast preparation. Yeast 31:61–66.

Forsburg, S. L. 2001. *S. pombe* strain maintenance and media. P. Supplement 64 *in* Current Protocols in Molecular Biology. John Wiley & Sons, Inc.

Gioti, A., J. E. Stajich, and H. Johannesson. 2013. *Neurospora* and the dead-end hypothesis: genomic consequences of selfing in the model genus. Evolution 67:3600–3616.

Giraud, T., R. Yockteng, M. Lopez-Villavicencio, G. Refregier, and M. E. Hood. 2008. Mating system of the anther smut fungus *Microbotryum violaceum*: Selfing under heterothallism. Eukaryot. Cell 7:765–775.

Glass, N. L., R. L. Metzenberg, and N. B. Raju. 1990. Homothallic Sordariaceae from nature: The absence of strains containing only the *a* mating type sequence. Exp. Mycol. 14:274–289.

Goldberg, E. E., J. R. Kohn, R. Lande, K. A. Robertson, S. A. Smith, and B. Igić. 2010. Species Selection Maintains Self-Incompatibility. Science 330:493–495.

Hadany, L., and S. P. Otto. 2009. Condition-Dependent Sex and the Rate of Adaptation. Am. Nat. 174:S71–S78.

Hadjivasiliou, Z., Y. Iwasa, and A. Pomiankowski. 2015. Cell–cell signalling in sexual chemotaxis: a basis for gametic differentiation, mating types and sexes. J. R. Soc. Interface 12:20150342.

Hadjivasiliou, Z., A. Pomiankowski, and B. Kuijper. 2016. The evolution of mating type switching. Evolution 70:1569–1581.

Hanschen, E. R., M. D. Herron, J. J. Wiens, H. Nozaki, and R. E. Michod. 2018. Repeated evolution and reversibility of self-fertilization in the volvocine green algae. Evolution 72:386–398.

Hanson, S. J., K. P. Byrne, and K. H. Wolfe. 2014. Mating-type switching by chromosomal inversion in methylotrophic yeasts suggests an origin for the three-locus *Saccharomyces cerevisiae* system. Proc. Natl. Acad. Sci. 111:E4851–E4858.

Hanson, S. J., and K. H. Wolfe. 2017. An evolutionary perspective on yeast mating-type switching. Genetics 206:9–32.

Heim, L. 1990. Construction of an *h+S* strain of *Schizosaccharomyces pombe*. Curr. Genet. 17:13–19.

Heitman, J., D. A. Carter, P. S. Dyer, and D. R. Soll. 2014. Sexual Reproduction of Human Fungal Pathogens. Cold Spring Harb. Perspect. Med. 4:a019281.

Hoekstra, R. F. 1987. The evolution of sexes. Pp. 59–91 *in* S. C. Stearns, ed. The evolution of sex and its consequences. Birkhauser Verlag, Basel.

Holsinger, K. E. 1987. Gametophytic Self-Fertilization in Homosporous Plants: Development, Evaluation, and Application of a Statistical Method for Evaluating Its Importance. Am. J. Bot. 74:1173–1183.

Hu, W., Z.-D. Jiang, F. Suo, J.-X. Zheng, W.-Z. He, and L.-L. Du. 2017. A large gene family in fission yeast encodes spore killers that subvert Mendel's law. eLife 6:e26057.

Jakočiūnas, T., L. R. Holm, J. Verhein-Hansen, A. Trusina, and G. Thon. 2013. Two portable recombination enhancers direct donor choice in fission yeast heterochromatin. PLOS Genet. 9:e1003762.

Jeffares, D. C. 2018. The natural diversity and ecology of fission yeast. Yeast 35:253–260.

Jeffares, D. C., C. Rallis, A. Rieux, D. Speed, M. Převorovský, T. Mourier, F. X. Marsellach, Z. Iqbal, W. Lau, T. M. K. Cheng, R. Pracana, M. Mülleder, J. L. D. Lawson, A. Chessel, S. Bala, G. Hellenthal, B. O'Fallon, T. Keane, J. T. Simpson, L. Bischof, B. Tomiczek, D. A. Bitton, T. Sideri, S. Codlin, J. E. E. U. Hellberg, L. van Trigt, L. Jeffery, J.-J. Li, S. Atkinson, M. Thodberg, M. Febrer, K. McLay, N. Drou, W. Brown, J. Hayles, R. E. C. Salas, M. Ralser, N. Maniatis, D. J. Balding, F. Balloux, R. Durbin, and J. Bähler. 2015. The genomic and phenotypic diversity of *Schizosaccharomyces pombe*. Nat. Genet. 47:235–241.

Klar, A. J. S., S. Moore, and K. Ishikawa. 2014. A unique DNA recombination mechanism of the mating/cell-type switching of fission yeasts: a review. Microbiol Spectr. 2:MDNA3-0003–2014.

Krassowski, T., J. Kominek, X.-X. Shen, D. A. Opulente, X. Zhou, A. Rokas, C. T. Hittinger, and K. H. Wolfe. 2019. Multiple Reinventions of Mating-type Switching during Budding Yeast Evolution. Curr. Biol. 29:2555-2562.e8.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. i. genetic models. Evol. Int. J. Org. Evol. 39:24–40.

Lee, S. C., M. Ni, W. Li, C. Shertz, and J. Heitman. 2010. The Evolution of Sex: a Perspective from the Fungal Kingdom. Microbiol. Mol. Biol. Rev. 74:298–340.

Leupold, U. 1949. Die vererbung von homothallie und heterothallie bei *Schizosaccharomyces pombe*. Universität Zürich, Zürich.

Leupold, U. 1955. Methodisches zur Genetik von *Schizosaccharomyces pombe*. Pathobiology 18:1141–1146.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv.

Lin, X., C. M. Hull, and J. Heitman. 2005. Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. Nature 434:1017–1021.

López Hernández, J. F., R. M. Helston, J. J. Lange, R. B. Billmyre, S. H. Schaffner, M. T. Eickbush, S. McCroskey, and S. E. Zanders. 2021. Diverse mating phenotypes impact the spread of *wtf* meiotic drivers in *Schizosaccharomyces pombe*. eLife 10:e70812. eLife Sciences Publications, Ltd.

Maekawa, H., and Y. Kaneko. 2014. Inversion of the chromosomal region between two mating type loci switches the mating type in *Hansenula polymorpha*. PLoS Genet 10:e1004796.

Maki, T., N. Ogura, J. E. Haber, H. Iwasaki, and G. Thon. 2018. New insights into donor directionality of mating-type switching in *Schizosaccharomyces pombe*. PLOS Genet. 14:e1007424.

McDaniel, S. F., J. Atwood, and J. G. Burleigh. 2013. Recurrent evolution of dioecy in bryophytes. Evolution 67:567–572.

Nagylaki, T. 1976. A Model for the Evolution of Self-fertilization and Vegetative Reproduction. J. Theor. Biol. 58:55–58.

Nieuwenhuis, B. P. S. 2017. Loss of Self-Incompatibility by Mating-Type Switching. Pp. 1–8 *in* John Wiley & Sons Ltd, ed. eLS. John Wiley & Sons, Ltd, Chichester, UK.

Nieuwenhuis, B. P. S., and S. Immler. 2016. The evolution of mating-type switching for reproductive assurance. BioEssays 38:1141–1149.

Nieuwenhuis, B. P. S., and T. Y. James. 2016. The frequency of sex in fungi. Philos. Trans. R. Soc. B Biol. Sci. 371:20150540.

Nieuwenhuis, B. P. S., S. Tusso, P. Bjerling, J. Stångberg, J. B. W. Wolf, and S. Immler. 2018. Repeated evolution of self-compatibility for reproductive assurance. Nat. Commun. 9:1639.

Nygren, K., R. Strandberg, A. Wallberg, B. Nabholz, T. Gustafsson, D. García, J. Cano, J. Guarro, and H. Johannesson. 2011. A comprehensive phylogeny of *Neurospora* reveals a link between reproductive mode and molecular evolution in fungi. Mol. Phylogenet. Evol. 59:649–663.

Ohtsuka, H., K. Imada, T. Shimasaki, and H. Aiba. 2022. Sporulation: A response to starvation in the fission yeast *Schizosaccharomyces pombe*. MicrobiologyOpen 11:e1303.

Oshima, Y., and I. Takano. 1971. Mating Types in *Saccharomyces*: Their Convertibility and Homothallism. Genetics 67:327–335.

Otto, S. P. 2009. The Evolutionary Enigma of Sex. Am. Nat. 174:S1–S14.

Perkins, D. D. 1987. Mating-type switching in filamentous ascomycetes. Genetics 115:215–216.

Quinlan, A. R., and I. M. Hall. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26:841–842.

Rhind, N., Z. Chen, M. Yassour, D. A. Thompson, B. J. Haas, N. Habib, I. Wapinski, S. Roy, M. F. Lin, D. I. Heiman, S. K. Young, K. Furuya, Y. Guo, A. Pidoux, H. M. Chen, B. Robbertse, J. M. Goldberg, K. Aoki, E. H. Bayne, A. M. Berlin, C. A. Desjardins, E. Dobbs, L. Dukaj, L. Fan, M. G. FitzGerald, C. French, S. Gujja, K. Hansen, D. Keifenheim, J. Z. Levin, R. A. Mosher, C. A. Müller, J. Pfiffner, M. Priest, C. Russ, A. Smialowska, P. Swoboda, S. M. Sykes, M. Vaughn, S. Vengrova, R. Yoder, Q. Zeng, R. Allshire, D. Baulcombe, B. W. Birren, W. Brown, K. Ekwall, M. Kellis, J. Leatherwood, H. Levin, H. Margalit, R. Martienssen, C. A. Nieduszynski, J. W. Spatafora, N. Friedman, J. Z. Dalgaard, P. Baumann, H. Niki, A. Regev, and C. Nusbaum. 2011. Comparative functional genomics of the fission yeasts. Science 332:930–936.

Ropars, J., K. S. Toro, J. Noel, A. Pelin, P. Charron, L. Farinelli, T. Marton, M. Krüger, J. Fuchs, A. Brachmann, and N. Corradi. 2016. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. Nat. Microbiol. 16033.

Roseaulin, L., Y. Yamada, Y. Tsutsui, P. Russell, H. Iwasaki, and B. Arcangioli. 2008. Mus81 is essential for sister chromatid recombination at broken replication forks. EMBO J. 27:1378–1387. John Wiley & Sons, Ltd.

Schlake, T., and H. Gutz. 1993. Mating configurations in *Schizosaccharomyces pombe* strains of different geographical origins. Curr. Genet. 23:108–114.

Seike, T., and H. Niki. 2017. Mating response and construction of heterothallic strains of the fission yeast *Schizosaccharomyces octosporus*. FEMS Yeast Res. 17. Oxford Academic.

Seike, T., C. Shimoda, and H. Niki. 2019. Asymmetric diversification of mating pheromones in fission yeast. PLOS Biol. 17:e3000101.

Shraim, R., and B. P. S. Nieuwenhuis. 2022. The search for *Schizosaccharomyces* fission yeasts in environmental metatranscriptomes. Yeast 39:83–94.

Sicard, A., and M. Lenhard. 2011. The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptation in plants. Ann. Bot. 107:1433–1443.

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

Speed, D., G. Hemani, M. R. Johnson, and D. J. Balding. 2012. Improved Heritability Estimation from Genome-wide SNPs. Am. J. Hum. Genet. 91:1011–1021. Elsevier.

Stebbins, G. L. 1957. Self Fertilization and Population Variability in the Higher Plants. Am. Nat. 91:337–354.

Szövényi, P., N. Devos, D. J. Weston, X. Yang, Z. Hock, J. A. Shaw, K. K. Shimizu, S. F. McDaniel, and A. Wagner. 2014. Efficient Purging of Deleterious Mutations in Plants with Haploid Selfing. Genome Biol. Evol. 6:1238–1252.

Takebayashi, N., and P. L. Morrell. 2001. Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. Am. J. Bot. 88:1143–1150.

Taylor, J. W., C. Hann-Soden, S. Branco, I. Sylvain, and C. E. Ellison. 2015. Clonal reproduction in fungi. Proc. Natl. Acad. Sci. 112:8901–8908.

Thon, G., T. Maki, J. E. Haber, and H. Iwasaki. 2019. Mating-type switching by homology-directed recombinational repair: a matter of choice. Curr. Genet. 65:351–362.

Tusso, S., B. P. S. Nieuwenhuis, F. J. Sedlazeck, J. W. Davey, D. C. Jeffares, and J. B. W. Wolf. 2019. Ancestral Admixture Is the Main Determinant of Global Biodiversity in Fission Yeast. Mol. Biol. Evol. msz126.

Tusso, S., F. Suo, Y. Liang, L.-L. Du, and J. B. W. Wolf. 2022. Reactivation of transposable elements following hybridization in fission yeast. Genome Res. 32:324–336.

Uyenoyama, M. K., and D. M. Waller. 1991. Coevolution of self-fertilization and inbreeding depression I. Mutation-selection balance at one and two loci. Theor. Popul. Biol. 40:14–46.

Whitehouse, H. L. K. 1949. Multiple-allelomorph heterothallism in the fungi. New Phytol. 48:212–244.

Wilson, A. M., R. Gabriel, S. W. Singer, T. Schuerg, P. M. Wilken, M. A. van der Nest, M. J. Wingfield, and B. D. Wingfield. 2021. Doing it alone: Unisexual reproduction in filamentous ascomycete fungi. Fungal Biol. Rev. 35:1–13.

Wilson, A. M., P. M. Wilken, M. A. van der Nest, E. T. Steenkamp, M. J. Wingfield, and B. D. Wingfield. 2015. Homothallism: an umbrella term for describing diverse sexual behaviours. IMA Fungus 6:207–214.

Wolfe, K. H., and G. Butler. 2022. Mating-Type Switching in Budding Yeasts, from Flip/Flop Inversion to Cassette Mechanisms. Microbiol. Mol. Biol. Rev. 0:e00007-21. American Society for Microbiology.

Wood, V., R. Gwilliam, M.-A. Rajandream, M. Lyne, R. Lyne, A. Stewart, J. Sgouros, N. Peat, J. Hayles, S. Baker, D. Basham, S. Bowman, K. Brooks, D. Brown, S. Brown, T. Chillingworth, C. Churcher, M. Collins, R. Connor, A. Cronin, P. Davis, T. Feltwell, A. Fraser, S. Gentles, A. Goble, N. Hamlin, D. Harris, J. Hidalgo, G. Hodgson, S. Holroyd, T. Hornsby, S. Howarth, E. J. Huckle, S. Hunt, K. Jagels, K. James, L. Jones, M. Jones, S. Leather, S. McDonald, J. McLean, P. Mooney, S. Moule, K. Mungall, L. Murphy, D. Niblett, C. Odell, K. Oliver, S. O'Neil, D. Pearson, M. A. Quail, E. Rabbinowitsch, K. Rutherford, S. Rutter, D. Saunders, K. Seeger, S. Sharp, J. Skelton, M. Simmonds, R. Squares, S. Squares, K. Stevens, K. Taylor, R. G. Taylor, A. Tivey, S. Walsh, T. Warren, S. Whitehead, J. Woodward, G. Volckaert, R. Aert, J. Robben, B. Grymonprez, I. Weltjens, E. Vanstreels, M. Rieger, M. Schäfer, S. Müller-Auer, C. Gabel, M. Fuchs, C. Fritzc, E. Holzer, D. Moestl, H. Hilbert, K. Borzym, I. Langer, A. Beck, H. Lehrach, R. Reinhardt, T. M. Pohl, P. Eger, W. Zimmermann, H. Wedler, R. Wambutt, B. Purnelle, A. Goffeau, E. Cadieu, S. Dréano, S. Gloux, V. Lelaure, S. Mottier, F. Galibert, S. J. Aves, Z. Xiang, C. Hunt, K. Moore, S. M. Hurst, M. Lucas, M. Rochet, C. Gaillardin, V. A. Tallada, A. Garzon, G. Thode, R. R. Daga, L. Cruzado, J. Jimenez, M. Sánchez, F. del Rey, J. Benito, A. Domínguez, J. L. Revuelta, S. Moreno, J. Armstrong, S. L. Forsburg, L. Cerrutti, T. Lowe, W. R. McCombie, I. Paulsen, J. Potashkin, G. V. Shpakovski, D. Ussery, B. G. Barrell, and P. Nurse. 2002. The genome sequence of *Schizosaccharomyces pombe*. Nature 415:871–880.

Wright, S. I., S. Kalisz, and T. Slotte. 2013. Evolutionary consequences of self-fertilization in plants. Proc. R. Soc. Lond. B Biol. Sci. 280:20130133.

Xu, K. 2023. Effects of selfing on the evolution of sexual and asexual reproduction. bioRxiv.

Yun, S.-H., M. L. Berbee, O. C. Yoder, and B. G. Turgeon. 1999. Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proc. Natl. Acad. Sci. U. S. A. 96:5592–5597.

Zanders, S. E., M. T. Eickbush, J. S. Yu, J.-W. Kang, K. R. Fowler, G. R. Smith, and H. S. Malik. 2014. Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast. eLife 3:e02630.

Table S1. The first two columns show the strain names as used in the [anonymize] lab or Bahler lab (UCL, London, UK), respectively. Thallism is assessed from the illumina from Jeffares et al. 2015, except for EBC662 and EBC663. Columns 'Mating efficiency' and 'Outcrossing' indicate the strains used for these measurements.

Acknowledgements

We thank Michaela Bauer and Bernadette Weissensteiner for help in the lab and Cristina Millanes Berenguer for providing fluorescent strains. We also thank past and current members of the Nieuwenhuis laboratory for discussions on the results and comments on the manuscript. Two anonymous reviewers are acknowledged for their critical and inspiring comments on a previous version of this manuscript. The Bähler lab is acknowledged for providing samples of the natural isolates.

Author contributions

HAG constructed strains and performed mating experiments. RS generated the bioinformatics pipeline and performed bioinformatics analyses and edited and contributed to finalizing the manuscript.. BN conceived the project, performed mating and outcrossing experiments, performed all data analyses and wrote the manuscript.

Data accessibility

All scripts and code used for analysis of the raw data, visualization and the simulations are available as electronic supplementary material through figshare under doi: 10.6084/m9.figshare.c.6514732.v1 https://doi.org/10.6084/m9.figshare.c.6514732.v1, along with the files containing the results from the analyses of the genomic data and our empirical measurements.

Fig. S1

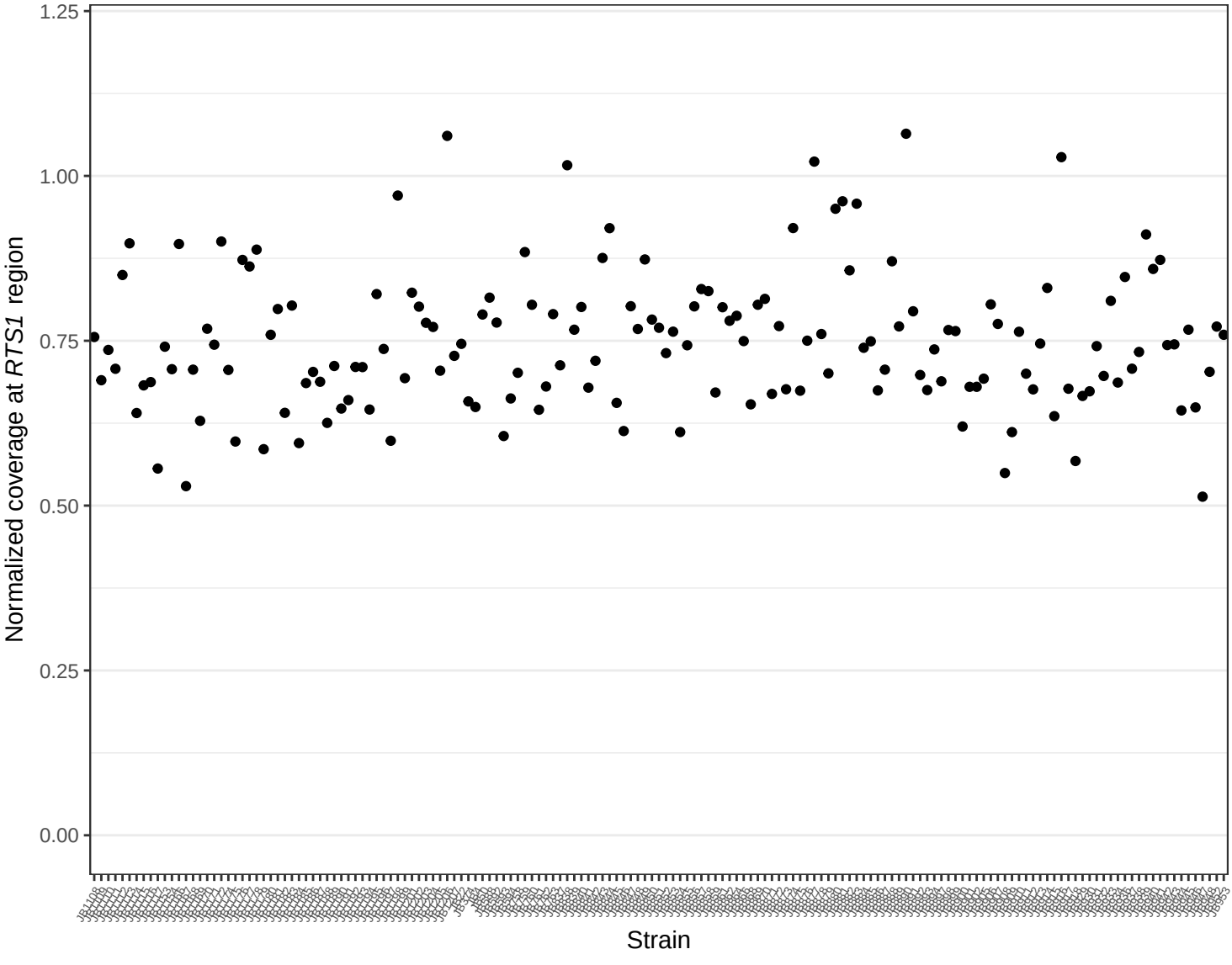

Fig S1. Coverage at the *Replication Termination Sequence 1* region centromere proximal of *mat1* relative to the 100kb region (ChrI:2.0−2.1Mb) shows that contrary to regions distal to *mat1* (Nieuwenhuis et al. 2018), the *RTS1* region is single copy.

Fig. S2

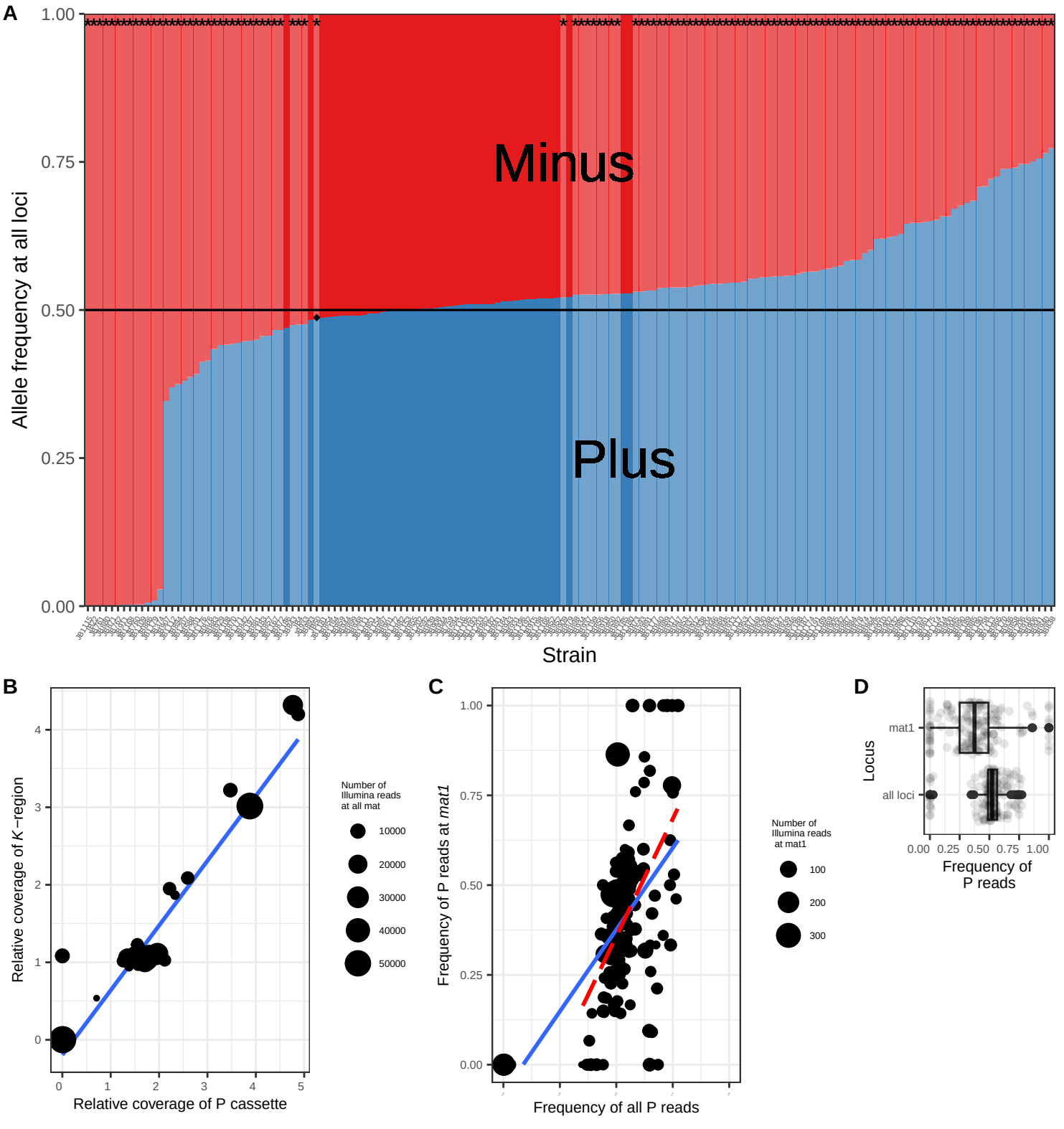

Fig S2. A) Frequency of the P and M mating−type cassettes per strain at all loci. The black line indicates the expected ratio of 1:1. The diamond gives the frequency of the *h90* lab strain. Light columns indicated with an asterisk are significantly deviation from 1:1 at the significance level of $p < 0.05$ using a Chi−square comparison on the numbers of raw reads, followed by a False Discovery Rate correction for multiple comparisons. ** B)** Correlation of relative coverage of the P cassette and the *K* region shows a strong correlation, suggesting that duplications of the P cassette are associated with duplication of the entire *mat2−K−mat3* region of the silent mating type region. **C)** The frequency of P reads at *mat1* covers the full range, though that for all P cassettes is between 0.35 and 0.77 – excluding the apparent *h−S* genotypes that lack P cassettes. The correlation between the frequency of reads at the P cassette and those counted at *mat1* (GLM binomial; including *h−S*, solid blue $z = 16.23$, $p < 10−16$; without *h−S*, dashed red * $z^* = 25.12$, $p < 10−16$) is more likely driven by the frequency of P reads at *mat1*, than by the copy number of the P cassette.

Fig. S3

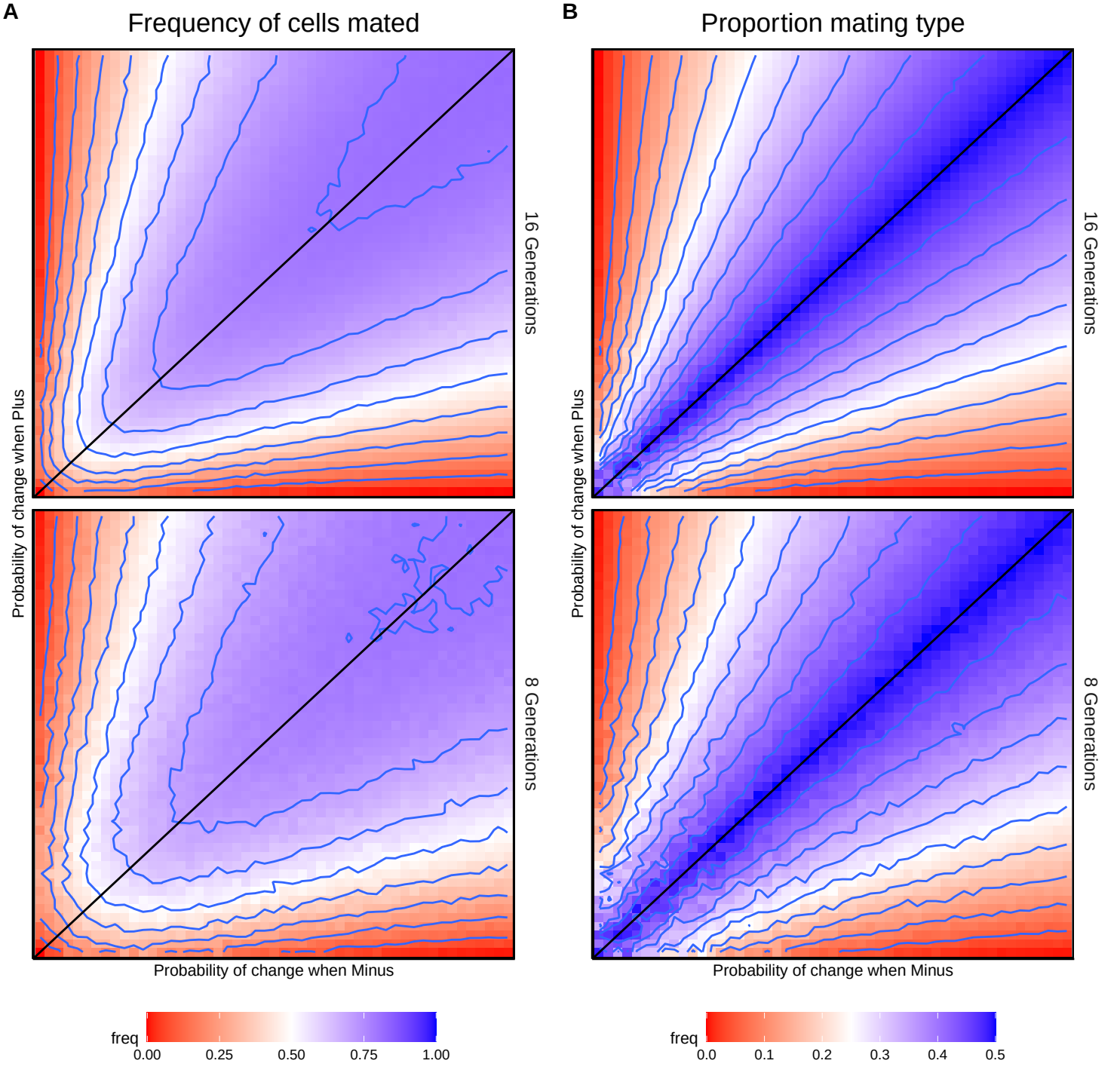

Fig S3. A) Mating efficiency (from 0 to 1 as indicated in colour scale) calculated as the proportion of cells mated for different frequencies of mating type switching from M to P on the x−axis and P to M on the y−axis for 16 generations (top) or 8 generations (bottom). The diagonal black line indicates symmetrical switching in both directions and blue lines indicate contours at tenths. **B)** Shows the mating−type frequencies for the same experiments in A, which indicate a deviation from 1:1 when asymmetry in mating−type switching increases. Each value is the mean value of 100 runs.

Fig. S4

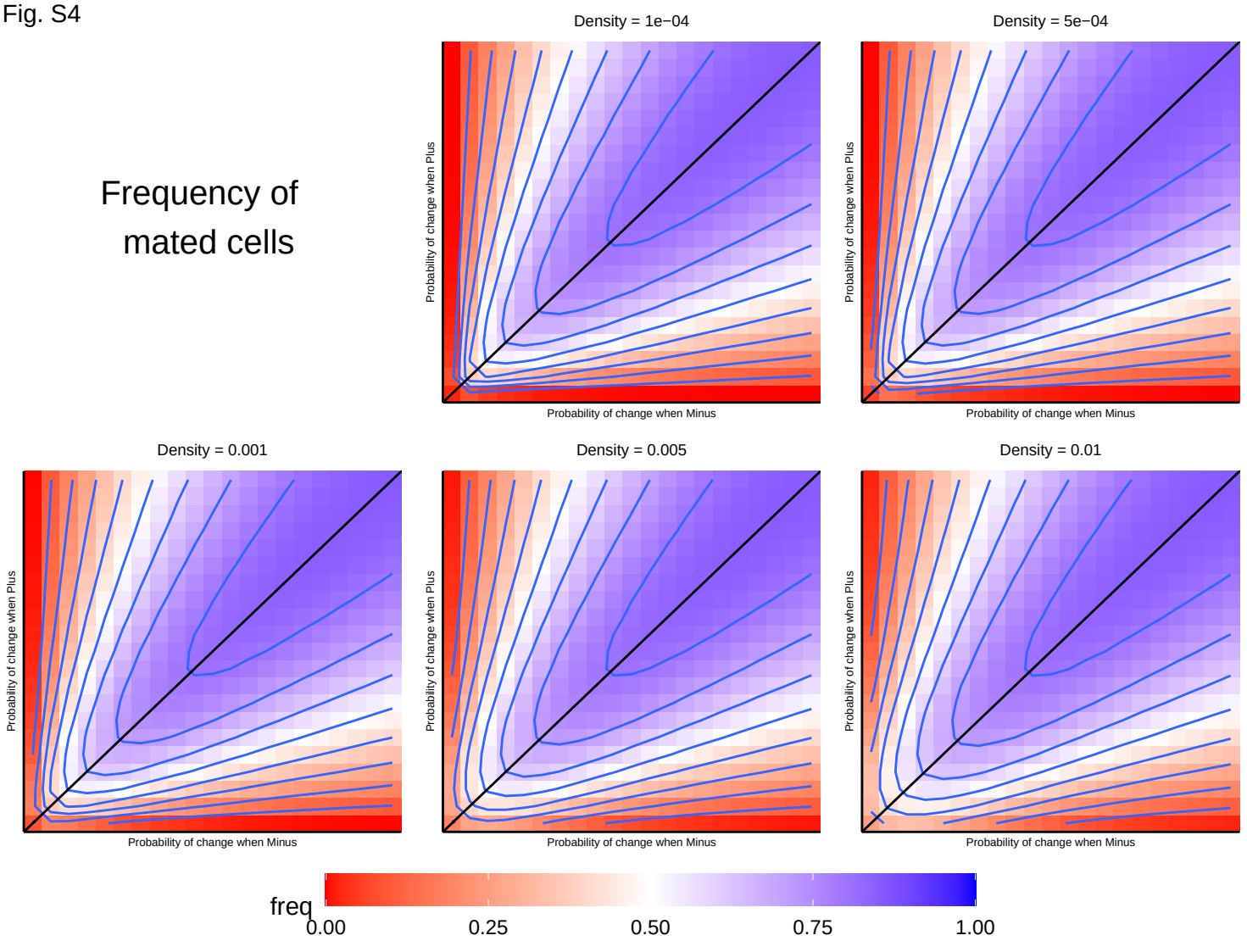

Fig S4. Mating efficiency (from 0 to 1 as indicated in colour scale) calculated as the proportion of cells mated for different frequencies of mating type switching from M to P on the x−axis and P to M on the y−axis. The different panels show a difference in initial density, affecting the size of the clonal patch each genotype occupies when the full grid is occupied (see methods for model details). The diagonal black line indicates symmetrical switching in both directions and blue lines indicate contours at tenths.

Fig. S5

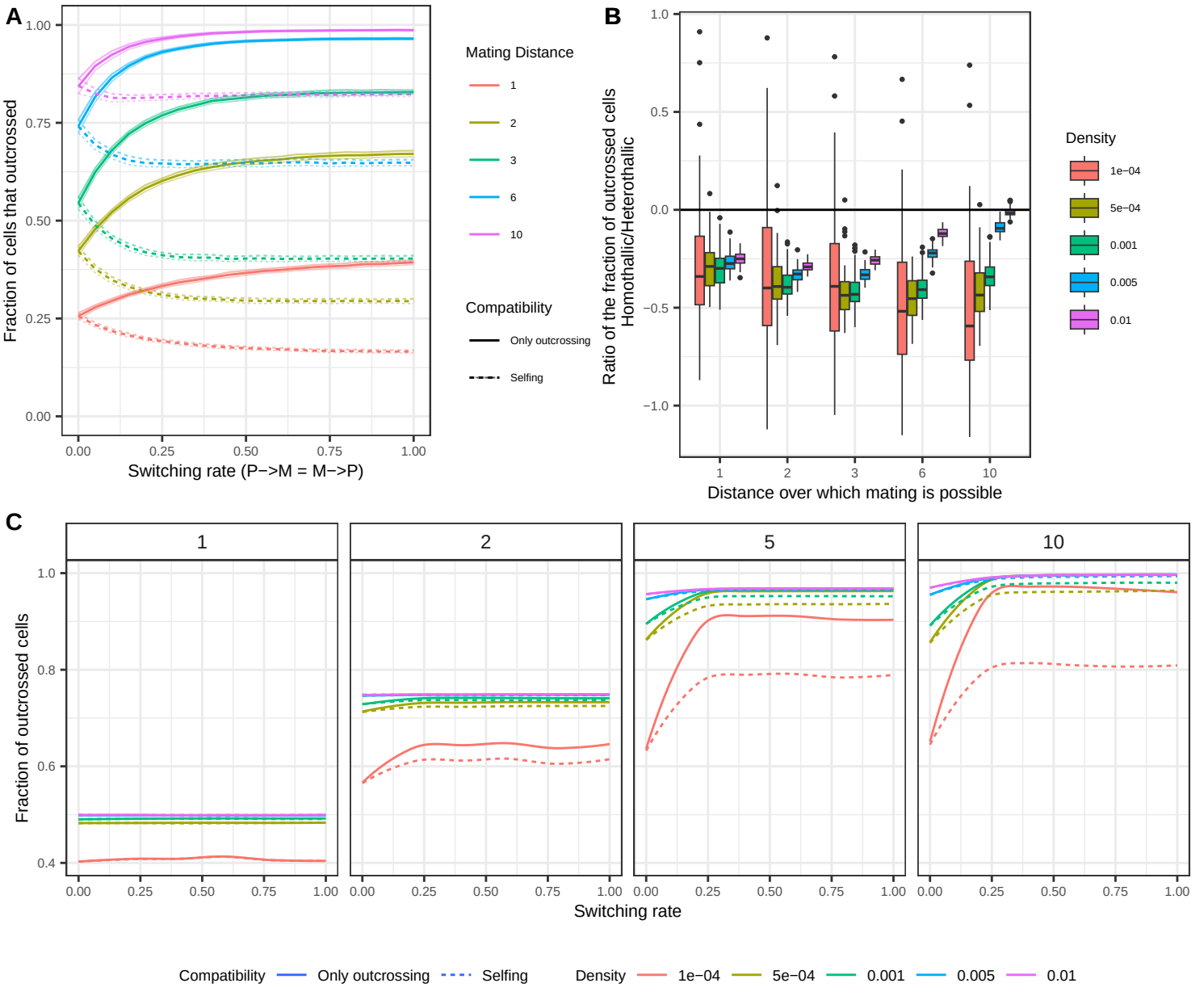

Fig S5. Effect of environmental structure on mating efficiency under switching. **A)** |Increasing the distance of potential partners in our cellular automaton simulations increases outcrossing of cells. With higher levels of switching, the fraction of cells that are able to mate increases when selfing is not possible (solid lines), but decreases when selfing is allowed (dashed lines). This shows that homothallism reduces outcrossing due to intra clonal selfing. Lines are average of 100 replicate simulations with thin lines indicating standard errors. Density is 0.001 in all simulations. **B)** Ratios of the fraction of cells that outcrossed under homothallism or heterothallism. Positive values indicate higher mating efficiency under homothallism. The ratio is calculated for one simulation of growth without switching, after which all cells were allowed to mate locally over different distances (as in A)) according to mating−type compatibility (heterothallic) or assuming all cells are compatible (homothallic). Boxplots represent 100 replicate simulations per parameter combination**. C)** Outcrossing under random mating for different number of mating opportunities (number above panels). When a single opportunity occurs, the fraction of mated cells nears 50%, which is the expected maximum outcrossing potential, because in the population half of the cells are of either mating type. The amount of switching does not affect this. When multiple opportunities occur, switching always increases the amount of outcrossing relative to no switching. Gamete discounting does play a role, as indicated by the line without selfing (solid), which is higher than the line with selfing (dashed), but both lines are higher than no−switching. Lines are average of 100 replicate simulations per parameter combination.