wtf genes are ancient meiotic drivers

Thesis

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Abstract

Meiotic drivers are selfish elements that bias their own transmission into more than half the progeny produced by a driver+/driver- heterozygote. Meiotic drivers are thought to exist for relatively short evolutionary timespans because drivers are generally found in single species, rather than being shared within a clade of close-related organisms. Additionally, drivers are predicted to decay both if they are suppressed and if they spread to fixation. In this study, we examine the evolutionary history of wtf meiotic drivers first discovered in the fission yeast Schizosaccharomyces pombe. We identify homologous genes in three other fission yeast species S. octosporus, S. osmophilus, and S. cryophilus, which are estimated to have diverged over 100 million years ago from S. pombe. Synteny evidence supports that wtf genes were present in the common ancestor of these four species. Moreover, the ancestral genes were likely drivers as wtf genes in S. octosporus cause meiotic drive. Our findings indicate that active meiotic drive systems can be maintained for long evolutionary timespans.

Director of Studies: SaraH Zanders, Ph. D
To my parents, Idalina Maria Pino Pantalião De Carvalho and Paulo Armando Almeida Azevedo De Carvalho that gift me the possibility to pursue whatever I wanted with their hard work. And I dedicate this thesis to Celine Alexandrine Lacroix De Carvalho that crossed the ocean during a worldwide pandemic just to be with me.
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# Table of Content

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>2</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>4</td>
</tr>
<tr>
<td>TABLE OF CONTENT</td>
<td>5</td>
</tr>
<tr>
<td>TABLE OF FIGURES</td>
<td>10</td>
</tr>
<tr>
<td>TABLE OF TABLES</td>
<td>11</td>
</tr>
<tr>
<td>1 CHAPTER 1: INTRODUCTION</td>
<td>12</td>
</tr>
<tr>
<td>1.1 LIST OF FIGURES</td>
<td>13</td>
</tr>
<tr>
<td>1.1 INTRODUCTION</td>
<td>14</td>
</tr>
<tr>
<td>1.2 MEIOTIC DRIVERS</td>
<td>15</td>
</tr>
<tr>
<td>1.2.1 What is meiotic drive?</td>
<td>15</td>
</tr>
<tr>
<td>1.2.2 Meiotic drive systems</td>
<td>15</td>
</tr>
<tr>
<td>1.2.3 Meiotic drive evolution</td>
<td>17</td>
</tr>
<tr>
<td>1.3 MEIOTIC DRIVER EXAMPLES.</td>
<td>20</td>
</tr>
<tr>
<td>1.3.1 Meiotic drivers in plants</td>
<td>20</td>
</tr>
<tr>
<td>1.3.1.1 Knobs bias chromosome segregation in maize</td>
<td>20</td>
</tr>
<tr>
<td>1.3.1.2 Meiotic drivers in rice use a variety of mechanisms</td>
<td>21</td>
</tr>
<tr>
<td>1.3.2 Meiotic drivers in Drosophila</td>
<td>24</td>
</tr>
<tr>
<td>1.3.2.1 Segregation Distorter, a killer-target meiotic drive in D. melanogaster</td>
<td>24</td>
</tr>
<tr>
<td>1.3.2.2 X chromosome drive in Drosophila simulans</td>
<td>26</td>
</tr>
<tr>
<td>1.3.3 t-haploidy a poison and antidote meiotic driver in Mus musculus</td>
<td>27</td>
</tr>
<tr>
<td>1.3.4 Spok genes in Podospora anserina</td>
<td>28</td>
</tr>
<tr>
<td>1.4 HOW MEIOTIC DRIVERS CAN PERSIST IN GENOMES</td>
<td>31</td>
</tr>
<tr>
<td>1.5 WTF GENES ARE A FAMILY OF MEIOTIC DRIVERS</td>
<td>33</td>
</tr>
<tr>
<td>1.6 DISCLOSURE</td>
<td>36</td>
</tr>
<tr>
<td>2 CHAPTER 2: WTF GENES HOMOLOGS OUTSIDE OF S. POMBE</td>
<td>37</td>
</tr>
</tbody>
</table>
Genes with homology to wtf drivers are found in S. octosporus, S. osmophilus and S. cryophilus. S. octosporus, S. osmophilus and S. cryophilus wtf genes share additional features with S. pombe genes. wtf genes in S. octosporus, S. osmophilus and S. cryophilus are associated with distributed 5S rDNA sequences. wtf genes were likely present in the common ancestor of S. octosporus, S. osmophilus, S. cryophilus and S. pombe. wtf genes show evolutionary signatures consistent with a history of genetic conflict. wtf genes duplicated to pre-existing 5S rDNA genes.

wtf genes are ancient meiotic drivers. S. octosporus wtf genes are transcriptionally active. Dispersed repetitive elements are associated with wtf genes. Common ancestor. Rapid evolution.

3 CHAPTER 3: WTF GENES CAUSE MEIOTIC DRIVE OUTSIDE OF S. POMBE

TABLE OF CONTENT CHAPTER 3

3.1 LIST OF FIGURES ................................................................. 80
3.2 LIST OF TABLES .................................................................... 80
3.3 INTRODUCTION .................................................................... 81
3.4 RESULTS ............................................................................. 83
3.4.1 *wtf* genes in *S. octosporus*, *S. osmophilus* and *S. cryophilus* encode poison and antidote proteins.

83

3.4.2 *wtf* genes seem to have high conservation of splicing site. .............................................. 88

3.4.3 *wtf* genes cause meiotic drive when heterozygous in *S. octosporus* .................................. 89

3.4.4 *S. octosporus* *wtf25* is a poison and antidote meiotic driver .................................................. 100

3.5 **DISCUSSION** ........................................................................................................................ 102

3.5.1 *wtf* genes are ancient meiotic drivers ..................................................................................... 102

3.5.2 Other old drive systems (also multicopy) .................................................................................... 104

3.6 **SUPPORTING INFORMATION** ................................................................................................ 106

4 **CHAPTER 4: DISCUSSION AND FUTURE DIRECTIONS** ......................................................... 121

4.1 **LIST OF FIGURES** .................................................................................................................. 122

4.2 **KEY CONCLUSIONS** ............................................................................................................... 123

4.2.1 *wtf* genes found outside of *S. pombe* ...................................................................................... 123

4.2.2 *wtf* active meiotic drivers over 119 Mya ................................................................................. 126

4.3 **FUTURE DIRECTIONS** ............................................................................................................ 127

4.3.1 New meiotic drivers in fission yeast ......................................................................................... 127

4.3.2 Investigating meiotic drive mechanism ..................................................................................... 128

4.3.3 Measuring fitness cost of *wtf* genes and inbreeding in other fission yeast species ................. 129

4.3.4 Repeat facilitated expansion of the *wtf* gene family ................................................................. 130

4.4 **OUTLOOK: MODEL FOR THE LONG-TERM PERSISTENCE OF WTF DRIVERS** ................. 135

4.5 **SUMMARY** .............................................................................................................................. 138

5 **CHAPTER 5: MATERIAL AND METHODS** ............................................................................. 140

**TABLE OF CONTENT CHAPTER 5** ............................................................................................... 141

5.1 **INTRODUCTION** ..................................................................................................................... 142

5.2 **NANOPORE SEQUENCING AND ASSEMBLY OF S. OSMOPHILUS** .................................. 143

5.3 RNA sequencing and Nanopore cDNA sequencing (Guo-Song Jia) .......................................... 143

5.3.1 Sample preparation .................................................................................................................... 143
5.3.2 RNA extraction ........................................................................................................ 144
5.3.3 RNA sequencing ..................................................................................................... 144
5.3.4 ONT cDNA sequencing ........................................................................................ 145
5.3.5 RNA-seq data processing ...................................................................................... 145
5.3.6 ONT cDNA data processing .................................................................................. 145
5.4 ANNOTATION ............................................................................................................ 146
5.4.1 S. osmophilus genome annotation ....................................................................... 146
5.4.2 Calculating amino acid identity between Schizosaccharomyces species .............. 146
5.4.3 Sequence homology search ................................................................................... 147
5.4.4 S. octosporus wtf and wag genes ......................................................................... 147
5.4.5 S. osmophilus wtf and wag genes annotations .................................................... 148
5.4.6 5S rDNA annotation ............................................................................................. 148
5.4.7 LTR annotation ..................................................................................................... 149
5.4.8 Identifying Mei4 binding sites (FLEX motif) outside of S. pombe ......................... 149
5.5 DNA SEQUENCE ALIGNMENTS AND PHYLOGENIC TREE CONSTRUCTION ........ 149
5.6 ANALYSES OF REPETITIVE REGIONS WITHIN WTF GENES ............................... 150
5.7 GARD ANALYSES OF RECOMBINATION WITHIN WTF GENE FAMILY. .............. 151
5.8 SYNTENIC ANALYSIS ............................................................................................... 151
5.9 MOLECULAR BIOLOGY .............................................................................................. 151
5.9.1 S. cerevisiae LExA-ER-AD β-estradiol inducible system ....................................... 151
5.9.2 Cloning S. octosporus, S. osmophilus and S. cryophilus wtf\textsuperscript{poison} and wtf\textsuperscript{antidote} alleles for expression in S. cerevisiae .................................................................................. 152
  5.9.2.1 Cloning S. octosporus wtf61poison (SOCG_04114) under the control of a β-estradiol inducible promoter. 152
  5.9.2.2 Cloning S. octosporus wtf61antidote (SOCG_04114) under the control of a β-estradiol inducible promoter. 153
  5.9.2.3 Cloning S. cryophilus wtf1poison (SPOG_03611) under the control of a β-estradiol inducible promoter. 153
5.9.2.4 Cloning S. cryophilus wtf1antidote (SPOG_03611) under the control of a β-estradiol inducible promoter. 154

5.9.2.5 Cloning S. osmophilus wtf41poison under the control of a β-estradiol inducible promoter. 154

5.9.2.6 Cloning S. osmophilus wtf41antidote under the control of a β-estradiol inducible promoter. 155

5.9.2.7 Cloning S. octosporus wtf25poison (SOCG_04480)-GFP under the control of a β-estradiol inducible promoter. 155

5.9.2.8 Cloning S. octosporus wtf25antidote (SOCG_04480) mCherry under the control of a β-estradiol inducible promoter. 155

5.9.3 Plasmid transformation in S. cerevisiae. 156

5.9.4 Spot assays in S. cerevisiae. 156

5.9.5 Imaging Wtf proteins expressed in S. cerevisiae. 157

5.9.6 S. octosporus strains (Guo-Song Jia). 157

5.9.7 Plasmids integrated into S. octosporus (Guo-Song Jia). 158

5.9.8 Spore viability analysis (Guo-Song Jia). 159

5.9.9 Crosses and random spore assay. 159

6. REFERENCES 161
Table of Figures

Figure 1.1: Models of meiotic driver mechanisms. .......................................................... 16
Figure 1.2 Meiotic drive in *Podospora anserina* ............................................................. 30
Figure 1.3: *wtf* produces a poison and antidote proteins ........................................... 34
Figure 1.4 Phylogeny of *Schizosaccharomyces* ............................................................. 35
Figure 2.1 *wtf* homologs are found outside of *S. pombe* ........................................ 42
Figure 2.2 Maps of the *wtf* gene family members in *S. octosporus, S. osmophilus, S. cryophilus*, and *S. pombe*. ............................................................... 45
Figure 2.3: Limited conservation of Wtf proteins ............................................................ 48
Figure 2.4 *S. pombe wtf* genes share features with other *wtf* genes outside of *S. pombe*. ..... 49
Figure 2.5 Transcription of predicted poison and antidote isoforms of *wtf* genes in *S. octosporus*. .............................................................................................................. 52
Figure 2.6 Genomic context of *wtf* genes ................................................................. 54
Figure 2.7: Distance between 5S rDNA and *wtf* genes .................................................. 56
Figure 2.8 Homology between distinct 5S rDNA-*wtf* units and *wag-wtf* units ............ 57
Figure 2.9 *S. octosporus wtf* gene units supported by maximum likelihood phylogeny. .... 58
Figure 2.10 *wtf* genes are found at the same syntenic locus in three fission yeast species..... 60
Figure 2.11 Synteny between *S. cryophilus wtf4* and *S. pombe wtf6* ......................... 62
Figure 2.12: Gene duplication and non-allelic gene conversion within *wtf* gene family. ..... 64
Figure 2.13: GARD analysis of *wtf* genes ..................................................................... 67
Figure 2.14: Contraction and expansion of repeat sequences in *wtf* genes ................. 69
Figure 2.15: Model of spread of *wtf* genes by duplication to preexisting 5S rDNA genes. .... 71
Figure 3.1: *wtf* genes can encode for poison and antidote proteins ............................ 84
Figure 3.2: Some *wtf* genes outside of *S. pombe* encode for poison and antidote proteins ... 85
Figure 3.3: Non-cognate Wtf*antidotes* fail to rescue cells from Wtf*poisons* ............... 86
Figure 3.4 *S. cryophilus wtf5* elongation of exon can decrease poison toxicity ............ 89
Figure 3.5: *wtf* genes are not required for meiosis or sporulation in *S. octosporus* ..........90

Figure 3.6: *S. octosporus wtf25* cause meiotic drive. .................................................................91

Figure 3.7: *S. octosporus wtf25* cause meiotic drive in a random spore assay like octad dissection......................................................................................................................92

Figure 3.8: Heterozygous diploid spore viability of *wtf68* .................................................................94

Figure 3.9: Heterozygous diploid spore viability of *wtf33* .................................................................95

Figure 3.10: Heterozygous diploid spore viability of *wtf46* ...............................................................96

Figure 3.11: Heterozygous diploid spore viability of *wtf60*. ..............................................................97

Figure 3.12 Heterozygous diploid spore viability of *wtf62* ...............................................................98

Figure 3.13: Heterozygous diploid spore viability of *wtf21* ..............................................................99

Figure 3.14: *S. octosporus wtf25* is a poison-and-antidote spore killer. ..............................................101

Figure 4.1: Model for long-term persistence of *wtf* meiotic drivers.....................................................125

Figure 4.2: *wtf* genes are unlikely to form extrachromosomal circles.................................................132

Table of Tables

Table 2.1 Percent identity of 1:1 orthologs in *Schizosaccharomyces* ................................................46

Table 2.2 *wtf* genes table prediction driver, antidote only, pseudogene and unknowns........47

Table 2.3 5S rDNA in *Schizosaccharomyces* genome .......................................................................57

Table 3.1: Yeast strains used in this Chapter. .........................................................................................106

Table 3.2: Oligos used in this Chapter..................................................................................................110

Table 3.3: Plasmid used in this Chapter................................................................................................119
1 Chapter 1: Introduction

Part of this Chapter are extracts from De Carvalho M, Zanders SE. 2019. A family of killers. *Elife* 8:8–10. doi:10.7554/eLife.49211
Table of content Chapter 1

1.1 List of Figures ................................................................. 13

1.1 Introduction ........................................................................ 14

1.2 Meiotic Drivers .................................................................. 15

1.2.1 What is meiotic drive? ...................................................... 15

1.2.2 Meiotic drive systems ..................................................... 15

1.2.3 Meiotic drive evolution ................................................... 17

1.3 Meiotic Driver Examples .................................................... 20

1.3.1 Meiotic drivers in plants .................................................. 20

1.3.1.1 Knobs bias chromosome segregation in maize .................. 20

1.3.1.2 Meiotic drivers in rice use a variety of mechanisms .......... 21

1.3.2 Meiotic drivers in Drosophila .......................................... 24

1.3.2.1 Segregation Distorter, a killer-target meiotic drive in D. melanogaster .................................................. 24

1.3.2.2 X chromosome drive in Drosophila simulans .................. 26

1.3.3 t-haplotype a poison and antidote meiotic driver in Mus musculus .................................................. 27

1.3.4 Spok genes in Podospora anserina ................................... 28

1.4 How Meiotic Drivers Can Persist in Genomes ......................... 31

1.5 WTF genes are a family of meiotic drivers ............................... 33

1.6 Disclosure ........................................................................... 36

1.1 List of Figures

Figure 1.1: Models of meiotic driver mechanisms ......................... 16

Figure 1.2 Meiotic drive in Podospora anserina .............................. 30

Figure 1.3: wtf produces a poison and antidote proteins ................ 34

Figure 1.4 Phylogeny of Schizosaccharomyces ............................... 35
1.1 Introduction

Some genes are born criminals. Mendel’s law of segregation states that the two alleles of a heterozygote are each transmitted into half of the gametes (Abbott and Fairbanks, 2016), but genes called meiotic drivers break this law by striving to be the only gene variant inherited (Sandler and Novitski, 1957; Zimmering et al., 1970). This selfish behavior can help meiotic drivers to spread in a population, but it can also decrease the fitness of organisms carrying the loci (Crow, 1991).

Meiotic drivers employ a variety of self-promoting mechanisms to bias their own transmission (Lindholm et al., 2016). Some are particularly ruthless because they kill the gametes or spores that do not inherit the driver gene. While other meiotic drivers bias chromosome segregation to their advantage by favoring the chromosome transmission that carry them into the egg, the viable gamete. Meiotic drivers occur throughout eukaryotes, including mammals, but relatively little is known about the genes responsible (Burt and Trivers, 2006; Lyon, 2005). For this thesis I studied meiotic drivers in fission yeasts of the Schizosaccharomyces genus. This thesis investigates the longevity of meiotic drivers over a long evolutionary timescale (i.e., across species) using the wtf gene family as meiotic driver model. This work combines both bioinformatics analyses (Chapter 2) and molecular biology approaches (Chapter 3) to determine how long wtf genes have been causing meiotic drive.

In this introduction, I define meiotic drivers and provide background on their evolution. I then discuss examples of meiotic drivers in different kingdoms and describe how old those meiotic drivers are predicted to be. Next, I explore the mechanisms that allow meiotic drivers to thrive.
in different species. Finally, I define the S. pombe wtf gene family used as a model for meiotic drive in this thesis.

1.2 Meiotic drivers

1.2.1 What is meiotic drive?

Meiosis is the process by which a diploid cell undergoes two divisions to generate haploid gametes. The first division allows segregation of the homologous chromosomes, the second division allows separation of the sister chromatids (Marston and Amon, 2004). In the case of a diploid (a/A), the progeny will carry a “a” or a “A” allele. In male meiosis, the four gametes produced from a diploid cell are viable (symmetric meiosis; “a” and “A” gametes). In female meiosis, only one haploid cell survives to develop into an egg (asymmetric meiosis; “a” or “A”). The rest of the cells, called polar bodies, will degenerate. Meiosis is the perfect process for a selfish element to increase in frequency in the population when heterozygous because it is spread in the population. Meiotic drivers favor their own transmission, as an example if “a” allele is the meiotic driver, “a” will favor its own transmission over “A”. “a” will be found in most of the gametes produced by the organism (Burt and Trivers, 2006; Sandler and Novitski, 1957; Zimmering et al., 1970).

1.2.2 Meiotic drive systems

Meiotic drivers will use different ways to cheat and increase their transmission (Figure 1.1) (Bravo Núñez et al., 2018b; Zanders and Unckless, 2019). In the case of helping to understand meiotic drivers cheating systems, we categorize them in different categories depending on when they act (during or after meiosis) and how they act (Segregation bias, Segregation sabotage, Killer-target, and Poison-antidote) (Figure 1.1). Meiotic drivers acting during meiosis can bias chromosome segregation in such a way that favors their transmission into the
female gamete, or they can sabotage segregation of the non-driver chromosome in male meiosis. If drivers act after meiosis, they can cheat in two different ways: killer-target or poison-antidote systems. Killer-target drive systems are composed by a killer (“a” in Figure 1.1C) that will act in trans on the target to kill the meiotic products that inherit the target (“A” in figure 1.1C). The target is found in an alternate haplotype of the killer, for example in the homologous chromosome. By killing the competition, the killer will be found in most of the gametes. Poison-antidote meiotic drivers are similar, but the poison and antidote genes are closely linked. The poison is produced and only the gametes that inherit the linked poison-antidote alleles will be rescue by the antidote. The other gametes that did not inherit the poison and antidote will be killed by the poison (Bravo Núñez et al., 2018b; Courret et al., 2019; Kruger and Mueller, 2021; Lindholm et al., 2016).

Figure 1.1: Models of meiotic driver mechanisms.

Figure modified from (Zanders and Unckless, 2019). Meiotic drivers are here separate in two broad categories depending on when they act during meiosis: during or after meiosis. During meiotic drive (A) Segregation bias in female meiosis, (B) Sabotage in male meiosis where the meiotic driver “a” disrupt segregation of allele “A”. After meiosis drive category encompass, (C) Killer-target systems where the killer kills the gametes that contain the target, and (D)
poison-antidote systems where linked alleles will encode for both poison and antidote proteins and only the gamete that inherit the meiotic drive “a” will be rescue from the poison toxicity.

1.2.3 Meiotic drive evolution

Natural selection can favor genes that promote fitness. However, meiotic drivers can favor their own transmission at the detriment of the host fitness and spread in population (Crow, 1991; Price and Wedell, 2008; Zanders and Unckless, 2019). As an example, heterozygotes males $t$-haplotype discussed below are not fully fertile (Manser et al., 2017; Sutter and Lindholm, 2016, 2015). Because of their detrimental effects, selection will favor genes that will suppress the driver (Helleu et al., 2015). This is the case of the driving chromosome $X^{SR}$ in the Paris meiotic driver system in Drosophila simulans, with Y and autosomal chromosome resistance arose in different parts of the world (Bastide et al., 2011; Cécile Courret, Pierre R. Gérard, David Ogereau, Matthieu Falque, Laurence Moreau et al., 2019; Helleu et al., 2016). Whereas selection will favor meiotic drivers that increase the drive phenotype and/or escape suppression. This tug of war between host genome and parasitic driver is known as genetic conflict. Genetic conflicts created by meiotic drivers are predicted to shape the evolution of genomes (Hamilton, 1967; Hartl, 1975; McLaughlin and Malik, 2017; Sandler and Novitski, 1957).

Meiotic drivers are widespread, they are found in plants, mammals, insects, and fungi (Beaghton et al., 2019; Bravo Núñez et al., 2018b; Burt and Trivers, 2006; Cocquet et al., 2012; Courret et al., 2019; Dawe et al., 2018; Didion et al., 2016; Finseth et al., 2021, 2015; Helleu et al., 2015; Hu et al., 2017; Jaenike, 2001; Kelemen and Vicoso, 2018; Koide et al., 2018; Kruger et al., 2019; Kruger and Mueller, 2021; Lindholm et al., 2016; Long et al., 2008; Nuckolls et al., 2020; Paczolt et al., 2017; Price et al., 2019; Shen et al., 2017; Svedberg et al., 2021; Tao et al., 2007a; Vogan et al., 2019; Yang et al., 2012). Many meiotic drivers identified
are unmapped in the genomes which bias our understanding of drive mechanism and drive evolution. Amongst the mapped meiotic drivers, many are species-specific meaning that they are found in only one species. The lack of identified meiotic drivers in multiple species could be due to the high number of unmapped meiotic drivers. Rapid evolution of the driver makes it even harder to identification of meiotic driver between different species. Meiotic drivers are under positive selection, meaning that selection will favor non-synonymous mutation that will change the amino acid sequence. Therefore, meiotic drivers are predicted to exist in species for a short time. Short period means here and throughout the thesis the time in one single species. The detrimental effects of drivers are so important that extinction of driver (or of the species carrying the driver in certain cases) is expected (Lyttle, 1977; Price et al., 2010). Meiotic drivers are associated with fitness cost. The cost observed can be associated with the destructions of gametes, off target effects and gamete viability (Dalstra et al., 2003; Grognet et al., 2014; Hu et al., 2017; Lin et al., 2018; Long et al., 2008; Nuckolls et al., 2017; Phadnis and Orr, 2009; Price and Wedell, 2008; Shen et al., 2017; Sutter and Lindholm, 2015; Tao et al., 2007a; Turner and Perkins, 1979; Xie et al., 2017; Yang et al., 2012; Zanders and Unckless, 2019).

Damocles sword in the Greek mythology tells the story of Damocles that sees above his head a sword that stands by only a single hair to keep the swords to fall on Damocles. Here, meiotic drivers are Damocles and extinction is the sword that can fall on the meiotic drivers. The Damocles sword is composed of three possible extinction pathways.

First, a driver can be fixed in the population (Carvalho and Vaz, 1999). When the meiotic driver is fixed in a population, the driver does not gain any more advantages compare to other loci
and can then accumulate inactivating mutations. In the case of a sexual chromosome drive or sterility of homozygous for the driver (driver+/driver+), fixation could result in species extinction (Carvalho and Vaz, 1999; Hamilton, 1967; Holman et al., 2015). For poison-antidote systems fixation of drivers is very difficult to identify. If the driver reaches fixation, selection would be predicted to maintain the antidote until the poison is extinct in the population (Hurst et al., 1996). Antidote before poison extinction causes death of the organism that will not have the antidote to rescue poison toxicity. In the case of the poison and antidote systems, the poison is highly costly for the organism if there is no antidote been expressed.

Another route for extinction is suppression. Genetic conflict between the meiotic driver and the genome creates an environment that favors alleles that suppress meiotic drivers (Van Valen, 1973). In the case of a total suppression of the driver, the driver is inactive, and the suppressor is fixed in the population. Complete suppression is predicted to make the driver go extinct if the driver is costly. High cost of the driver will favored the rapid evolution of suppression (Bull and Charnov, 1988; Fisher, 1930). The Paris Sex Ratio (SR) system in *D. simulans*, the driver is totally suppressed in African populations and the frequency of the chromosome X^{SR} (chromosome X that carry the meiotic driver SR) decrease very rapidly in frequency and replaced by a chromosome X without driver X^{ST} (Bastide et al., 2011). When the driver is extinct the suppressor are predicted to go extinct too by drift (Burt and Trivers, 2006).

Finally, drivers that have a difficulty to drive and accumulate recessive mutations on linked genes can cause detrimental fitness effects. This is decay that can also result in extinction. For example, the X^{D} X-chromosome allele in *D. recens* causes death of Y-bearing sperm. Suppression of X^{D} recombination has led to linkage of the whole chromosome in one haplotype
with very low polymorphism which increased the probability of deleterious mutations. \(X^D\) in *Drosophila recens* causes sterility in homozygous females because of the linked mutations and heterozygous females have low fecundity (Dyer et al., 2007; Dyer and Hall, 2019). It is predicted that such chromosomes will decay rapidly.

The dogma of meiotic driver evolution states that driver are short-lived because the only road for the driver is a road leading to extinction but this is not an universal rule (Hamilton, 1967; Hatcher, 2000; Price et al., 2019). There is a Damocles sword above the meiotic driver head that need to escape fixation, suppression, and decay. If meiotic driver does not escape, the Damocles sword falls, and the meiotic driver goes extinct.

### 1.3 Meiotic driver examples.

#### 1.3.1 Meiotic drivers in plants

Meiotic drivers in plants are variable, I choose here to present only two organisms of plants that have a mapped meiotic driver loci, rice, and maize. Other meiotic driver loci have been identified in other plants species (Burt and Trivers, 2006; Finseth et al., 2021, 2015; Rick, 1966; Taylor and Ingvarsson, 2003).

#### 1.3.1.1 Knobs bias chromosome segregation in maize

Female meiosis gives meiotic drivers the opportunity to favor their chromosome segregation into the egg. Some meiotic drivers like the knobs in *Zea mays mays* (maize) will favor the segregation of an abnormal chromosome 10 (Ab10) into the viable gamete (Figure 1.1A). Knobs are well studied cases of meiotic drive in plants that show increased frequency of Ab10
in the egg production (Rhoades, 1942). The meiotic driver region, called knob, is situated in the telomere of Ab10 and creates a strong force to segregate in the viable cell in meiosis I and II. The knob structure recruits proteins that make it motile and promote knob movement toward the spindle poles at meiosis I and meiosis II. Because the knob is present in the telomeric region, the probability of crossing over between centromere and knob with N10 (normal chromosome 10) is high. Within the knob region, recombination is suppressed by inversion and repeats in Ab10. Recombination suppression within the knob region avoids the breakage of the meiotic driver systems. Knob structure is very repetitive with two types of repeats identified that are converted in neocentromeres, 350-bp TR1 and 180 bp repeats. To drive, knobs recruit Kindr, part of specialized kinesin gene family, that bind the knobs and convert those regions in neocentromeres (Dawe et al., 2018; Swentowsky et al., 2020). Kindr, a member of the kinesin family, is implicated the spindle pole assembly. The binding of Kindr in the 180 bp knob causes drive. TR1 is not necessary for the drive (Mroczek et al., 2006). Other Ab10 chromosomes have been identified in other subspecies of maize, like Zea mays mexicana and Zea mays parviglumis which will make Ab10 one of the oldest meiotic drive identified of 12 Mya corresponding to the last common ancestor of the species cited above (Dawe et al., 2018; Kanizay et al., 2013). Still the meiotic driver as not been shown active in the other maize species and the evolution of Ab10 not fully understood yet.

1.3.1.2 Meiotic drivers in rice use a variety of mechanisms

Our knowledge about meiotic drivers in rice stems from scientists trying to understand sterility that can arise in hybrids. This is important because rice is one of the most predominant cereals consumed in the world and so making hybrids may allow stronger, healthier and/or quicker growing plants. Rice is an interesting model that shows different meiotic drive systems with different mechanisms of drive. In Oryza sativa, 5 meiotic driver systems have been identified.
Many hybrids like *Oryza sativa japonica-indica*; *O. sativa* and *O. glaberrima*; and *O. sativa japonica* and *O. meridionalis* are sterile (Koide et al., 2018; Long et al., 2008; Ouyang et al., 2010; Shen et al., 2017; Yang et al., 2012; Yu et al., 2018).

For example, *japonica-indica* hybrids have three loci implicated in meiotic drive (Chen et al., 2008; Long et al., 2008; Yang et al., 2012). The *S5* and *Sa* drivers cause female and male sterility, respectively (Chen et al., 2008; Long et al., 2008). *S5* is a poison and antidote system in female rice gametes, called megaspore, located in chromosome 6. *S5-i* (*S5 indica*) will be found in most of the cells, whereas *S5-j* (*S5 japonica*) megaspore. The poison and antidote mechanism implicates 3 alleles *ORF3*, *ORF4* and *ORF5*. *S5-i* is composed of *ORF3*+, *ORF4*- and *ORF5*+. *S5-j* is composed of *ORF3*-, *ORF4*+ and *ORF5*-. *ORF5*+ combines with *ORF4*+ to form a poison that kills the cells that did not inherit *ORF3*, the antidote (Yang et al., 2012). *S5* is located on chromosome 6 of *O. sativa* and *S5-I* will be transmitted to more than 90% of the progeny. Three genes are necessary for the drive.

*Sa* is a killer-target meiotic drive locus located in chromosome 1 of *O. sativa*. *Sa* locus is composed of *SaF* and *SaM*, which encodes a F-box protein (mediate protein-protein interactions) and a small ubiquitin modified E3 ligase-like protein, respectively. Depending on the subspecies of *O. sativa, indica* and *japonica* do not present the same alleles. *O. japonica* contains *SaF*- and *SaM-*, whereas *indica* expresses *SaF+* and *SaM+*. In *indica-japonica* hybrid, *SaF+* and *SaM+* form a killer that targets pollen that inherits the *SaM-* locus. In this case the *Sa-i* will be found in almost all the viable gametes.
Sc is an essential pollen gene present in different copy number between indica and japonica. In indica, Sc-i is found in tandem repeats of 28 kb in the genome. In japonica, only one copy of Sc is present. Sc-i (Sc indica) is the killer that targets Sc-j (Sc japonica), which cannot develop pollen, the Sc drive system is similar to the Killer-target system (Shen et al., 2017) (Figure 1.1C). Sterility of the hybrid indica-japonica is explained by Sc-i repeats repressing expression of Sc-j in pollen causing abortion of Sc-j gametes. Another drive system S1 is also implicated in reproductive isolation of O. sativa and O. glaberrima with a gamete killer system (killer-target; Figure 1.1C) during male and female meiosis (Xie et al., 2017). S1-g (S1 allele of O. glaberrima) kills the gametes of O. sativa by activation of peptidase molecules that are implicate in the killing of the competitor gamete (Antalis et al., 2010; “Rpf2p, an Evolutionarily Conserved Protein, Interacts with Ribosomal Protein L11 and Is Essential for the Processing of 27 SB Pre-rRNA to 25 S rRNA and the 60 S Ribosomal Subunit Assembly in Saccharomyces cerevisiae * | Elsevier Enhanced Reader,” n.d.).

Finally, the qHMS27 locus, poison-antidote system, is responsible for the sterility found in the hybrid meridionalis-sativa (Yu et al., 2018) (Figure 1.1D). Two open reading frames (ORF) contribute to the sterility observed between O. meridionalis and O. sativa. The first, named ORF2, produces a poison. The second, ORF3, encodes for an antidote that rescues the poison toxicity of ORF2. ORF3 is a non-essential gene. ORF2 seems to be active in presence of ORF3 and in other lineages it is thought that ORF2 is inactive acquire poison toxicity in O. rufipogon and O. sativa. ORF2 seems to have originated in the ancestor of wild rice species because ORF2 is found in many species such as O. meridionalis, O. barthii, O. rufipogon, and O. sativa, making of ORF2 a 17 million years old gene but maybe not active in those ancestral species (Wambugu et al., 2015). ORF3 (antidote) is present in O. sativa and O. rufipogon only. ORF3 is present only in sativa and rufipogon, taking the divergence between those two species ORF3
is predicted to be 12 million years old (Wambugu et al., 2015). Of course, we do not account for the possibility of introgression (transfer of genetic material between hybrids) between different rice populations when calculating the probable age of drivers (Chen, 2004). Introgression would mean that ORF3 was born in only one species and by introgression inserted in another one which could be more recent event than the phylogenetic divergence between the two species.

1.3.2 Meiotic drivers in *Drosophila*

In insects, *Drosophila* meiotic drivers may have been the most studied organisms with 19 meiotic driver systems identified (Courret et al., 2019; Helleu et al., 2015; Jaenike, 2001). Many of the meiotic drivers in *Drosophila* are on sex chromosomes (Dyer et al., 2007; Fuller et al., 2020; Jaenike, 2001; Larner et al., 2019; Lin et al., 2018; Tao et al., 2007a). This bias of identification is explained by the apparent phenotypes seen by the scientist when sex ratio than the expected 1:1 (Hamilton, 1967). Here we report only two of the most studied meiotic driver systems: *Sd* in *D. melanogaster* and *SR* in *D. simulans*.

1.3.2.1 Segregation Distorter, a killer-target meiotic drive in *D. melanogaster*

In *Drosophila melanogaster*, The *Segregation distorter (SD)* is a killer-target meiotic driver (Figure 1.1C) that promotes its own biased transmission in male meiosis. The driver is encoded in a 10.4kb locus on chromosome 2 and drive is caused by two genetic components (Larracuente and Presgraves, 2012). *SD* is composed by the driver *Sd* that targets *Responder (Rsp)* in the homologous chromosome 2 of *Sd. Rsp*, a repetitive satellite DNA, is composed by A-T rich nucleotides sequences, with each repeat size of 120 bp. Interestingly, *Sd* drive is dependent on the number of *Rsp* repeats; high number of repeats (*Rsp > 2000*) are super
sensitive to *Sd*, whereas lower number of repeats (*Rsp* <200) are not sensitive to the drive of *Sd*. *Rsp* repeats are also present in the driving SD locus, but with only 20 occurrences, so no drive is observed for SD chromosome 2. *Sd* is a truncated duplication of *RanGAP*. *RanGAP* are proteins that transport RNAs from the cytosol to the nucleus. In the case of *Sd*, the truncated version of *RanGAP* still encodes an enzymatically active protein, but it is mislocalized in the nuclei instead of the cytosol. This mislocalization contributes to the distortion in the spermatocytes (Kusano et al., 2001; Larracuente and Presgraves, 2012). *SD* is transmitted to 95-100% of the offspring of a heterozygous *SD/SD*+. The *Sd*, truncated *RanGAP*, creates a delay in the transition of histones to protamines (nuclear proteins arginine rich in sperm) in *SD*+ sperm (*Rsp* rich). The transition from histones to protamines is essential for the reorganization of the spermatid nuclei during spermatogenesis. Depending on the number of *Rsp* repeats present in *SD*+ chromosome 2, the sperm quality of a *SD/SD*+ varies (Herbette et al., 2021). The *SD* system is complex and contains unidentified modifies of drive (that will increase the drive) and unidentified suppressors that reduce the drive. Those elements are strong indications of ongoing genetic conflict in *D. melanogaster* (Larracuente and Presgraves, 2012).

*RanGAP* is an essential gene share in the *Drosophila* clade but the *SD-RanGAP* arose only 16,000 years to give birth of a meiotic driver corresponding to the divergence between European and African lines of *D. melanogaster* (Brand et al., 2015). The other component of the drive system *SD*, *Rsp-like* are predicted to have been born ~240,000 years ago (Larracuente, 2014). Both *Sd* and *Rsp* are rapidly evolving in a genetic arms race in *Drosophila melanogaster*. 

25
1.3.2.2 X chromosome drive in *Drosophila simulans*

In *Drosophila simulans*, two genetically distinct meiotic driver systems have been identified, the Paris and Winters/Durham Sex-ratio (SR) systems. In both cases a driver in the chromosome X kills the Y-bearing sperm of a heterozygote (driver+/driver-).

The *D. simulans* Paris SR males carrying the X$^{SR}$ chromosomes sire more females than males (81-96%) (Cazemajor et al., 2000; Montchamp-Moreau and Cazemajor, 2002). During Anaphase II, SR will cause missegregation of the Y chromosome (Sabotage; Figure 1.1 B) (Cazemajor et al., 2000; Helleu et al., 2016). Two distortion loci have been identified in Paris meiotic driver: Dp$^{SR}$ and wlasta. One component of the distortion is a duplication of a heterochromatin protein, called HP1D2, located in wlasta duplication in the X chromosome of *D. simulans* Paris. HP1D2 binds heterochromatic on the Y chromosome and favors the missegregation (Helleu et al., 2016). The other distorter in the Dp$^{SR}$ (duplication of SR) is still unknown.

The other meiotic driver system called Winters/Durham was initially thought to be two different meiotic driver systems, but the discovery of common suppressors raises the idea of one single system (Lin et al., 2018). Two meiotic driver genes, called *Dox* and *MDox* (Distorter on the X and Mother of Distorter on the X), fail to transition from histones to protamines in the Y-bearing sperm spermatocytes. The mechanism is like the SD system in *D. melanogaster* but the exact system is unknown (Lin et al., 2018). In both cases, meiotic driver *Dox* and *MDox* are suppressed in the genome of *D. simulans* by hairpin RNAs (hpRNA) (Lin et al., 2018; Tao et al., 2007b). These hairpin RNAs, Nmy (*Not much yang*) and Tmy (*Too much yang*) are able to degrade transcripts of *Dox* and *MDox* (Lin et al., 2018). Interestingly, *Dox* and *MDox* are
found in two species, *D. mauritiana* and *D. simulans*. Gene flow between *D. mauritiana* and *D. simulans* has been shown to occur. The important gene flow implicated chromosome X introgression between *D. mauritiana* and *D. simulans* which contains meiotic driver Dox and *MDox* sequences (Meiklejohn et al., 2018).

### 1.3.3 t-haplotype a poison and antidote meiotic driver in *Mus musculus*

The t-haplotype is a complex of different *Mus musculus* genes on chromosome 17 that bias Mendelian segregation in male mouse by a poison-antidote mechanism (Figure 1.1D). The t-haplotype locus contains four inversions that suppress recombination between a wild-type chromosome 17 and the t-complex. In heterozygous males (*t/+*), 99% of the progeny inherit the t-haplotype (Herrmann and Hermann, 2012; Schimenti, 2000). The t-haplotype is found in wild populations at a maximum frequency of 10-25%. The frequency of t-haplotype in wild populations is explained by a very high cost and the sterility of homozygous t-haplotype male mice *t/t* that may prevent fixation of the meiotic driver in the population (Kelemen and Vicoso, 2018). Four factors contributing to drive have been identified in the t-haplotype complex, three poison genes, called *Tcd*, and an antidote, called *SmokTcr*. The poison produced by the t-haplotype loci reduces sperm motility. *SmokTcr*, an allele of a sperm motility kinase, acts as an antidote and will rescue the sperm motility induce by meiotic driver. During gametogenesis, a heterozygous diploid mouse *t/+* produces *t*-sperm and +-*sperm*. The *t*-sperm have an increased motility that allows them to fertilize eggs more efficiently (Amaral and Herrmann, 2021). When sperm from both wild-type mice and t-haplotype heterozygotes are mixed (*t*-sperm and +-*sperm*), the +-*sperm* from +/+ mouse is better at fertilizing than the *t*-sperm from the heterozygote (Olds-Clarke, 1989). This result shows that there is an important fitness cost for organism to carry t-haplotype, because wild type sperm outcompete t-haplotype sperm when mating.
The t-haplotype is present in *M. m. musculus*, *M. m. domesticus*, and *M. m. castaneus*. The divergence between *M. musculus* subspecies is predicted to be 2.9 million years, which would be an approximation for t-haplotype birth (Harr et al., 2016). Gene flow between different subspecies seems to be an important factor of the t-haplotype evolution. For example the phylogeny of the t-haplotype between *M. m. domesticus*, and *M. m. castaneus* indicates gene flow between the two subspecies (Kelemen and Vicoso, 2018). That would mean that 2.9 million years may be a overestimation the t-haplotype gene birth (Kelemen and Vicoso, 2018). The divergence between the different species of *M. musculus* seems to be less than 0.5 Mya. Still t-haplotype is present in three species and has been maintained by gene flow between the *castaneus* and *domesticus* genomes (Kelemen and Vicoso, 2018).

1.3.4 Spok genes in *Podospora anserina*

This part is an extract from De Carvalho M, Zanders SE. 2019. A family of killers. Elife 8:8–10. doi:10.7554/eLife.49211. This is a good example of poison and antidote mechanism in fungi like *S. pombe wtf* genes.

Hanna Johannesson and co-workers at Uppsala University, the University of Bordeaux and Wageningen University – including Aaron Vogan and Lorena Ament-Velasquez, both from Uppsala, as joint first authors – report on the genetic architecture underlying a series of killer meiotic drive phenotypes in the fungus *Podospora anserina* (Vogan et al., 2019).

*P. anserina* is an excellent model organism for studying meiotic drive, largely because the phenotype is easy to observe: the two spores that inherit the drive locus are viable and
pigmented, while the two spores that do not are dead and unpigmented (Figure 1.2A; Padieu and Bernet, 1967; Turner and Perkins, 1991, Espagne et al., 2008). *P. anserina* strains carrying spore killing loci have been sorted into seven types (*Psks*) based on their killing phenotypes, but the genes underlying these phenotypes were unknown (Van Der Gaag et al., 2000). Recently, *Spok2* was identified as an autonomous single-gene meiotic driver in *P. anserina*, and it was shown that the *Spok* genes comprise a gene family found in many copies in diverse fungal lineages (Grognet et al., 2014). These discoveries suggested the *Spok* genes as candidates for causing the *Psk* phenotypes.

Vogan et al. started by sequencing and assembling the genomes of six *P. anserina* strains representing the distinct spore killing phenotypes. These assemblies showed that *Spok2* is present in most *P. anserina* isolates and facilitated the discovery of two new *Spok* genes (*Spok3* and *Spok4*) that are both spore killers. Curiously, these two genes are found within a block of sequence that is found in single copy at different locations within the various genomes. In some, the block contains only *Spok3* or *Spok4*, but in others, it contains both. How the block has moved during the evolutionary history of *P. anserina* is a mystery. All the *Psk* phenotypes can be explained by just three genes (*Spok2*, *Spok3* and *Spok4*). Spore killing depends on the number of *Spok* genes inherited by each pair of spores after meiosis. Generally, the pair inheriting the largest number of different *Spok* genes will kill the other pair (Figure 1A and 1B). Strains with no *Spok* genes are considered naïve and spore killing will occur when these strains are crossed to *Psk* strains, but not when they are crossed to other naive strains. The most dominant *Psk* phenotypes, *Psk-1*, and *Psk-7*, contain all three *Spok* genes. These strains cause killing when crossed to all other types. Interestingly, killing is also observed when *Psk-1* and *Psk-7* are crossed to each other because the *Spok* block is found at different loci in the two strains, which means that some spores do not inherit it (Figure 1.2B). The other strains that
cause killing contain one or two Spok genes. Again, their phenotypes are explained by the identity and location of their Spok genes.

Figure 1.2 Meiotic drive in Podospora anserina.

Figure from (De Carvalho and Zanders, 2019). A) A heterozygous diploid generated by mating a naïve strain to one carrying Spok2 undergoes meiosis and generates four spores. One possible outcome of meiosis is depicted. The spores that inherit Spok2 are alive (pigmented), while the spores that do not inherit the Spok2 gene are destroyed (unpigmented, hazard signs). B) A heterozygous diploid generated by mating the Psk-1 strain (purple) to the Psk-7 strain (green) undergoes meiosis and generates four spores. Note that the Spok block (black oval) is on different chromosomes in the two isolates. Two possible outcomes are depicted: (left) all four spores survive because they all inherited Spok2 and the block that contains Spok3 and Spok4; (right) two of the four spores do not survive because they did not inherit the Spok block.

Previous work demonstrated that these genes encode both poison and antidote functions in genetically separable domains and predicted a kinase domain in one of these regions (Grognet et al., 2014). The new work shows that Spok3 can be expressed outside of spore formation, and Vogan et al. exploit this observation to better characterize how single Spok genes carry out
their dual roles. A cysteine-rich domain and a nuclease domain are both required for resistance and killing functions, respectively. How the nuclease domain promotes killing and how the kinase domain neutralizes that killing are unknown.

*Spok* genes are present in different species of filamentous fungi (Grognét et al., 2014). Other *Spok* genes are found in *Sordariales* (that includes *Podospora anserina*) and *Dorthideomycetes*, which diverged between 430 and 400 million years ago (Beimforde et al., 2014). It seems, though, that *Spok* genes have been transmitted to different species of fungi by horizontal gene transfer (Grognét et al., 2014). The best hypothesis for now is the presence of palindromic terminal repeats in the *Spok* block, similar to transposons’ repetitive sequences (Vogan et al., 2021). A transposon, called *Entreprise* in *P. comata*, has been identified with similarities to the *Spok* block. It is then possible that the *Spok* genes have not been segregating in filamentous fungi over more than 400 million years but have been transmitted horizontally to different species.

1.4 How meiotic drivers can persist in genomes

Meiotic driver can be maintained in genomes by increasing its allelic transmission in the population. If a driver is fixed, suppressed, or decayed, the meiotic driver will be inactivated by mutations and go extinct. The meiotic driver will need for that to escape all these mechanisms to continue cheating natural selection. The meiotic driver is under the Damocles sword of the genome that will find all the possible ways to make the driver extinct in a genetic conflict.
The first possible mechanism to escape extinction will be gene flow. Gene flow is the passage of alleles from one population to another population that mate to form hybrids and share in this way genetic material. Gene flow will give the opportunity for a driver to find another background without suppressors which will allow the driver to act. A meiotic driver could be found in two different species for each gene flow happens, for example the Dox and MDox genes found in D. mauritiana and D. simulans (Meiclejohn et al., 2018) or the t-haplotyppe for domesticus and castaneus (Kelemen and Vicoso, 2018). Other examples, notably the Neurospora Spore killer Sk-1 has been found to originate in the genome of N. sitophila from N. hispaniola (Svedberg et al., 2021).

Another way for drivers to cross the species barrier is horizontal gene transfer between different species. This may be the case of Spok genes that are found in different filamentous fungi and where we Spok genes are found in a patchy phylogenetic distribution between very diverge species for which gene flow is very unlikely (Grogné et al., 2014). The identification of a possible transposable element in Podospora comata supports this hypothesis (Vogan et al., 2021).

In this thesis, I propose a model of evolution for meiotic drivers that will implicate duplication events. The Damocles sword above meiotic driver can fall on the driver, but by duplicating and accumulate mutations, the driver can create a paralog copy that will be unseen by the genome. The new copy of the meiotic driver can continue cheating meiosis with the first copy from which the new driver originated can be lost. If the new copy is in the brink of extinction, new duplication event can make the driver reborn again. To duplicate in the genome the driver will need to favor non-allelic gene conversion. Non-allelic gene conversion is the result of a repair
event after double strand break of non-homologous chromosomes. During meiosis double strand break could occur near the driver. The repair process can then involve a non-allelic sequence elsewhere in the genome that do not contain the driver that will result in a duplicated copy of the driver. This mechanism is known to contribute concerted evolution of 5S rDNA elements where mutation in one copy spread in other copies (Eickbush and Eickbush, 2007). Other example, like Rsp-elements in D. melanogaster has been shown to form DNA extrachromosomal circles that carry copies of Rsp (Sproul et al., 2020). Those DNA extrachromosomal circle is then integrated in the genome by recombination and can allow increase of copies in the genome of the Rsp elements. It is possible that drivers increase their number of copies in the genome in this way. It is important to note that many drivers are found in many copies and/or implicate repetitive elements (Bravo Núñez et al., 2020a; Eickbush et al., 2019; Hu et al., 2017; Meiklejohn et al., 2018; Vogan et al., 2019).

The cycle of birth and death may allow meiotic drivers to survive in genomes for longer time periods than the expectation of the dogma. To study how old meiotic driver can survive in the genome, we looked at wtf genes in Schizosaccharomyces a very tractable organism.

1.5 wtf genes are a family of meiotic drivers

A new family of meiotic drivers, the wtf (with Tt transposons) genes, were recently discovered in the fission yeast Schizosaccharomyces pombe (Nuckolls, Bravo Núñez et al., 2017; Hu et al., 2017). One member of this family, wtf4, was demonstrated to cause drive using and poison-antidote system (Figure 1.3). wtf4 is a single gene with two transcriptional start sites. The long transcript includes six exons and encodes an antidote protein, while the short transcript has five exons and encodes a poison protein. The antidote transcript is under the control of a promotor
in the region upstream of exon 1. The short transcript is under the control of a promoter found within intron 1 of the antidote transcript.

Figure 1.3: *wtf* produces a poison and antidote proteins.

Model for meiotic drive of *wtf* genes in *S. pombe*, modified from (Nuckolls et al., 2017). All spores are exposed to poison protein, but those that inherit the *wtf* driver is rescued by the antidote protein.

When *S. pombe* undergoes meiosis, four meiotic products (spores) are formed within an ascus (sack) (Davis and Smith, 2001). The Wtf4 poison protein is first produced prior to the meiotic divisions, and it spreads throughout the entire ascus (Figure 1.2). The antidote protein produced by *wtf* is spore-specific, meaning it is produced and maintained predominantly within the spores that inherit *wtf*. The spores that do not inherit the *wtf* locus therefore do not have the antidote to protect them from the poison. Because of this, the *wtf*-spores produced by a *wtf*/- heterozygote diploid cell is destroyed and the *wtf* allele is found in more than 90% in the viable spores. This decreases fertility to around 50% because instead of four spores, *wtf*/- heterozygotes tend to make two. *wtf* has no fertility phenotype in homozygotes. In *wtf*/*wtf* homozygotes, all the spores will inherit *wtf* and there will not die because each spore produces an antidote. For *wtf*/- homozygotes, there is no death because no poison is produced (Hu et al., 2017; Nuckolls et al., 2017). *wtf* genes could spread in the population by outcrossing which will reduce the probability of driver fixation (López Hernández et al., 2021).
Currently, \textit{wtf} genes are reported only in isolates of \textit{S. pombe}. The \textit{Schizosaccharomyces} genus is composed of four other species, in addition to \textit{S. pombe}: \textit{S. japonicus}, \textit{S. cryophilus}, \textit{S. octosporus} and \textit{S. osmophilus} (Figure 1.4) (Brysch-Herzberg et al., 2019; Rhind et al., 2011). The diversity and the rapid evolution of \textit{wtf} genes in \textit{S. pombe} suggests that \textit{wtf} genes could be present in other species of fission yeast. I predict the presence of \textit{wtf} genes may have been missed outside of \textit{S. pombe} because they are more diverged between two species than the average genes. \textit{S. octosporus} and \textit{S. pombe} diverged 119 Mya (Rhind et al., 2011). The genomic divergence between the two species is comparable to the one found between humans and cephalochordates. We can expect that if \textit{wtf} genes are present in other fission yeast species, they will be so diverged that identification will be difficult.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{phylogeny.png}
\caption{Phylogeny of \textit{Schizosaccharomyces}.}
\end{figure}

The phylogeny is modified from (Brysch-Herzberg et al., 2019; Rhind et al., 2011). Nodes dates from (Rhind et al., 2011).

I propose in this thesis to study the origin of the \textit{wtf} meiotic drivers by analyzing \textit{wtf} genes in different fission yeast species. In \textbf{Chapter 2}, I identify \textit{wtf} genes candidates in \textit{S. octosporus}, \textit{S. cryophilus}, and \textit{S. osmophilus} and annotate the genomes with the help of RNA-seq data from Guo-Song Jia (Li-Lin Du lab). In \textbf{Chapter 3}, Guo-Song Jia and I prove that \textit{wtf} genes in other fission yeast use a poison-antidote mechanism to favor their allelic transmission and that they are meiotic drivers when heterozygous. In \textbf{Chapter 4}, I discuss the main conclusions and
outcomes of finding and characterizing \textit{wtf} genes outside of \textit{S. pombe}. Finally, in Chapter 5, I explain all the methods and material used in this thesis.

1.6 Disclosure

This thesis is supported by unpublished data from Guo-Song Jia and Me (Mickael De Carvalho). Both of us worked in parallel in this project and some parts of this thesis are exclusively my work. Other parts include his work but are included to give the reader an overview of all the research made. The main bioinformatic analysis was performed by me with support of RNA-seq data generate by Guo-Song Jia. For the molecular biology approaches I worked on the budding yeast testing of \textit{wtf} genes and Guo-Song Jia worked on \textit{S. octosporus}. Finally, some results presented in the discussion are my work. I will specify throughout the thesis when Guo-Song Jia’s work is presented.
2 Chapter 2: wtf genes homologs outside of S. pombe

Parts of this paper are in collaboration with Guo-Song Jia from the Li-Lin Du lab. The contributions are discussion on the wtf annotation and performing RNA-seq during meiosis of S. octosporus. Figure 2.4B, Figure 2.5 and Figure 2.11A were modified from Guo-Song Jia work.
CHAPTER 2: WTF GENES HOMOLOGS OUTSIDE OF S. POMBE

2.1 List of figures

2.2 List of Tables

2.3 Introduction

2.4 Results

2.4.1 Genes with homology to WTF drivers are found in S. octosporus, S. osmophilus and S. cryophilus

2.4.2 S. octosporus, S. osmophilus and S. cryophilus WTF genes share additional features with S. pombe genes.

2.4.3 WTF genes in S. octosporus, S. osmophilus and S. cryophilus are associated with distributed 5S rDNA sequences.

2.4.4 WTF genes were likely present in the common ancestor of S. octosporus, S. osmophilus, S. cryophilus and S. pombe.

2.4.5 WTF genes show evolutionary signatures consistent with a history of genetic conflict.

2.4.6 WTF genes duplicated to pre-existing 5S rDNA genes.

2.5 Discussion

2.5.1 WTF genes are ancient meiotic drivers.

2.5.2 S. octosporus WTF genes are transcriptionally active.

2.5.3 Dispersed repetitive elements are associated with WTF genes.

2.5.4 Common ancestor.

2.5.5 Rapid evolution.

2.6 Summary
Figure 2.1 wtf homologs are found outside of S. pombe .................................................. 42
Figure 2.2 Maps of the wtf gene family members in S. octosporus, S. osmophilus, S. cryophilus, and S. pombe. ................................................................. 45
Figure 2.3: Limited conservation of Wtf proteins. ................................................................. 48
Figure 2.4 S. pombe wtf genes share features with other wtf genes outside of S. pombe. ...... 49
Figure 2.5 Transcription of predicted poison and antidote isoforms of wtf genes in S. octosporus .......................................................................................................... 52
Figure 2.6 Genomic context of wtf genes. ................................................................................ 54
Figure 2.7: Distance between 5S rDNA and wtf genes ............................................................ 56
Figure 2.8 Homology between distinct 5S rDNA-wtf units and wag-wtf units. .................... 57
Figure 2.9 S. octosporus wtf gene units supported by maximum likelihood phylogeny. ....... 58
Figure 2.10 wtf genes are found at the same syntenic locus in three fission yeast species ...... 60
Figure 2.11 Synteny between S. cryophilus wtf4 and S. pombe wtf6 ..................................... 62
Figure 2.12: Gene duplication and non-allelic gene conversion within wtf gene family. ....... 64
Figure 2.13: GARD analysis of wtf genes ................................................................................ 67
Figure 2.14: Contraction and expansion of repeat sequences in wtf genes ......................... 69
Figure 2.15: Model of spread of wtf genes by duplication to preexisting 5S rDNA genes. .... 71

2.2 List of Tables

Table 2-1 Percent identity of 1:1 orthologs in Schizosaccharomyces ................................. 46
Table 2-2 wtf genes table prediction driver, antidote only, pseudogene and unknowns. ....... 47
Table 2-3 5S rDNA in Schizosaccharomyces genome .......................................................... 57
2.3 Introduction

During meiosis, the two alleles at a given locus segregate from each other and are each packaged into an equal number of gametes produced by a heterozygous organism. This fundamental rule of genetics is known as Mendel’s Law of Segregation (Abbott and Fairbanks, 2016). Most genetic loci follow this law, which facilitates natural selection by allowing alternate variants to compete on an even playing field (Crow, 1991). Meiotic drivers, however, are genetic loci that manipulate gametogenesis to gain an unfair transmission into gametes. Rather than being transmitted to 50% of the gametes produced by a driver+/driver- heterozygote, meiotic drivers are transmitted to most or all the functional gametes (Sandler and Novitski, 1957; Zimmering et al., 1970).

Meiotic drivers are found in diverse eukaryotes including plants, fungi and animals (Bravo Núñez et al., 2018b; Burt and Trivers, 2006; Helleu et al., 2015; Lindholm et al., 2016). Despite their broad phylogenetic distribution, drivers in different systems are not thought to share common evolutionary origins. Instead, empirical observations combined with theoretical work indicate that drivers will be evolutionarily short-lived (Burt and Trivers, 2006). Specifically, new drivers are born, and old drivers go extinct leading to lineage-specific drive systems. Understanding the birth of a driver is conceptually straightforward; if a sequence acquires the ability to drive, it can spread in the population (Crow, 1991). The paths to driver extinction are more complex. One route to driver destruction is through suppression (Bastide et al., 2011; Bravo Núñez et al., 2018a; Cécile Courret, Pierre R. Gérard, David Ogereau, Matthieu Falque, Laurence Moreau et al., 2019; Lin et al., 2018; Tao et al., 2007b; Unckless et al., 2015). Drive is generally costly to fitness, so natural selection is thought to favor the evolution of drive suppressors unlinked to the drive locus (Cazemajor et al., 2000; Crow, 1991; Fishman and Saunders, 2008; Zanders and Unckless, 2019). Suppressed drivers have no transmission
advantage and are expected to accumulate inactivating mutations and be lost (Helleu et al., 2015). In a second path to driver destruction, the driver evades suppression and spreads to fixation. If the driver is on a sex chromosome or the driving haplotype acquires strongly deleterious mutations, driver fixation can lead to driver extinction via host extinction (Pinzone and Dyer, 2013). If the fixed driver is autosomal, it does not acquire transmission advantage and can accumulate inactivating mutations like suppressed drivers.

The limited number of mapped meiotic drivers largely support the idea that drivers have limited evolutionary lifespans (Akera et al., 2017; Bauer et al., 2012, 2007; Cocquet et al., 2012, 2009; Crow, 1988; Didion et al., 2015; Fuller et al., 2020; Helleu et al., 2016; Hu et al., 2017; Jaenike, 2001; Kelemen and Vicoso, 2018; Koide et al., 2018; Larracuente and Presgraves, 2012; Long et al., 2008; Lorenzen et al., 2008; Lyon, 1984; Paczolt et al., 2017; Phadnis and Orr, 2009; Price et al., 2019; Rathje et al., 2019; Riek and Saupe, 2016; Schimenti, 2000; Seuring et al., 2012; Shen et al., 2017; Svedberg et al., 2021; Yang et al., 2012). In Drosophila, for example, the sister species *D. melanogaster* and *D. simulans* shared a common ancestor only 5.4 million years ago (Tamura, 2003), but the species both contain distinct meiotic drive systems (Cazemajor et al., 1997; Helleu et al., 2016; Larracuente and Presgraves, 2012; Lin et al., 2018; Tao et al., 2007b). There are a few known exceptions where the same drivers are found in closely related or more distantly related species (Grognét et al., 2014; Meiklejohn et al., 2018). In the *Dox* drive system recent introgression between *Drosophila* species or horizontal gene transfer of the *spok* genes in filamentous fungi may explain the phylogenetic distribution of the drivers better than shared decent from a common ancestor (Grognét et al., 2014; Meiklejohn et al., 2018; Vogan et al., 2020). The oldest described drive system may be the ‘knobs’ found in maize that are found in both *Zea mays* and *Zea mays mexicana* and thus predicted to be 12 million years old (Dawe et al., 2018; Kanizay et al., 2013).
Figure 2.1 *wtf* homologs are found outside of *S. pombe*.

Modified from Figure 1.1 and 1.2. (A) Model for meiotic drive of *wtf* genes in *S. pombe*, modified from (Nuckolls et al., 2017). All spores are exposed to poison protein, but those that inherit the *wtf* driver is rescued by the antidote protein. (B) Phylogeny of *Schizosaccharomyces* species including the number of hits from a PSI-BLAST search for *wtf* homologs. MYA represents million years ago. * The *S. osmophilus* genome is not fully assembled, so the number represents the *wtf* PSI-BLAST hits within the assembled contigs. The phylogeny is based on published reports (Brysch-Herzberg et al., 2019; Rhind et al., 2011) and our own analyses with the added partial assembly of *S. osmophilus*.

The *wtf* genes are found in the fission yeast *Schizosaccharomyces pombe* (Nuckolls, Bravo Núñez et al., 2017; Hu et al., 2017). Different meiotic drivers in *S. pombe* use a system of poison-antidote system. Meiotic driver, *wtf* is one gene with two transcriptional start sites. The antidote protein is encoded by the first start site, the second one encodes the poison protein. Overlapping sequences between the two proteins allows perfect recognition of the poison by the antidote and strong linkage with impossibility to separate the poison and antidote (Bravo Núñez et al., 2018a; Nuckolls et al., 2020).

Here, we analyzed the phylogenetic distribution of *wtf* genes and found highly diverged but homologous *wtf* genes in *Schizosaccharomyces octosporus, Schizosaccharomyces osmophilus*
and *Schizosaccharomyces cryophilus*. Analyses of synteny supports that the genes were inherited from a common ancestor. Like the *S. pombe wtf* genes, the newly described *wtf* genes exhibit evolutionary signatures of genetic conflict, namely rapid evolution. We conclude that *wtf* drivers were present in the ancestor of these species and have been active for over 100 million years.

### 2.4 Results

#### 2.4.1 Genes with homology to *wtf* drivers are found in *S. octosporus, S. osmophilus* and *S. cryophilus*

As a first step in understanding the long-term evolution of the *wtf* meiotic drivers, we analyzed the phylogenetic distribution of the *wtf* gene family. There are four described *Schizosaccharomyces* species in addition to *S. pombe*: *S. octosporus, S. osmophilus, S. cryophilus* and *S. japonicus* (Figure 2.1B). These species are thought to have had a common ancestor 200 million years ago; and the amino acid divergence between 1:1 orthologs between in *S. pombe* and *S. japonicus* is 55%, similar to that observed between humans and lancelets (a cephalochordate) (Rhind et al., 2011).

At the time this work was initiated, genome assemblies were available for all species except *S. osmophilus*, which was only recently discovered (Brysch-Herzberg et al., 2019). We therefore sequenced *S. osmophilus* using both Illumina 100 bp paired end reads and Oxford nanopore reads (see Chapter 5.2). We used these reads to assemble a draft of the *S. osmophilus* genome consisting of 11 contigs. We predicted the coding sequences of *S. osmophilus* genes using the Augustus algorithm trained with gene data from an *S. octosporus* annotation (Figure 2.2; (Tong et al. 2019; Hoff & Stanke, 2018). We predicted 1:1 orthologs between the species
using OrthoVenn2 and found that *S. osmophilus* proteins are on average 88.9% identical to their 1:1 orthologs in *S. octosporus* and 85.2% to 1:1 orthologs in *S. cryophilus* (Xu et al., 2019) (Table 2.1). Our results are consistent with the previously proposed phylogeny of the fission yeasts that used limited sequencing data from *S. osmophilus* (Brysch-Herzberg et al., 2019), Figure 2.1B).

Using the identified proteins by orthoVenn2 we perform two BLAST searches to find the percentage of identity between all the common proteins between each paired species.
Figure 2.2 Maps of the wtf gene family members in *S. octosporus*, *S. osmophilus*, *S. cryophilus*, and *S. pombe*.

Genome map of wtf genes from (A) *S. octosporus*, (B) *S. osmophilus*, (C) *S. cryophilus*, and (D) *S. pombe*. Genes on the forward strand are shown above each chromosome, whereas genes on the reverse strand are shown below chromosomes. Genes we predict to be intact drivers because they contain an alternate translational start site near the beginning of exon 2 are shown in purple. Genes lacking the potential alternate start site that we predict to be drive suppressors are shown in black. Unknown wtf genes with no predicted functions are shown in light blue text. Predicted pseudogenes are indicated with an asterisk*. The *S. pombe* map is modified from (Eickbush et al., 2019). Annotations of the novel wtf genes can be found in (Appendix Tables A1-A3).
Table 2.1 Percent identity of 1:1 orthologs in *Schizosaccharomyces*

<table>
<thead>
<tr>
<th>Percent amino acid Identity</th>
<th>S. cryophilus</th>
<th>S. pombe</th>
<th>S. osmophilus</th>
<th>S. japonicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. octosporus</td>
<td>83.6</td>
<td>65.3</td>
<td>88.9</td>
<td>56.6</td>
</tr>
<tr>
<td>S. cryophilus</td>
<td>65.6</td>
<td></td>
<td>85.2</td>
<td>56.7</td>
</tr>
<tr>
<td>S. pombe</td>
<td></td>
<td>65.2</td>
<td></td>
<td>57.3</td>
</tr>
<tr>
<td>S. osmophilus</td>
<td></td>
<td></td>
<td></td>
<td>56.5</td>
</tr>
</tbody>
</table>

We next searched all the fission yeast genome assemblies for *wtf* gene homologs. Even within *S. pombe*, the *wtf* genes are diverse and a standard BLAST (Basic Local Alignment Search Tool) search using one *wtf* gene as a query does not identify all members of the family (Altschul et al., 1990). Because of this, we carried out our search for homologs using PSI-BLAST (Position-Specific Iterated BLAST) (Altschul et al., 1997). PSI-BLAST uses the results from an initial search to create a profile of the multi-alignment between the query protein and the best hits. This profile is then used to find other proteins and the iterative process continues until no more significant hits are found. Using *wtf4* from *S. pombe* as an initial query, we were able to find potential *wtf* homologs in all species except *S. japonicus* (Figure 2.1B). We refined and repeated our PSI-BLAST searches using candidate *wtf* proteins from other species (*S. octosporus wtf25, S. cryophilus wtf1 and S. osmophilus wtf14*) until our search was saturated. Searches initiating with the *S. octosporus, S. cryophilus* and *S. osmophilus* genes all identified *S. pombe wtf* genes as hits. None of our searches found candidate *wtf* homologs in *S. japonicus* or outside of fission yeasts (see Chapter 5.4.3).

We then used the nucleotide sequence of individual *wtf* candidate genes to perform additional BLASTn searches to find potential pseudogenes missed by our PSI-BLAST results. For example, we used the nucleotide sequences of all the *S. octosporus wtf* genes identified by
the PSI-BLAST search as queries to search for homologous pseudogenes within *S. octosporus*. Only hits more than 200 bp long were considered as pseudogenes *wtf* genes, although there were additional shorter hits that are likely homologous but highly degraded. We then used sequence alignments of *wtf* candidate genes within each species, and sometimes between species, to refine the predicted coding sequences. In *S. octosporus*, The Li-Lin Du lab generated RNA sequencing data sets from vegetative and meiotic samples. We used those sequences to further improve the coding sequences predictions of *S. octosporus*. Overall, I identified 48 predicted *wtf* genes and 35 predicted *wtf* pseudogenes in *S. octosporus*, 32 predicted *wtf* genes and 10 predicted *wtf* pseudogenes in *S. osmophilus* and 2 predicted *wtf* genes and 3 predicted *wtf* pseudogenes in *S. cryophilus* (Figure 2.2; Table 2.2). Previously, 16 intact *wtf* genes and 9 pseudogenes were described in the reference isolate of *S. pombe* (Eickbush et al., 2019; Hu et al., 2017).

<table>
<thead>
<tr>
<th>Species</th>
<th><em>S. pombe</em></th>
<th><em>S. octosporus</em></th>
<th><em>S. cryophilus</em></th>
<th><em>S. osmophilus</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Driver</td>
<td>4</td>
<td>48</td>
<td>1</td>
<td>30</td>
<td>83</td>
</tr>
<tr>
<td>Antidote</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Pseudogene</td>
<td>9</td>
<td>35</td>
<td>3</td>
<td>11</td>
<td>58</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>83</td>
<td>5</td>
<td>42</td>
<td>155</td>
</tr>
</tbody>
</table>

2.4.2 *S. octosporus*, *S. osmophilus* and *S. cryophilus* *wtf* genes share additional features with *S. pombe* genes.

The homology between the *S. pombe* *wtf* genes and those found in the other *Schizosaccharomyces* species is low (Figure 2.3). For example, the most similar *wtf* gene pair
between *S. pombe* (*Sk wtf2*) and *S. octosporus* (*S. octosporus wtf16*) share only 19% amino acid identity, compared to an average of 65.3% amino acid identity between orthologous gene pairs (table 2.1). Given this high divergence, we examined features outside of the coding sequences not considered in our PSI-BLAST searches to further test if our candidate genes are members of the wtf gene family.

![Figure 2.3: Limited conservation of Wtf proteins.](image)

The percent identity shared amongst all 113 Wtf predicted antidote proteins from *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe* (isolate FY29033) aligned with MAFFT (L-INS-I; BLOSSUM62 scoring matrix/k=2; Gap open penalty of 2; offset of 0.123). The data are shown in 10 amino acid sliding windows.

We first looked for similarities in overall gene structure between the *S. pombe* wtf genes and the candidate wtf genes in *S. octosporus*, *S. osmophilus* and *S. cryophilus*. The wtf genes of *S. pombe* have been classified into three broad categories: predicted meiotic drivers (4-14 per isolate), predicted suppressors of drive that encode only antidote proteins (9-17 per isolate) and genes of unknown function (4 in each isolate) (Bravo Núñez et al., 2020b, 2020a, 2018a; Hu et al., 2017; Nuckolls et al., 2017). We found that the overall gene structure of the candidate wtf
homologs in all three species was similar to 5-exon \textit{wtf} drivers and 5-exon \textit{wtf} suppressors in \textit{S. pombe} (Figure 2.4). Specifically, the relative size of the corresponding exons and introns are remarkably similar between the species, even though the actual sequences are generally quite different (Figure 2.3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{\textit{S. pombe} \textit{wtf} genes share features with other \textit{wtf} genes outside of \textit{S. pombe}.}
\end{figure}

\textbf{(A)} Schematic \textit{wtf} loci of the \textit{Schizosaccharomyces} species. Orange boxes correspond to exons (E1 indicates exon 1, etc.), the red boxes represent 5S rDNA genes, the blue box represents a pseudogenized \textit{wag} gene and the yellow box is an LTR from a Tf transposon. The predicted translational start sites for the antidote (ATG in exon 1) and poison (ATG in exon 2) proteins are indicated, as is the FLEX transcriptional regulatory motif. \textbf{(B)} Long read RNA sequencing reads from \textit{S. octosporus} aligned to \textit{wtf25} illustrating two main transcriptional start sites, presumably encoding an antidote and a poison protein, respectively. Raw data is shown in pink, the blue lines indicate the absence of that sequence in the reads due to splicing (modified from Guo-Song Jia).
We next looked for similarities between regulatory elements that have been defined in the \textit{S. pombe} \textit{wtf4} driver and the potential promoters of the candidate \textit{wtf} homologs. The \textit{wtf4} promoters in \textit{S. pombe} are highly like and thus, likely representative of, the other \textit{wtf} drivers and suppressors of \textit{S. pombe} (Nuckolls et al., unpublished). The Wtf4\textsuperscript{antidote} protein is encoded on exons 1-6, with the promoter found upstream of exon 1. The cis regulatory elements controlling the expression of the \textit{wtf}\textsuperscript{antidote} transcripts are unclear, but we found no shared homology between the \textit{S. pombe} \textit{wtf}\textsuperscript{antidote} promoter sequences and sequences upstream of exon 1 in the candidate \textit{wtf} homologs found in the other species.

The Wtf4\textsuperscript{poison} protein is encoded on exons 2-6 and the promoter is found within what is intron 1 of the \textit{wtf}\textsuperscript{antidote} transcript. The \textit{S. pombe} \textit{wtf4} poison promoter contains a cis-regulatory FLEX motif that is bound by the Mei4 master meiotic transcription factor and is essential for expression of the Wtf4\textsuperscript{poison} protein (Nuckolls et al., unpublished, Figure 2). The FLEX motif consists of a seven base pair core sequence (G/A)TAAA(C/T)A). Mei4 tends to bind an extended FLEX motif with AACA on the 3’ end (Alves-Rodrigues et al., 2016; Horie et al., 1998; Ioannoni et al., 2016). All verified \textit{S. pombe} \textit{wtf} drivers contain the complete 11 base pair FLEX motif (Nuckolls et al., unpublished).

To test if Mei4 was potentially regulating the expression of the candidate \textit{wtf} genes outside of \textit{S. pombe}, we first predicted the Mei4 binding motif in \textit{S. octosporus} using a list of 227 conserved genes that are Mei4 targets in \textit{S. pombe} (see Chapter 5.4.8). This identified a seven base-pair (G/A)TAAA(C/T)A putative \textit{S. octosporus} FLEX motif. We then looked for this motif in intron 1 of the candidate \textit{wtf} orthologs and found that only 4 (\textit{wtf13}, \textit{wtf15}, \textit{wtf59}, and \textit{wtf81}) out of 48 of the intact \textit{wtf} candidate genes in \textit{S. octosporus} and one (\textit{wtf39}) out of 31...
intact wtf candidate genes in *S. osmophilus* lack the core seven base pair FLEX motif within intron 1 (Appendix A1-A2). In *S. cryophilus*, both intact wtf candidate genes contain the core FLEX motif in intron 1 (Appendix A3). Interestingly, most of the genes with the core FLEX motif also have an in-frame alternate translational start site near the beginning of exon 2, similar to the wtf drivers of *S. pombe* (Eickbush et al., 2019; Hu et al., 2017; Nuckolls et al., 2017). The two remaining intact wtf candidate genes, *S. osmophilus* wtf16 and *S. cryophilus* wtf2, appear analogous to the *S. pombe* suppressor wtf genes in that they are very similar to the drivers, but they lack the alternate translational start site in exon 2 (Figure 2.4) (Bravo Núñez et al., 2018a; Eickbush et al., 2019). We found no genes that appeared similar to the unknown class of *S. pombe* wtf genes (Table 2.2) (Bravo Núñez et al., 2020a; Eickbush et al., 2019).

The *S. pombe* wtf meiotic drivers and wtf drive suppressors are transcriptionally upregulated during meiosis (Kuang et al., 2017; Lock et al., 2018; Marguerat et al., 2012). The presence of the FLEX motif in the wtf candidate genes outside of *S. pombe* suggested these genes could also be expressed during meiosis. We tested this idea by further analyses of the *S. octosporus* long-read RNA sequencing data sets described above. We found that the candidate wtf genes were expressed during meiosis (Figure 2.4B, Figure 2.5, Appendix A1). In addition, our data show that there are two major transcripts for some of the candidate wtf genes. Analogous to *S. pombe* drivers, one transcript starts upstream of exon 1 and one transcript starts within intron 1 (Figure 2B, Figure 2.5) (Eickbush et al., 2019; Hu et al., 2017; Kuang et al., 2017; Nuckolls et al., 2017).
Figure 2.5 Transcription of predicted poison and antidote isoforms of *wtf* genes in *S. octosporus*.

Long-read (Oxford Nanopore) RNA sequencing reads from *S. octosporus* after 19 hours on sporulation media. All *wtf* genes are listed with the read count of the long isoform (predicted antidote) in grey and the short isoform (predicted poison) in black. The bold underlined genes were all analyzed by deletion (Figures 3.5 to 3.13) and predicted pseudogenes are shown in red. The read counts of the two isoforms can be found in Appendix A1. (Modified from Guo-Song Jia.)
Based on homology and the presence of the FLEX motif, we speculate that the candidate wtf genes of *S. cryophilus* and *S. osmophilus* are likely to have two transcripts and are likely to be meiotically expressed as well. We did not test this directly because protocols for efficient induction of meiosis in *S. cryophilus* and *S. osmophilus* have not yet been developed.

We conclude based on the amino acid conservation, the conserved gene structure, conserved promoter features, and transcriptional timing, that candidate genes are members of the wtf gene family. We, therefore, will henceforth refer to them as wtf genes.

### 2.4.3 wtf genes in *S. octosporus, S. osmophilus and S. cryophilus* are associated with distributed 5S rDNA sequences.

The *S. pombe* wtf genes derive their name from their association with the Tf transposon sequences (with Tf). Most *S. pombe* wtf genes are flanked on at least one side by a Tf LTR (long terminal repeat; Figure 2.6). There are over 200 Tf LTRs (averaging about 320 base pairs) throughout the *S. pombe* reference genome (Bowen et al., 2003). Tf transposons were previously discovered in *S. cryophilus* and we found them in our *S. osmophilus* assembly (Rhind et al., 2011). *S. octosporus*, however, does not contain recognizable Tf-derived sequences (Rhind et al., 2011). In *S. cryophilus*, there are 10 LTRs but none are associated with wtf genes. In *S. osmophilus*, there are 36 LTRs and four wtf genes are LTR-associated. (Appendix A.4)
The *wtf* genes are found in a limited number of genomic contexts. The *wtf* genes are represented as orange boxes, *wag* genes are in blue, and LTRs are in yellow. NA indicates not applicable as *wag* genes absent from *S. pombe* and LTRs are absent from *S. octosporus*.

Instead of close association with transposon sequences, we found that most the *wtf* genes outside of *S. pombe* are associated with distributed 5S rDNA genes (Figure 2.6). There are 112
5S rDNA genes in *S. octosporus* and 104 of them (92%) are associated with *wtf* genes. In *S. osmophilus*, 59 out of 107 5S rDNA genes (55%) that are associated with *wtf* genes. In *S. cryophilus*, there are 117 5S rDNA genes and 4 of them (3.4%) are associated with *wtf* genes (Table 2.3). When the *wtf* genes and 5S rDNA genes are immediately adjacent to each other, the distance between the genes is less than 1kb (Figure 2.7). In some cases, there is a gene from an uncharacterized gene family between the *wtf* gene and the 5S rDNA gene. We named this new gene family *wag* for *wtf*-associated gene. We found that the genomic context of *wtf* genes could be described by a limited number of patterns, including those first identified in *S. pombe* that are specific to that species (Figure 2.9). These patterns likely reflect a few genomic contexts that were duplicated multiple times during the expansion of the gene family as the intergenic sequences within a given type of *wtf*-5S rDNA or 5S rDNA-*wag*-wtf gene block are also highly similar within blocks of the same type within a species (Figure 2.8). The patterns of 5S rDNA regions between the different units show that non allelic gene conversion can occur with different block shared by different *wtf*-5S rDNA units (Figure 2.9).
Figure 2.7: Distance between 5S rDNA and wtf genes.

The distance in base pairs between 5S rDNA and the coding sequence of a wtf gene in (A) *S. osmophilus* and (B) *S. octosporus*. Only wtf genes with a flanking 5S rDNA were considered. The wtf gene is collapsed at 0 and the flanking sequences were considered in 100 base pair bins.
<table>
<thead>
<tr>
<th>Species</th>
<th>Number of 5S rDNA</th>
<th>Number of wtf loci associated with 5S rDNA</th>
<th>wtf genes associated with 5S rDNA</th>
<th>5S rDNA associated with wtf</th>
<th>wtf loci number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. octosporus</em></td>
<td>112</td>
<td>57 out 67 (85.1%)</td>
<td>72</td>
<td>104 (92%)</td>
<td>67</td>
</tr>
<tr>
<td><em>S. osmophilus</em></td>
<td>107</td>
<td>31 (81.6%)</td>
<td>33</td>
<td>59 (55.1%)</td>
<td>38</td>
</tr>
<tr>
<td><em>S. cryophilus</em></td>
<td>117</td>
<td>2 (40%)</td>
<td>2</td>
<td>4 (3.4%)</td>
<td>5</td>
</tr>
<tr>
<td><em>S. pombe</em></td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td><em>S. japonicus</em></td>
<td>7</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
<td>Na</td>
</tr>
</tbody>
</table>

Na is for Non applicable there is no *wtf* genes in *S. japonicus*.

Figure 2.8 Homology between distinct 5S rDNA-*wtf* units and *wag*-*wtf* units.
The regions containing \textit{wtf} genes with the indicated genetic contexts were aligned with MAFFT to find the percent sequences identity. The percent identity is shown in 50 base pair sliding windows. (A) The percent identity shared amongst 37 \textit{wtf-5S} rDNA units from \textit{S. octosporus}. (B) The percent identity shared amongst 17 \textit{wtf-wag} units from \textit{S. octosporus}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.9.png}
\caption{\textit{S. octosporus} \textit{wtf} gene units supported by maximum likelihood phylogeny.}
\end{figure}

The regions flanking the \textit{wtf} genes in \textit{S. octosporus} were sorted into the color-coded groups shown based on maximum phylogenies shown in Appendix A5 to A7. Orange boxes correspond to \textit{wtf} genes, the red boxes represent 5S rDNA genes, and the blue box represents a \textit{wag} gene. Genetic contexts lacking (A) and including a \textit{wag} gene (B) are shown.
2.4.4 *wtf* genes were likely present in the common ancestor of *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe*

We next wanted to determine if the *wtf* genes were present in the common ancestor of *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe*. The alternate hypothesis is that the *wtf* genes were more recently transferred between the species by horizontal gene transfer or by more recent introgression. Horizontal gene transfer does occur in fission yeast (Rhind et al., 2011), but the introgression hypothesis may be unlikely as the species are likely reproductively isolated (Jeffares et al., 2017; Seike et al., 2019, 2015; Sipiczki, 1979; Sipiczki et al., 1982).

If the gene family was vertically inherited from a common ancestor, it is possible that we find a syntenic *wtf* locus inherited from the ancestor in *S. pombe* and in at least one species from the *S. octosporus*, *S. osmophilus*, and *S. cryophilus* clade. Overall, synteny is limited between the four species (Rhind et al., 2011). We therefore manually inspected the genes flanking *S. pombe* *wtf* to look for orthologous gene pairs on the same loci of *wtf* in one or more additional species (Appendix A8). We found a *wtf* gene or genes between the *clr4* and *met17* genes in *S. octosporus*, *S. osmophilus*, and *S. pombe* (Figure 2.10.A). This shared synteny could reflect that the common ancestor contained a *wtf* gene between *clr4* and *met17*, but it could also mean that those loci were co-transferred with a *wtf* gene via horizontal gene transfer or introgression.
Figure 2.10 *wtf* genes are found at the same syntenic locus in three fission yeast species.

(A) The syntenic region between *clr4* and *met17* in *S. octosporus*, *S. osmophilus*, *S. cryophilus*, and *S. pombe* is shown. The *S. pombe* locus shown is from the *S. kambucha* isolate. The orange boxes represent *wtf* genes, the blue boxes represent *wag* genes, the red arrows represent 5S rDNA, the green arrow represents *trNA-his*, the grey boxes represent genes without a homolog in this region in the species shown and the black boxes represent genes that are syntenic between the species. The phylogenetic relationship between species is shown to the left of the DNA representation. The orthologs of *clr4* (B) and *met17* (C) were aligned and used to build neighbor-joining trees that were midpoint rooted with *S. japonicus* designated as the outgroup. Branch support (0-100) was calculated using bootstrap.

To distinguish these possibilities, we analyzed the divergence of the locus between the species. Superficially, the loci appear quite diverged, including gains and/or losses of additional genes in different lineages. This supports a long period of divergence between these loci that would...
be expected if the locus descended from the common ancestor of these species. We next analyzed the divergence more precisely. Given the extremely rapid evolution of the wtf genes (Eickbush et al., 2019), we thought the flanking genes would prove most informative. If the genes were recently transferred between lineages by horizontal gene transfer, it was possible there may be two copies of clr4 and/or met17 in the recipient genome. met17 has a paralogous (SPAC23A1.14c) found in S. pombe with a very low identity (20% with met17) that can be found in other fission yeast species. In any case, because of the high divergence between the two met17 paralogous we could easily discriminate between the different copies. No clr4 duplication with S. pombe was found. We also reconstructed phylogenies of the fission yeast clr4 and met17 genes and found that the gene trees matched the species trees (Figure 2.10B and 2.10C). If the genes had been transferred between species, for example from the lineage leading to S. pombe to the lineage leading to S. octosporus and S. osmophilus, the gene tree should reflect that pattern. In this example, the S. octosporus and S. osmophilus clr4 and met17 genes should group with the S. pombe genes on trees rather than with the S. cryophilus genes as we observed. In addition, the percent amino acid divergence we observed in pairwise comparisons between the orthologs revealed divergences like the average percent divergences between the species, except for met17 of S. octosporus, which may have gained an intron and diverged extensively (Figure 2.10.B). Together, our analyses are consistent with vertical transmission of clr4 and met17 and the wtf genes between them. This suggests the ancestor of S. octosporus, S. osmophilus, S. cryophilus, and S. pombe had a wtf gene between clr4 and met17 and that the wtf locus was lost in the lineage leading to S. cryophilus. We found additional shared synteny between S. pombe wtf6 and S. cryophilus wtf4 (figure 2.11). This suggests the ancestor of the species also contained a wtf gene at that locus, which was lost in the lineage leading to S. octosporus and S. osmophilus.
Figure 2.11 Synteny between S. cryophilus wtf4 and S. pombe wtf6.

(A) The syntenic region containing cyp9 and ago1 is show from all fission yeast species. An inversion in the S. pombe lineage separated cyp9 and ago1. There is a wtf gene upstream ago1 in both S. pombe and S. cryophilus. The orange boxes represent the wtf genes. Five genes are numbered and shown in green to illustrate that the ancestor of S. pombe and S. cryophilus likely had a wtf gene between cyp9 and ago1. The black boxes represent additional orthologous genes in synteny (modified from Guo-Song Jia). The orthologs of cyp9 (B) and ago1 (C) were aligned and used to build neighbor-joining trees that were midpoint rooted with S. japonicus designated as the outgroup. Branch support (0-100) was calculated using bootstrap.

There were additional cases where an S. pombe wtf gene was flanked on one side by a gene whose ortholog was also flanked by a wtf in one of the other species. We designate this partial
synteny. We found three *S. pombe* wtf loci (*wtf*27, the *wtf*30+*wtf*31+*wtf*10 locus, and *wtf*33 in the *S. kambucha* isolate) with partial synteny with *wtf* genes in *S. octosporus* (*wtf*4, *wtf*31 and *wtf*13) (Appendix A8). Amongst those three loci, two were also in partial synteny with *wtf* genes in *S. osmophilus* (*wtf*5 and *wtf*15). Altogether, our analyses indicate *S. pombe*, *S. octosporus*, *S. osmophilus*, and *S. cryophilus* inherited *wtf* genes from their common ancestor.

2.4.5  *wtf* genes show evolutionary signatures consistent with a history of genetic conflict

We next wanted to determine if the *wtf* genes are ancient meiotic drive genes or if the genes more recently acquired the ability to drive in the lineage leading to *S. pombe*. To address this, we first analyzed the evolutionary history of the gene family in more depth. Meiotic drivers are predicted to be rapidly evolving and the *S. pombe* wtf genes support this prediction (Eickbush et al., 2019; Hu et al., 2017; McLaughlin and Malik, 2017). This rapid evolution is thought to be driven by the genetic conflict predicted to exist between meiotic drivers and the rest of the genome. The conflict arises because the best interest of the driving haplotype (i.e., drive) is at odds with the best interest of the rest of the genome, which is Mendelian allele transmission (Crow, 1991). The driving haplotype gains an evolutionary advantage by driving, but this is generally bad for the fitness of the organism (Zanders and Unckless, 2019). The rest of the genome therefore gains an evolutionary advantage by suppressing drive. This thus leads to rapid evolution due to an evolutionary arms race between the drive locus and unlinked genomic suppressors where each side must constantly innovate (McLaughlin and Malik, 2017).

In *S. pombe*, the evolutionary innovation of *wtf* genes stem from gene duplications, expansion, and contraction of repetitive coding sequences, and extensive non-allelic gene conversion within the family (Eickbush et al., 2019; Hu et al., 2017). We looked for similar evidence of
rapid evolutionary innovation in the *wtf* genes outside of *S. pombe*. As a first step, we built a maximum likelihood phylogeny of intact *wtf* genes from all four species (Figure 2.12). For *S. pombe*, we used the genes from the FY29033 isolate, as it contains more intact *wtf* genes than the reference genome strain. We also excluded genes from the unknown functional class of *S. pombe* (*wtf7*, *wtf11*, *wtf14* and *wtf15*) because these genes are widely diverged from each other and all other *wtf* genes. We observed that the *S. pombe* *wtf* genes grouped together in a well-supported clade (Figure 2.12).

![Phylogenetic tree]

Figure 2.12: Gene duplication and non-allelic gene conversion within *wtf* gene family.
All the predicted intact Wtf antidote amino acid sequences were aligned using MAFFT from Figure 2.3 and used to build a maximum likelihood tree using PhyML. The *S. pombe* sequences were from the FY29033 isolate as it has more *wtf* genes than the reference genome. The *S. pombe* genes are shown in black, *S. octosporus* genes are in red, *S. osmophilus* genes are dark blue, and the *S. cryophilus* genes are cyan. The triangles represent multiple genes with the precise number indicated on the right. The branch support values (0-1) were calculated using aLRT SH-like and are shown at each node.

The genes from the other three species did not group together in supported clades that excluded genes from other species (Figure 2.12). The *S. cryophilus* genes were found distributed within clades of *S. osmophilus* genes. However, 37 *S. octosporus* genes grouped together in a well-supported clade. The remaining 11 *S. octosporus* genes grouped together within a well-supported clade that includes 2 *S. osmophilus* genes (Figure 2.12). Interestingly, this clade of 13 genes contains most (11/14) of the *S. octosporus* wag-associated intact *wtf* genes and two *S. osmophilus* genes in the clade are also wag-associated. Overall, these patterns are consistent with a history of species-specific duplications and non-allelic gene conversion, which we explore in more depth below.

We next explored the variation levels of gene duplication between lineages, or a more complex combination of gene gains and losses of *wtf* genes diverged from a common ancestor. To explore these possibilities, we first returned to our analyses of synteny. If gene loss was the predominant driver of variation in *wtf* gene number, we would expect to find that the *wtf* genes at a site within one or more additional species. Novel *wtf* gene duplications are more likely to be lineage specific. As described above, there are only five *wtf* loci found in *S. pombe* that are shared in at least one other species. Similarly, there are 32, 12, and 2, lineage-specific *wtf* loci in *S. octosporus*, *S. osmophilus*, and *S. cryophilus*, respectively (Appendix A9). These observations are consistent with novel gene duplications occurring in the lineages leading to all four species. Independent expansions are additionally supported by the different genomic
contexts of the *wtf* genes in *S. pombe* (Tf-association) and the other species (*wag* and or 5S rDNA-association). Gene losses are also likely within all lineages, as mentioned above, for the lost ancestral *wtf* locus between *met17* and *clr4* in *S. cryophilus*.

We next looked for signatures of non-allelic gene conversion within the newly discovered *wtf* genes. We started with genes found in synteny with a *wtf* gene in another lineage. These genes should be orthologous and group together in a well-supported clade. Non-allelic gene conversion, however, can overwrite genes and thus cause them to be more similar to different members of the gene family found at ectopic sites (Daugherty and Zanders, 2019). We focused on *S. octosporus*, and *S. osmophilus*, as those two species are most closely related and share 26 *wtf* loci. We found that none of the genes from syntenic loci group together with the orthologous locus. This suggests gene conversion has frequently over-written genes in one or both of those lineages.

We next analyzed all the genes within *S. octosporus* and *S. osmophilus* for signatures of gene conversion using the GARD (Genetic Algorithm Recombination Detection) program (Kosakovsky Pond et al., 2006a). This program builds multiple phylogenetic trees using different segments of genes. If the entire gene shares the same evolutionary history, the trees constructed from different parts of the genes should be the same. Ectopic gene conversion, however, can shuffle variation within a gene family and lead to differences between trees constructed from different parts of the genes. Consistent with the patterns described above, GARD detected evidence of non-allelic gene conversion within both *S. octosporus* and *S. osmophilus* (Figure 2.13, Appendix A10, and A11).
We used GARD (genetic algorithm for the detection of recombination (Kosakovsky Pond et al., 2006b)) analysis to look for evidence of gene conversion within the \textit{wtf} genes of (A) \textit{S. octosporus}, (B) \textit{S. osmophilus} and (C) \textit{S. pombe}. We considered only genes predicted to be meiotic drivers or suppressors. This analysis found that a hypothesis allowing multiple trees for different segments of the alignment is $>100$ times more likely than a hypothesis allowing only a single tree, supporting that non-allelic recombination has occurred within \textit{wtf} genes. The analysis identified two likely breakpoints in each species. For \textit{S. pombe} the analysis is from (Eickbush et al., 2019).

Finally, we looked for potential evolutionary innovation due to expansion and contraction of repetitive coding sequences in the newly identified \textit{wtf} genes. Exon 6 of some \textit{S. pombe} \textit{wtf} genes encodes a 7 amino acid sequence that can be repeated in tandem multiple times (Eickbush et al., 2019). A \textit{wtf} gene can drive without this sequence (Bravo Nunez et al 2020), but the number of repeat units found can be important for conferring specificity between a \textit{Wtf}^{poison} protein and the \textit{Wtf}^{antidote} protein that neutralizes it (Bravo Núñez et al., 2018a; Nuckolls et al., 2020). The sequence is thus important for functional innovation of drivers and suppressors. We looked for amino acid repeats in our candidate \textit{wtf} homologs and found a 7 amino acid sequence
that was repeated a variable number of times in tandem in exon 4 of genes from *S. octosporus* and *S. osmophilus*. We generated sequence logos to describe both the codons and amino acids underlying the repeat in both species (Figure 2.14, Appendix A12). We found that the repeat sequences were similar in all three species, consistent with shared ancestry. For example, the *S. pombe* and *S. osmophilus* repeats both have IGNXXNG as the most common amino acid sequence. So, like the *S. pombe wtf* drivers, the *wtf* drivers of *S. octosporus* and *S. osmophilus* show signatures of evolutionary innovation via expansion and contraction of a repetitive coding sequence. Together with previous analyses of *S. pombe*, our analyses demonstrate an extensive history of evolutionary innovation within the *wtf* genes. This is consistent with the hypothesis that these genes have a long history as meiotic drivers.
Figure 2.14: Contraction and expansion of repeat sequences in *wtf* genes.

The *wtf* genes of *S. octosporus (A)*, *S. osmophilus (C)*, and *S. pombe (E)* can contain the indicated repetitive sequences. The DNA (top) and amino acid (bottom) sequences logos representing the repeat regions are shown for each species. The size distribution of the repeat regions for all *S. octosporus (A)*, *S. osmophilus (C)*, and *S. pombe (E)* *wtf* genes is shown. The sizes are presented in base pairs instead of repeat units because the terminal repeats are not always full length. The *S. pombe* data are from (Eickbush et al., 2019). The repeat count in exon 4 of *S. octosporus wtf* genes and *S. osmophilus wtf* genes is shown in Appendix A12.

2.4.6 *wtf* genes duplicated to pre-existing 5S rDNA genes.

Given their association with distributed 5S rDNA genes, we hypothesized that the *wtf* genes in the lineages leading to *S. octosporus*, *S. osmophilus* and *S. cryophilus* could be duplicating to
pre-existing 5S rDNA genes. We propose two recombination models by which this could happen, ectopic gene conversion and integration of ectopic DNA circles (Figure 2.15A-2.15B) (Daugherty and Zanders, 2019). Under both models, lineage-restricted wtf loci associated with at least one 5S rDNA gene (e.g., Species A in Figure 2.15C) are predicted to be at loci containing a 5S rDNA gene in other species (e.g., Species B in Figure 2.15C). To test this, we first looked at sites where the *S. octosporus* and *S. osmophilus* locus contains a wtf gene, but the locus in *S. cryophilus* does not. There are 21 such wtf loci. In 86% (18 out of 21) of those sites, the *S. cryophilus* locus contains a 5S rDNA gene. We looked at 5S rDNA presence in all three species (*S. octosporus*, *S. osmophilus*, and *S. cryophilus*) with only *S. octosporus* having 5S rDNA wtf units, out of 12 loci 7 showed the presence of 5S rDNA without wtf genes. This is consistent with wtf genes duplicating adjacent to pre-existing 5S rDNA genes.
Figure 2.15: Model of spread of wtf genes by duplication to preexisting 5S rDNA genes.

(A) Model of duplication via non-allelic gene conversion: 1) A double strand DNA break (DSB) occurs near a 5S rDNA (red box). 2) The DSB is repaired via recombination with an ectopic locus containing 5S rDNA and a wtf gene. 3) The repair template containing the wtf gene is copied to the site of the initiating DSB. (B) Crossing-over between 5S rDNA repeats flanking a wtf gene can generate an extrachromosomal circular DNA. This circle that can recombine with ectopic locus containing a 5S rDNA to generate a new wtf locus. (C) Both models predict spread of wtf loci to preexisting 5S rDNA genes. This can be addressed by testing if lineage restricted wtf genes occur at sites where the ancestral species is inferred to have had a 5S rDNA gene. An example of this situation is illustrated in the phylogeny where species A has a wtf gene and species B has a 5S rDNA gene at the syntenic locus. (D) Table of number of wtf+5S rDNA loci species A (any of the gene layouts illustrated in (C) with 5S rDNA at the syntenic locus in species B. This analysis only considers wtf loci in species A where no wtf is found in species B.
We saw similar evidence of wtf gene duplication to pre-existing 5S rDNA genes when we considered lineage-specific wtf genes. We found that in 11 of 17 lineage-specific S. octosporus wtf loci associated with a 5S rDNA gene, the syntenic locus contains a 5S rDNA gene in S. osmophilus (Figure 2.15C). Similarly, 3 of 7 lineage-specific S. osmophilus wtf genes associated with 5S rDNA are at a site where the syntenic locus in S. octosporus contains a 5S rDNA gene (Figure 2.15C). Overall, these analyses support the hypothesis that wtf genes spread to pre-existing 5S rDNA genes in the lineages leading to S. octosporus and S. osmophilus. It is important to note, however, that lineage-specific loss of 5S rDNA-associated wtf genes could, and likely do, also contribute to the patterns described above even thought we did not detect the sequences. It is also possible that wtf genes could duplicate in sites without a homology with 5S rDNA but with their homology in the case of gene conversion that do not implicate dispersed repetitive elements or that the 5S rDNA was lost.

2.5 Discussion

2.5.1 wtf genes are ancient meiotic drivers.

Most meiotic drivers identified are predicted to exist for a short evolutionary period because of their detrimental effects on the organisms, and, they are species specific (Helleu et al., 2015). Even though meiotic drivers are predicted to have a short lifespan, some meiotic drivers can be found in different species as Dox and MDox have been found in Drosophila Mauritiania and D. simulans (Meiklejohn et al., 2018). Likewise, Spok genes in Podospora anserina are present in different filamentous fungi species (Grognet et al., 2014). Other meiotic drivers not yet identified are present for a long time in population like the SR in D. pseudoobscura that seem to have segregated for 1 Million year (Price et al., 2019). Here we show wtf genes in S. pombe to be present in Schizosaccharomyces species making, the wtf genes the oldest meiotic drive systems identified (119 MYA).
By combining sequencing and protein prediction tools, we identified *wtf* homologs in three other species: *S. octosporus*, *S. osmophilus*, and *S. cryophilus* (Figure 2.1; Figure 2.2). They have a similar exon-intron structure with two start sites that we predict to encode for antidote and poisons, like *S. pombe wtf* genes (Figure 2.4). The overall structure of *wtf* genes seem to be conserved amongst the four species.

### 2.5.2 *S. octosporus wtf* genes are transcriptionally active

Combining multi sequencing alignment and RNA-seq, Guo-Song Jia and I annotated *S. octosporus wtf* genes with more accuracy (Figure 2.4). Furthermore, the long-read RNA-seq datasets showed *S. octosporus wtf* genes expression during meiosis and expression of two transcripts one encoding the antidote, the other one encoding the poison similar to *S. pombe wtf* genes (Bowen et al., 2003; Kuang et al., 2017). Mei4 a transcription factor implicated in expression of early meiotic genes, binds a conserved FLEX motif on the promoter regions of meiotic genes (Alves-Rodrigues et al., 2016). *S. octosporus* and *S. osmophilus wtf* genes contain a FLEX motif in intron 1 allowing the binding of Mei4 and expression of *wtf* short transcript (poison) (Figure 2.4) (Nuckolls, unpublished). The presence of a FLEX motif and meiotic expression in *S. octosporus wtf* genes suggest that the poison transcription is regulated by a similar process, and it is an indication that *S. octosporus wtf* genes could be meiotic drivers. In any case, more molecular analysis will be necessary to show drive in *S. octosporus* with the possibility that the actual *wtf* genes are not meiotic driver in *S. octosporus* (Chapter 3).
2.5.3 Dispersed repetitive elements are associated with wtf genes.

Dispersed repetitive sequences are known to increase the frequency of non-allelic gene conversion (Eickbush and Eickbush, 2007; Krasileva, 2019). Dispersed repetitive elements are short sequences found in multiple copies in genomes. Repair seems to be the key for increase of decrease of the number of copies of those dispersed repetitive elements (Figure 2.15). For example, 5S rDNA has been shown to increase the possibility of replication fork stalling that cause breaks, that are then repaired which creates an increase number of copies of 5S rDNA in budding yeast (Hull et al., 2017; Salim et al., 2017). It has been shown that S. pombe wtf genes are associated with LTR sequences and that they are near meiotic double strand break hotspots (Bowen et al., 2003; Eickbush et al., 2019). During meiosis, double strand breaks (DSB) are introduced throughout the genome to allow homologous recombination. The repair process involves a search for homology found in the allelic site of the break. When the break occurs next to a dispersed repetitive element (LTR or 5S rDNA) the repair can involve non allelic locus and result in non-allelic gene conversion (Figure 2.15). In this case gene conversion can duplicate or lose DNA sequences. We found that S. octosporus wtf genes are associated with 5S rDNA another type of dispersed repetitive elements (Figure 2.6-2.7-2.9, Table 2.3). Dispersed repetitive elements could be hotspots of duplication events in fission yeast (see Chapter 4).

2.5.4 Common ancestor

The finding of wtf genes outside of S. pombe could mean wtf genes were present a common ancestor of S. pombe, S. octosporus, S. osmophilus, and S. cryophilus. The other hypothesis will be that wtf genes are the result of a horizontal gene transfer or an introgression event. Meiotic drivers are generally found to be species-specific though some exceptions like the Spok genes in Podospora anserina, that show possible horizontal gene transfer between different
filamentous fungi species (Groget et al., 2014). We rule out this hypothesis for \textit{wtf} genes because we can find one single event for the birth of meiotic driver (119 Mya), because of the presence of all the species in the lineages of \textit{S. pombe}, \textit{S. cryophilus}, \textit{S. octosporus}, and \textit{S. osmophilus}.

Introgression of meiotic drivers between species could be an explanation for the finding of \textit{wtf} genes in other fission yeast species. Introgression of the \textit{Dox} system between \textit{Drosophila simulans} and \textit{D. mauritiana} is due to gene flow between those different species (Meiklejohn et al., 2018). In regards of \textit{wtf} genes, gene flow seems rare and reproductive isolation between species is important (Seike et al., 2019; Tusso et al., 2019). There is one horizontal gene transfer identified between \textit{S. pombe} and \textit{S. octosporus} (Vassiliadis et al., 2020). We found two syntenic \textit{wtf} loci between the different species supporting the common ancestor hypothesis (Figure 2.10-2.11). Our syntenic analysis highlight some partial syntenies that have only one gene upstream or downstream in common between different \textit{wtf} genes loci. This would mean that \textit{wtf} genes were born \textit{de novo} in the lineage leading to \textit{S. pombe}, \textit{S. octosporus}, \textit{S. osmophilus}, and \textit{S. cryophilus}. Our extensive search in \textit{S. japonicus} were unfruitful which support the \textit{de novo} gene birth (See Chapter 5.4.3). However, we found many \textit{wtf} genes in synteny between \textit{S. osmophilus} and \textit{S. octosporus}. The limited number of loci in synteny could be understand by an increased number of chromosomal rearrangements between \textit{S. octosporus} and \textit{S. pombe}.

Furthermore, there is likely more synteny breaks between 5\textit{S} \textit{rDNA} in \textit{S. octosporus} for example than \textit{S. pombe} which could indicate that 5\textit{S} \textit{rDNA} sequences are near hotspots of recombination (Ács-Szabó et al., 2018). The association with LTR and 5\textit{S} \textit{rDNA} sequences could erase synteny by increase of the loss or duplication events in the different lineage. It is important to note that the divergence between \textit{S. octosporus} and \textit{S. pombe} is 119 Mya which is comparable to the divergence between humans and lancelets. This long time may have erase syntenies after each lineage has evolve for a so long evolutionary period.
2.5.5 Rapid evolution

Meiotic drivers are in a constant genetic arms race illustrated by the red queen hypothesis (Van Valen, 1973). The red queen hypothesis states that genes in conflict will be under positive selection, accumulating mutations to escape suppression or even fixation in a population. I show that *S. octosporus* and *S. osmophilus* wtf genes show signatures of rapid evolution like non-allelic gene conversion (Figure 2.1), lineage specific wtf genes (Appendix A9), and sequence repeats size variation between different wtf genes (Figure 2.14). GARD analyses shows that *S. octosporus* wtf and *S. osmophilus* wtf genes have a possible breakage in exon 2 which correspond to the first exon of the poison coding sequence, suggesting that poison suppression is targeted by the genome because change in the antidote sequence could cause a poison only allele that will never be observe in the population (all spores dead) (Figure 2.13, Appendix A10-A11). Repeats in the sequences of *S. pombe* wtf genes are important for the activity of the poison and the antidote (Bravo Núñez et al., 2018a). I observed similar repeats in *S. octosporus* and *S. osmophilus* wtf genes and size variation, suggesting that repeats in *S. octosporus* and *S. osmophilus* may have a similar function and are rapidly evolving to escape suppression. Those elements indicate rapid evolution of wtf genes in *S. octosporus* and *S. osmophilus*.

2.6 Summary

In chapter I, I prove that *S. octosporus* wtf genes are homologs of *S. pombe* wtf genes. They are similar in structure with 5 exons, two start sites, transcription during meiosis and show signatures of rapid evolution. wtf genes seem to have evolved differently between the *S. pombe* and the other clade (*S. octosporus*, *S. osmophilus* and *S. cryophilus*) spreading with a close
association with LTR or 5S rDNA, respectively. Further analysis will be needed to prove the increase of non-allelic gene conversion near those repeats. Our data suggest that the common ancestor of *S. pombe, S. octosporus, S. osmophilus*, and *S. cryophilus* had already a *wtf* genes in the genome. The idea of a meiotic driver exists over 119 Mya is striking and new. To fully answer that question, we need to answer if those meiotic driver in *S. octosporus* are actual meiotic driver or if they evolved another function.
3 Chapter 3: *wtf* genes cause meiotic drive outside of *S. pombe*

Parts of this chapter are in collaboration with Guo-Song Jia from the Li-Lin Du lab. The contributions are performing RNA-seq during meiosis of *S. octosporus* and generation of deletion in *S. octosporus*. 
CHAPTER 3: WTF GENES CAUSE MEIOTIC DRIVE OUTSIDE OF S. POMBE ........................................... 78

TABLE OF CONTENT CHAPTER 3 ........................................................................................................ 79

3.1 LIST OF FIGURES .......................................................................................................................... 80
3.2 LIST OF TABLES ............................................................................................................................ 80
3.3 INTRODUCTION ............................................................................................................................. 81
3.4 RESULTS ......................................................................................................................................... 83
  3.4.1 wtf genes in S. octosporus, S. osmophilus and S. cryophilus encode poison and antidote proteins. 83
  3.4.2 wtf genes seem to have high conservation of splicing site ......................................................... 88
  3.4.3 wtf genes cause meiotic drive when heterozygous in S. octosporus ......................................... 89
  3.4.4 S. octosporus wtf25 is a poison and antidote meiotic driver ..................................................... 100
3.5 DISCUSSION .................................................................................................................................. 102
  3.5.1 wtf genes are ancient meiotic drivers ..................................................................................... 102
  3.5.2 Other old drive systems (also multicopy) ................................................................................. 104
3.6 SUPPORTING INFORMATION ........................................................................................................ 106
3.1 List of Figures

Figure 3.1: *wtf* genes can encode for poison and antidote proteins. ........................................84
Figure 3.2: Some *wtf* genes outside of *S. pombe* encode for poison and antidote proteins. ...85
Figure 3.3: Non-cognate Wtf$^\text{antidotes}$ fail to rescue cells from Wtf$^\text{poisons}$ .........................86
Figure 3.4 *S. cryophilus* *wtf5* elongation of exon can decrease poison toxicity. .........................89
Figure 3.5: *wtf* genes are not required for meiosis or sporulation in *S. octosporus* ......................90
Figure 3.6: *S. octosporus* *wtf25* cause meiotic drive. .................................................................91
Figure 3.7: *S. octosporus* *wtf25* cause meiotic drive in a random spore assay similar to octad dissection.................................................................................................................................92
Figure 3.8: Heterozygous diploid spore viability of *wtf68*. ..........................................................94
Figure 3.9: Heterozygous diploid spore viability of *wtf33* ............................................................95
Figure 3.10: Heterozygous diploid spore viability of *wtf46* ..........................................................96
Figure 3.11: Heterozygous diploid spore viability of *wtf60*. ........................................................97
Figure 3.12 Heterozygous diploid spore viability of *wtf62* ...........................................................98
Figure 3.13: Heterozygous diploid spore viability of *wtf21* ........................................................99
Figure 3.14: *S. octosporus* *wtf25* is a poison-and-antidote spore killer. .....................................101

3.2 List of Tables

Table 3.1: Yeast strains used in this Chapter. ..................................................................................106
Table 3.2: Oligos used in this Chapter. .........................................................................................110
Table 3.3: Plasmid used in this Chapter. ......................................................................................119
3.3 Introduction

Meiosis is the biological process by which a diploid cell produces haploid cells called gametes. During this process, an allele presents on one chromosome will segregate away from the corresponding gene copy on the homologous chromosome. This process is generally assumed to be random, so each allele is seen at an equal frequency (50%) in the gamete pool. This equality of allele transmission was first noted by Gregor Mendel and was thought to be so universally true, it was dubbed “Mendel’s law of segregation.” However, Mendel’s law of segregation is not without its exceptions.

Some elements of the genome can promote their own transmission. That is the case of meiotic drivers that disturb meiosis to increase their own allelic transmission. Those selfish genetic elements, like meiotic drivers are detrimental for genomes, they decrease fertility, cause infertility, or result in species extinction (Hamilton, 1967; Helleu et al., 2015; Price and Wedell, 2008; Zanders and Unckless, 2019). Some meiotic drivers can increase their own transmission more than 50% in the viable gamete population by killing any gametes that do not inherit them, directly decreasing the fertility of the organism. An allele variant not linked to a given driver will be favored by selection when they suppress the driver. As for the meiotic driver, variants that escape from suppression are expected to spread in the population as they have a transmission advantage. This conflict of interest is predicted to create an evolutionary ‘arms race’ between the selfish gene and the rest of the genome. This genetic arms race is thought to promote rapid evolution of genes involved in meiotic drive. In this way, the genetic conflict between drivers and the rest of the genome can play a significant role in shaping genome evolution and in promoting speciation (Arvid Ågrenid et al., 2018; Bravo Núñez et al., 2020b; Jaenike, 2001; McLaughlin and Malik, 2017; Price et al., 2010; Rice, 2013; Van Valen, 1973)
Despite their harmful effects, meiotic drivers are observed in all the eucaryotic taxa from plants to mammals (Bravo Núñez et al., 2018b; Courret et al., 2019; Jaenike, 2001; Kruger and Mueller, 2021; Lindholm et al., 2016). For example, in *Drosophila simulans* the Paris Sex Ratio driver can be found on the X chromosome (X<sup>SR</sup>) in African populations. X<sup>SR</sup> prevents segregation of the Y chromosome sister chromatids during anaphase of meiosis II in males (Cazemajor et al., 2000). This disrupts the production of spermatids containing the Y chromosome leading to an increase in X-bearing sperm and causing males carrying the Sex ratio locus to father 90% female offspring.

Drivers are predicted to rapidly evolve but go extinct over short evolutionary timespans. This idea as not been yet tested because the majority of known meiotic drive systems are genetically complex and require many genes (Bravo Núñez, Nuckolls, & Zanders, 2018; Helleu, Gérard, & Montchamp-Moreau, 2015). In most cases, some or all the genes have not been identified. Even if drivers have a transmission advantage only slightly above 50%, they can spread in a population. If a meiotic driver spreads to fixation (i.e., the driver is shared by all the population), it may decay because it no longer confers a competitive advantage. If the meiotic driver is effectively suppressed, it may also decay by accumulating deleterious mutations or be lost by genetic drift (Helleu, Gérard and Montchamp-Moreau, 2015).

In Chapter 2, we found *wtf* genes homologs in, *S. octosporus, S. cryophilus, and S. osmophilus*. The presence of *wtf* genes in those species suggest that *wtf* genes were born in the common ancestor of *S. pombe, S. octosporus, S. cryophilus, and S. osmophilus*. It is possible that the ancestral function of *wtf* genes is not meiotic drive and in this case *S. octosporus wtf* genes may
not drive. To prove that \textit{wtf} are 119 Mya old meiotic driver genes, we show here that \textit{wtf} genes in all the species produces poison and antidote proteins in \textit{Saccharomyces cerevisiae}. Finally, we show that \textit{S. octosporus} \textit{wtf} genes drive when heterozygote in the endogenous species.

3.4 Results

3.4.1 \textit{wtf} genes in \textit{S. octosporus}, \textit{S. osmophilus} and \textit{S. cryophilus} encode poison and antidote proteins.

We next wanted to test if there was functional conservation between the \textit{wtf} genes. There are very few genetic tools available outside of \textit{S. pombe}, especially in \textit{S. osmophilus} and \textit{S. cryophilus}. We therefore tested the function of the genes outside of their endogenous species. We previously demonstrated that the \textit{S. pombe} \textit{Wtf}4\textsuperscript{poison} and \textit{Wtf}4\textsuperscript{antidote} proteins can act in the budding yeast, \textit{Saccharomyces cerevisiae}. Specifically, expression of \textit{Wtf}4\textsuperscript{poison} protein kills vegetative \textit{S. cerevisiae}, and co-expression of the \textit{Wtf}4\textsuperscript{antidote} neutralizes the toxicity of the \textit{Wtf}4\textsuperscript{poison} (Nuckolls et al., 2020). We used this system to test if the \textit{wtf} genes from the other fission yeast species also encode poison and antidote proteins.

Based on the \textit{S. pombe} \textit{wtf} drivers, we predicted that the \textit{wtf} genes encode a poison protein in exons 2-5 and an antidote protein in exons 1-5. We cloned the putative poison protein and antidote protein coding sequences of \textit{S. octosporus} \textit{wtf}25 and \textit{wtf}61, \textit{S. osmophilus} \textit{wtf}19 and \textit{wtf}41 and \textit{S. cryophilus} \textit{wtf}1 under the control of a \(\beta\)-estradiol-inducible promoter on separate plasmids. We then introduced the plasmids into \textit{S. cerevisiae} and analyzed the phenotypes of the resulting strains. We found that induction of each of the putative \textit{Wtf}\textsuperscript{poison} proteins, except \textit{S. osmophilus} \textit{wtf}19, caused cell death in \textit{S. cerevisiae} (Figure 3.1 A-C, Figure 3.2). Moreover, the toxicity of each functional \textit{Wtf}\textsuperscript{poison} protein was partially neutralized by co-expression of the cognate (i.e., encoded on the same gene) \textit{Wtf}\textsuperscript{antidote} proteins (Figure 3.1A-C; Figure 3.1B).
Figure 3.1: *wtf* genes can encode for poison and antidote proteins.

Spot assay of serial dilutions of *S. cerevisiae* cells on non-inducing (SC -His -Trp -Ura) and inducing (SC -His -Trp -Ura +500 nM β-estradiol) media. Each strain contains [TRP1] and [URA3] ARS CEN plasmids that are either empty (EV) or carry the indicated β-estradiol inducible *wtf* alleles. (A) *S. octosporus* *wtf25*<sup>poison</sup> -GFP and *wtf25*<sup>antidote</sup> -mCherry (B) *S. osmophilus* *wtf41*<sup>poison</sup> and *wtf41*<sup>antidote</sup>, and (C) *S. cryophilus* *wtf1*<sup>poison</sup> and *wtf1*<sup>antidote</sup>. The dilution factor is 0.2 starting at OD=1. (D) A cell carrying a [URA3] plasmid with β-estradiol inducible *S. octosporus* *wtf25*<sup>poison</sup> -GFP (green). (E) A cell carrying a [TRP1] plasmid with β-estradiol inducible *S. octosporus* *wtf25*<sup>antidote</sup> -mCherry (magenta). (F) A representative *S. cerevisiae* cell carrying a [URA3] plasmid with β-estradiol inducible *S. octosporus*
**wtf25**\textsuperscript{toxin}-**GFP** (green) and [**TRP1**] plasmid with β-estradiol inducible **S. octosporus wtf25**\textsuperscript{antidote}-mCherry (magenta). In all the experiments, the cells were imaged approximately 4 hours after induction in 500 nM β-estradiol. TL= transmitted light.

Figure 3.2: Some wtf genes outside of S. pombe encode for poison and antidote proteins.

Spot assay of serial dilutions of **S. cerevisiae** cells on non-inducing (SC -His -Trp -Ura) and inducing (SC -His -Trp -Ura +500 nM β-estradiol) media. Each strain contains [**TRP1**] and [**URA3**] ARS CEN plasmids that are either empty (EV) or carry the indicated β-estradiol inducible alleles. (A) **S. osmophilus wtf19**\textsuperscript{toxin} and wtf19\textsuperscript{antidote} (B) **S. octosporus wtf61**\textsuperscript{toxin} and wtf61\textsuperscript{antidote}. The dilution factor is 0.1 for (A) 0.2 for (B) with a starting OD=1 for both panels.

In **S. pombe**, Wtf\textsuperscript{antidote} proteins generally do not neutralize the Wtf\textsuperscript{toxin} proteins encoded by distinct drivers (Bravo Núñez et al., 2020b, 2018a; Hu et al., 2017). Instead, a high level of sequence identity appears to be required for Wtf\textsuperscript{antidote} proteins to co-assemble with and neutralize Wtf\textsuperscript{toxin} proteins (Nuckolls et al., 2020). We tested if this feature was shared with wtf genes outside of **S. pombe**. We tested proteins from five pairs of wtf genes. Excluding the antidote protein-specific residues encoded in exon 1, the proteins encoded by those genes share from 13-76% amino acid identity. Like our previous observations in **S. pombe**, we found that Wtf\textsuperscript{antidote} proteins did not neutralize non-cognate Wtf\textsuperscript{toxin} proteins (Figure 3.3A-E).
Figure 3.3: Non-cognate Wtf<sup>antidote</sup> fail to rescue cells from Wtf<sup>poison</sup>.

Spot assay of serial dilutions of <i>S. cerevisiae</i> cells on non-inducing (SC -His -Trp -Ura) and inducing (SC -His -Trp -Ura +500 nM β-estradiol) media. Each strain contains [TRP1] and [URA3] ARS CEN plasmids that are either empty (EV) or carry the indicated β-estradiol inducible Wtf<sup>poison</sup> and wtf<sup>antidote</sup> alleles. (A) <i>S. octosporus</i> wtf<sup>61</sup> and <i>S. osmophilus</i> wtf<sup>41</sup> (B) <i>S. cryophilus</i> wtf<sup>1</sup> and <i>S. osmophilus</i> wtf<sup>41</sup> (C) <i>S. octosporus</i> wtf<sup>61</sup> and <i>S. cryophilus</i> wtf<sup>1</sup> (D) <i>S. cryophilus</i> wtf<sup>1</sup> and <i>S. octosporus</i> wtf<sup>25</sup> and (E) <i>S. pombe</i> wtf<sup>4</sup> and <i>S. octosporus</i> wtf<sup>25</sup>. In C-E, the Wtf<sup>25poison</sup> protein was tagged with GFP and the Wtf<sup>25antidote</sup> protein was tagged with mCherry. The percent identity between the coding sequences of the wtf<sup>poison</sup> alleles and the percent amino acid identity shared by the Wtf<sup>poison</sup> proteins is shown at the top of each panel. The dilution factor for all plates is 0.2 starting at OD=1.
We imaged tagged versions of the *S. octosporus* Wtf25 proteins to see if the localization of the proteins *in S. cerevisiae* was like we previously observed for *S. pombe*. We found that *S. octosporus* Wtf25\textsuperscript{poison}-GFP and Wtf25\textsuperscript{antidote}-mCherry were both functional (Figure 3.1A). We observed *S. octosporus* Wtf25\textsuperscript{poison}-GFP and distributed throughout the cytoplasm, which is like what we previously observed for *S. pombe* Wtf4\textsuperscript{poison}-GFP (Figure 3.1D). The *S. octosporus* Wtf25\textsuperscript{antidote} largely localized within the vacuole, which is different from our previous observations with *S. pombe* Wtf4\textsuperscript{antidote} protein (Figure 3.1E). The *S. pombe* Wtf4\textsuperscript{antidote} was also trafficked to the vacuole, but that protein mostly accumulated outside the vacuole in the insoluble protein deposit, although we did observe some localization of Wtf4\textsuperscript{antidote} protein within the vacuole as well (Nuckolls et al., 2020).

When the *S. octosporus* Wtf25\textsuperscript{poison}-GFP and Wtf25\textsuperscript{antidote}-mCherry proteins were co-expressed, we observed some colocalization of the proteins (Figure 3.1F). The colocalized proteins appear to be trafficked to vacuole. The mCherry signal persists within the vacuole, whereas we do not detect GFP within the vacuole. We speculate that both proteins enter the vacuole, but that we do not observe the GFP because the fluorophore is more sensitive than mCherry to the acidity of the vacuole (Kimura et al., 2007; Shinoda et al., 2018). These localization patterns are like our previous observations of *S. pombe* Wtf4 proteins where the Wtf4\textsuperscript{antidote} co-assembles with the Wtf4\textsuperscript{poison} and causes a change of localization of the Wtf4\textsuperscript{poison} protein. With *S. pombe* Wtf4 proteins, however, the co-expressed poison and antidote proteins mostly accumulate outside the vacuole at the insoluble protein deposit, with less protein entering the vacuole (Nuckolls et al., 2020). Overall, our results are consistent with broad, but not absolute, functional conservation of the Wtf proteins, despite extensive amino acid divergence.
3.4.2  *wtf* genes seem to have high conservation of splicing site.

During our analysis, we notice that *S. cryophilus wtfs* was predicted to be a 4 exons coding gene by the Augustus prediction. After multi sequence alignment, I noticed a mutation in the 5’ splicing site with a TT instead of a GT nucleotide sequence. The splicing site mutation allows elongation of the fourth exon in the fourth introns until a stop codon at 47 bp (Figure 3.4A). *S. cryophilus* *wtf* 4 exons is toxic to the cells compared to the cognate antidote (Figure 3.4B). *S. cryophilus* *wtf* with 5 exons correspond to the construct if the splicing site was not mutated. In that case the poison kills more efficiently than *S. cryophilus* *wtf* 4 exons. No rescue is observed for *S. cryophilus* *wtf* 4 exons antidote of *S. cryophilus* *wtf* 4 exons poison. For *S. cryophilus* *wtf* 5 exons some rescue is observed from *S. cryophilus* *wtf* 5 exons antidote (Figure 3.4B). In Hu, et al. 2017, deletion of the last 10 amino acid of *S. pombe* *wtf* (from wild isolate CBS5556) causes *wtf* to lose is ability of poison but not its capacity of antidote. Here we show that the last exon (27 amino acids) may be important for the poison toxicity and maybe the poison and antidote interaction is between those 27 amino acids. The fact that poison toxicity is lost in the last 10 amino acid if deleted, it is possible that between 27-10 last amino acid an important poison region is responsible for the poison toxicity. Overall, this allele could help investigate *wtf* poison and antidote functions.
Figure 3.4 *S. cryophilus* wtf5 elongation of exon can decrease poison toxicity.

(A) Coding sequences of two predict *S. cryophilus* wtf1 annotation with 4 exons or 5 exons. *S. cryophilus* wtf5 as a mutation in exon 4 in a splicing site mutated AT to TT. This transversion elongate exon 4 coding sequences. In orange are the different exons from E1 to E5. *S. cryophilus* wtf5 4E is depicted with only 4 exons; *S. cryophilus* wtf5 5E is depicted with 5 exons. (B) Spot assay of serial dilutions of *S. cerevisiae* cells on non-inducing (SC -His -Trp -Ura) and inducing (SC -His -Trp -Ura +500 nM β-estradiol) media. Each strain contains [TRP1] and [URA3] ARS CEN plasmids that are either empty (EV) or carry the indicated β-estradiol inducible wtf alleles. The dilution factor is 0.2 starting at OD=1.

### 3.4.3 wtf genes cause meiotic drive when heterozygous in *S. octosporus*.

We next wanted to formally test if wtf genes could cause meiotic drive outside of *S. pombe* using *S. octosporus*. According to our RNA-seq data analysis, only a small fraction of wtf genes in *S. octosporus* have detectable levels of the short transcript isoform (poison isoform) initiated from within intron 1 (Figure 2.5). We preferentially tested those genes as we reasoned that expression of the poison would be essential for drive. We successfully deleted seven wtf genes in haploid strains of both mating types and analyzed whether any of the deletions affected viability of spores derived from homozygous and heterozygous crosses using octad dissection analysis (*S. octosporus* generates eight spores per meiosis due to a post-meiotic mitosis prior...
to spore packaging). The spore’s viability is measured by the formation of colonies after octad dissection.

Figure 3.5: wtf genes are not required for meiosis or sporulation in S. octosporus.

Heterozygous deletion but not homozygous deletion of wtf25, wtf68 or wtf33 in crosses caused significant spore viability loss. Spore viability was measured using octad dissection analysis (see methods). Representative octads are shown in Figure 9, Figure 9-supplements 1-6 and source data 1. Numerical data are provided in Table S30. p-values (Fisher’s exact test) for crosses with > 5% spore viability reduction compared to the wild-type control are shown. Guo-Song Jia data.

In homozygous crosses, none of the deletions significantly altered spore viability comparing to the wild-type control (Figure 3.5, Appendix A13). Thus, like previous observations in S. pombe, those seven wtf genes are not required for mating, meiosis, or sporulation in S. octosporus (Bravo Núñez et al., 2018a; Hu et al., 2017; Nuckolls et al., 2017). In heterozygous crosses, deletion of wtf25, wtf68 or wtf33 caused notable and significant spore viability reduction (> 5% spore viability reduction and p < 0.05, Fisher’s exact test) and resulted in significant allele transmission bias against the wtf deletion allele relative to the wild-type wtf+
allele ($p < 0.05$, exact binomial test; Appendix A13-A16, Figure 3.5-3.6-3.8-3.9-3.10). These results indicate that $wtf25$, $wtf68$, and $wtf33$ are active meiotic drivers.

Interestingly, we found that $wtf46$ and $wtf60$ genes also exhibited a weak but significant allele transmission bias in heterozygotes, but the spore killing was so weak the decrease in viability was not significant (Figure 3.5, Figure 3.6; Figure 3.8-3.9, Appendix A15-A17).

Figure 3.6: *S. octosporus* $wtf25$ cause meiotic drive.

(A) Representative octads dissected from asci produced from a $wtf25$ heterozygous diploid. The coordinates A to H indicate the 8 spores dissected from one ascus, rows 1 to 11 represent eleven octad asci analyzed. The genotypes of clones were determined by replica plating. Raw data of this figure can be found in source data 1. (B) The percentage of spores that were viable and with indicated genotypes produced by $wtf25^+/wtf25\Delta$ diploids. The $p$-value was calculated using exact binomial test and
Numerical data are provided in Table S31. (C) Classification of octads derived from \textit{wtf25}+/\textit{wtf25}Δ diploids according to the number of spores with and without a \textit{wtf} gene deletion. The \( p \)-values were calculated using an exact binomial test for the pairs of octad types with more than five total octads (a significant \( P \) value is not possible when the number of total octads of a pair of octad types \( \leq 5 \)). (D) Correlation between transmission distortion ratio and poison isoform expression level. The transmission distortion ratio represents the proportion of \textit{wtf} containing spores in total viable spores produced by a \textit{wtf}+/\textit{wtf}Δ heterozygote and the read count of the putative poison isoform was calculated according to the classification in Figure 2.5. Numerical data of transmission distortion ratio of each \textit{wtf} gene can be found in Appendix A14. Guo-Song Jia data.

Another way to find distortion bias is to perform a random spore assay where all the spores are recovered from a cross. After many tests, we succeeded in performing this assay in \textit{S. octosporus} (see Chapter 5 Material and methods). In this assay, we cross \textit{S. octosporus} \textit{wtf25}Δ with \textit{wtf25}+ strain. Depending on the position of the antibiotic marker, 1kb upstream gene or 1 kb downstream of the \textit{S. octosporus} \textit{wtf25} genes; we found a difference between 64.6 \% and 70.2\% \textit{wtf25}+ allele transmission from diploid \textit{wtf25}Δ/\textit{wtf25}+, respectively (Figure 3.7). Similar transmission bias to \textit{S. octosporus} \textit{wtf25}+ seems to be observed with the tetrads dissection by Guo-Song Jia (66.5\%).

Figure 3.7: \textit{S. octosporus} \textit{wtf25} cause meiotic drive in a random spore assay like octad dissection.

Allelic transmission assays from heterothallic strains of \textit{S. octosporus}. Cross (1) has a clonNat upstream of \textit{S. octosporus} \textit{wtf25} in allele1, in allele 2 \textit{wtf25}Δ is deleted with G418 resistance gene (\textit{kanMx}). Cross...
(2) has a clonNat downstream of \textit{S. octosporus wtf25} in allele 1, in allele 2 \textit{wtf25}Δ is deleted with G418 resistance gene (\textit{kanMx}). Cross (3) has a clonNat upstream of \textit{S. octosporus wtf25} in allele 1, in allele 2 wild type allele. Cross (4) has a clonNat downstream of \textit{S. octosporus wtf25} in allele 1, in allele 2 wild type allele. We sporulated on PMG media and performed a modified random spore analysis (Smith, 2009). The expected percentage of each spore genotype and the observed percentages are shown, as well as the raw data (*=p < 0.0001, G-test; NS = not significant). Numerical data of transmission distortion ratio of each \textit{wtf} gene can be found in Appendix A14.

To further explore the octad dissection data, we classified octads derived from heterozygous crosses according to the number of viable spores with a \textit{wtf} gene deletion (“R”, antibiotic resistant) and the number of viable spores without a \textit{wtf} gene deletion (“S”, antibiotic sensitive) in an octad. For example, an octad with 7 viable spores can be classified as either the 4R3S type or the 3R4S type. If spore viability is not affected by a \textit{wtf} gene deletion, the ratios of 4R3S to 3R4S, 4R2S to 2R4S, 4R1S to 1R4S, and 4R0S to 0R4S should be about 1:1. For \textit{wtf25}, \textit{wtf68}, and \textit{wtf33}, the three genes deemed as active meiotic drivers based on the analysis of overall spore data, most of these octad type ratios significantly deviate from 1:1 (\(P < 0.05\), exact binomial test; Figure 3.6, Figure 3.8-3.9). The 4R3S to 3R4S ratio of \textit{wtf46} and the 4R2S to 2R4S ratio of \textit{wtf60} also significantly deviate from 1:1 (Figure 3.10-3.11), suggesting that \textit{wtf46} and \textit{wtf60} have weak spore killing activities. \textit{Wtf62 (SOCG_04077)} and \textit{wtf21 (SOCG_02322)} did not cause significant deviation of octad type ratios (Figure 3.12-13), consistent with the low expression levels of the putative poison isoforms of these two genes (Figure 2.5). In fact, the levels of allele transmission favoring the \textit{wtf+} allele appear to be correlated with the expression levels of the putative poison isoforms (Figure 3.6D).
Figure 3.8: Heterozygous diploid spore viability of wtf68.

(A) Representative octads dissected from asci produced from a wtf68 heterozygous diploid. The coordinates A to H stands for 8 spores dissected from one ascus, rows 1 to 11 represent 11 octad asci analyzed. The genotypes of clones were determined by replica plating. (B) The percentage of spores that were viable and with indicated genotypes in wtf68+/wtf68Δ diploids. The p-value was calculated using exact binomial test and numerical data are provided in Appendix A15. (C) Classification of octads derived from wtf68+/wtf68Δ diploids according to the number of spores with and without a wtf gene deletion. The p-values were calculated using an exact binomial test for the pairs of octad types with more than five total octads (a significant P value is not possible when the number of total octads of a pair of octad types ≤ 5).
Figure 3.9: Heterozygous diploid spore viability of *wtf33*

(A) Representative octads dissected from asci produced from a *wtf33* heterozygous diploid. The coordinates A to H stands for 8 spores dissected from one ascus. Rows 1 to 11 represent 11 octad asci analyzed. The genotypes of clones were determined by replica plating (B) The percentage of spores that were viable and with indicated genotypes in *wtf33*+/*wtf33*Δ diploids. The p-value was calculated using exact binomial test and numerical data are provided in Appendix A17 (C) Classification of octads derived from *wtf33*+/*wtf33*Δ diploids according to the number of spores with and without a *wtf* gene deletion. The p-values were calculated using an exact binomial test for the pairs of octad types with more than five total octads (a significant P value is not possible when the number of total octads of a pair of octad types ≤ 5). Guo-Song Jia data.
Figure 3.10: Heterozygous diploid spore viability of wtf46

(A) Representative octads dissected from asci produced from a wtf46 heterozygous diploid. The coordinates A to H stands for 8 spores dissected from one ascus rows 1 to 11 represent 11 octad asci analyzed. The genotypes of clones were determined by replica plating (B) The percentage of spores that were viable and with indicated genotypes in wtf46+/wtf46Δ diploids. The p-value was calculated using exact binomial test and numerical data are provided in Appendix A18 (C) Classification of octads derived from wtf46+/wtf46Δ diploids according to the number of spores with and without a wtf gene deletion. The p-values were calculated using an exact binomial test for the pairs of octad types with more than five total octads (a significant P value is not possible when the number of total octads of a pair of octad types ≤ 5). Guo-Song Jia data.
Figure 3.11: Heterozygous diploid spore viability of wtf60.

(A) Representative octads dissected from asci produced from a wtf60 heterozygous diploid. The coordinates A to H stands for 8 spores dissected from one ascus rows 1 to 11 represent 11 octad asci analyzed. The genotypes of clones were determined by replica plating. (B) The percentage of spores that were viable and with indicated genotypes in wtf60+/wtf60Δ diploids. The p-value was calculated using exact binomial test and numerical data are provided in Appendix A19. (C) Classification of octads derived from wtf60+/wtf60Δ diploids according to the number of spores with and without a wtf gene deletion. The p-values were calculated using an exact binomial test for the pairs of octad types with more than five total octads (a significant P value is not possible when the number of total octads of a pair of octad types ≤ 5). Guo-Song Jia data.
Figure 3.12 Heterozygous diploid spore viability of wtf62

(A) Representative octads dissected from asc us produced from a wtf62 heterozygous diploid. The coordinates A to H stands for 8 spores dissected from one asc us rows 1 to 11 represent 11 octad asci analyzed. The genotypes of clones were determined by replica plating. (B) The percentage of spores that were viable and with indicated genotypes in wtf62+/wtf62Δ diploids. The p-value was calculated using exact binomial test and numerical data are provided in Appendix A20 (C) Classification of octads derived from wtf62+/wtf62Δ diploids according to the number of spores with and without a wtf gene deletion. The p-values were calculated using an exact binomial test for the pairs of octad types with more than five total octads (a significant P value is not possible when the number of total octads of a pair of octad types ≤ 5). Guo-Song Jia data.
Figure 3.13: Heterozygous diploid spore viability of wtf21

(A) Representative octads dissected from ascci produced from a wtf21 heterozygous diploid. The coordinates A to H stands for 8 spores dissected from one ascus rows 1 to 11 represent 11 octad asci analyzed. The genotypes of clones were determined by replica plating. (B) The percentage of spores that were viable and with indicated genotypes in wtf21+/wtf21Δ diploids. The p-value was calculated using exact binomial test and numerical data are provided in Appendix A21 (C) Classification of octads derived from wtf21+/wtf21Δ diploids according to the number of spores with and without a wtf gene deletion. The p-values were calculated using an exact binomial test for the pairs of octad types with more than five total octads (a significant P value is not possible when the number of total octads of a pair of octad types ≤ 5). Guo-Song Jia data.
3.4.4 S. octosporus wtft25 is a poison and antidote meiotic driver

To determine whether an active wtft gene in S. octosporus can cause spore killing at an ectopic genomic locus, we constructed an integrating plasmid carrying a 2.5-kb genomic region containing wtft25 together with its upstream and downstream flanking 5S rDNA genes and integrated the plasmid at the leu1 locus in the wtft25 deletion background. Octad dissection analysis indicated that wtft25 integrated at the leu1 locus can act as a meiotic driver in a heterozygous cross (leu1Δ::wtft25/leu1) and the level of meiotic drive was comparable to that caused by the endogenous wtft25 gene (Figure 10B, left panel). This result indicates that wtft25 integrated at leu1 locus can act as a model for detailed dissection of molecular mechanism of S. octosporus wtft genes.

wtft25 can express a long transcript isoform and a short transcript isoform through alternative transcriptional initiation (Figure 2.4B). Based on the S. pombe wtft genes and our analyses in S. cerevisiae (Figure 3.1), we hypothesized that the long and short isoforms encode antidote and poison proteins, respectively (Hu et al., 2017; Nuckolls et al., 2017). We introduced point mutations into the predicted start codons of the long and short isoforms of wtft25 integrated at the leu1 locus and analyzed the effects of the mutations on spore killing. The mutation of the predicted start codon in the short transcript isoform (ATG to GCG, methionine to alanine) referred to as wtft25antidote-only, was unable to kill spores not inheriting it in a wtft25 deletion background (Figure 10A and 10B, right panel, Table S38). This supports our hypothesis that the short transcript encodes a spore-killing poison.
Figure 3.14: *S. octosporus* wtf25 is a poison-and-antidote spore killer.

(A) Schematic of the wtf25 alleles integrated at the *leu1* (SOCC_02003) locus. The black stars indicate start codon mutations. The start codon for the putative *wtf25\textsuperscript{poison}* coding sequence is mutated in the *wtf25\textsuperscript{antidote-only}* allele and the start codon for the putative *wtf25\textsuperscript{antidote} coding sequence is mutated in the *wtf25\textsuperscript{poison-only}* allele. (B) The wild-type *wtf25* allele integrated at the *leu1* locus can act as a spore killer while *wtf25\textsuperscript{antidote-only}* mutant allele integrated at the same locus was unable to kill spores not inheriting it in heterozygous cross. P values were calculated with a binomial test of goodness-of-fit (C) The *wtf25\textsuperscript{poison-only}* allele integrated at *leu1* can cause self-killing in spores that do not inherit wild-type *wtf25* at the endogenous locus. The effects of the *wtf25\textsuperscript{poison-only}* allele were compared to a control cross in which an empty vector was integrated at *leu1*. Numerical data are provided, and the p value is calculated in Appendix A22. Guo-Song Jia data.
Analogously, we mutated the predicted start codon in the long transcript isoform and generated the \textit{wtf25}^{poison-only} mutant allele (Figure 3.13A). We could not obtain transformants with a plasmid carrying this mutant allele in the \textit{wtf25} deletion background, possibly due to self-killing. Instead, we integrated the plasmid at the \textit{leu1} locus in the wild-type background and crossed the resulting strain to a \textit{wtf25}Δ strain. As a control, we integrated an empty vector at the \textit{leu1} locus in the wild-type background and crossed the resulting strain to a \textit{wtf25}Δ strain. Compared to the control, \textit{wtf25}Δ spores (spores not inheriting the wild-type \textit{wtf25} at the endogenous locus) suffered markedly more severe viability loss and the \textit{wtf25}Δ spores that also inherited the \textit{wtf25}^{poison-only} mutant allele at the \textit{leu1} locus were all inviable (Figure 3.13C). These results further support the model that the short isoform encodes a poison protein that confers killing but not protection. In addition, they demonstrate that the long isoform is required for protection against spore killing.

This data confirms the role of \textit{S. octosporus} \textit{wtf} genes to be active meiotic drivers in \textit{S. octosporus}.

### 3.5 Discussion

#### 3.5.1 \textit{wtf} genes are ancient meiotic drivers

In Chapter 2, I show that \textit{wtf} genes are present in \textit{S. octosporus}, \textit{S. osmophilus}, and \textit{S. cryophilus}. In this chapter Guo-Song Jia and I demonstrate that \textit{wtf} genes can act as meiotic drivers when heterozygous. \textit{S. octosporus} \textit{wtf} genes encode for both poison and antidote proteins, like \textit{S. pombe} \textit{wtf} genes, to increase the allelic frequency in the population. However, the allelic bias toward \textit{S. octosporus} \textit{wtf} genes seem lower than \textit{S. pombe} \textit{wtf} genes (70% against 90%) ([Hu et al., 2017; Nuckolls et al., 2017]). Even using two different assays to access allelic transmission, we saw that the drive of \textit{S. octosporus} \textit{wtf25} is between 64% and 70%.
Viability of the heterozygous diploid, *S. octosporus* wtf25+/wtf25Δ is 68.7% corresponds to 5.5 out of 8 spores survive after meiosis, meaning 1.6 spores did not inherit the *wtf* allele and still survive the poison toxicity. Contrary, for *S. pombe* wtf4 0.2 spores that survive without inheriting the *wtf* allele with a drive of 90%. The difference in spores *wtf*- that survive could be possible because *S. octosporus* produce between 4 and 8 spores depending of the media use to induce meiosis (Seike and Niki, 2017). To induce meiosis in *S. octosporus* we use PMG plates, which allow more than 70% of cells to produce between 7 to 8 spores (Seike and Niki, 2017). The octad dissection perform by Guo-Song Jia was only including 8 spores (~70% of total spores produced). My random spore assay allows a broader selection of spores from all the asci (from tetrad to octad), yet the rate of allelic transmission for wtf25- is similar (around 70%). Furthermore, Guo-Song Jia shows that there is a positive correlation between expression and allelic bias in *S. octosporus* wtf genes (figure 3.6). That could mean expression is a key factor in *S. octosporus* wtf genes poison toxicity in regards. Additionally, it is not excluded that an ascus of 8 spores is more challenging in the spread of the toxic molecules.

We notice that *wtf* genes were present in more copies in *S. octosporus* than *S. pombe* genome. *S. pombe* wtf genes are mainly presented in the third chromosome. The presence of *wtf* genes in the third chromosome is thought to be the consequence of *wtf* genes competition when outcrossing. When *wtf* genes compete, selection favors disomic chromosomes with two copies of chromosome 3. Those two copies can carry different *wtf* genes that will encode both poison and antidote proteins. Those proteins could maintain two copies in the spores. Once the spores germinate, the cell can lose one of the two copies of the chromosome three. Chromosome 1 and 2 cannot be disomic in spores (Niwa et al., 2006). In *S. octosporus*, it is unclear if disomes can be found for all three chromosomes.
Drivers are expected to be short lived because of their detrimental effects. Evolution of suppression to counteract meiotic drive is predominant for many driver systems (Carvalho and Vaz, 1999; Jaenike, 2001). \textit{wtf} genes suppressors are \textit{wtf} genes that lost the poison activity and only produce an antidote that could share high amino acid identity to rescue a ectopic poison toxicity (Bravo Núñez et al., 2018a). Our data suggest that transcription of \textit{wtf} genes is an important factor of the drive (increases allelic transmission). Interestingly, in \textit{S. octosporus}, they are only a subset of poison transcribe (Figure 2.5, Figure 3.6). This implicates that decrease poison expression could be a suppression mechanism. Strong drivers like \textit{S. octosporus wtf}25, is going against a global suppression mechanism may appeared over 119 million years. Because \textit{wtf} genes are so different suppression mechanism of all the copies could be costly for the genome.

3.5.2 Other old drive systems (also multicopy)

Knobs are heterochromatic regions regulated by kinases in Maize. The drive is then characterized by a bias of segregation for the neocentromere that will be found in almost all progenies. Ab10 is found in the genomes of \textit{Zea mays} and other teosinte which have diverged 12 MYA from \textit{Sorghum}. The knob genes Ab10 show meiotic drive in the two species (Dawe et al., 2018; Kanizay et al., 2013). Interestingly, Kanizay and colleagues found that the frequency of Ab10 was 75\% in the teosinte population which show an increase in frequency of the Ab10 chromosome compared to McClintock B, Yamakake T, (1981) for which Ab10 was found at 35 \% in the population. Increase in frequency of the Ab10 chromosome may indicate that Ab10 is a meiotic driver in those other wild maize species, but not formal driver proof has been shown yet. Furthermore, many copies of Ab10 are found (Ab10-I, Ab10-II and Ab10-III),
making of the Knobs genes in Maize a multicopy drive family that may have been around for more than 12 Mya, but formal proof has not been shown yet. The different copies of Ab10 could be the result of hybridization between maize species.

Spok genes in *Podospora anserina* have been shown to cause meiotic drive (Grognet et al., 2014; Van Der Gaag et al., 2000; Vogan et al., 2019). *spok* genes have been found outside of *Podospora* in other taxa (Grognet et al., 2014). *spok* genes are scattered throughout filamentous fungi, but the phylogeny does not seem to be consistent with one single origin of *spok* genes because many species do not carry *spok* genes. The presence with the *spok* genes of a transposon called *Entreprise* that may favor horizontal gene transfer between filamentous fungi species (Vogan et al., 2021).

*wtf* genes in *Schizosaccharomyces* persist for longer period of times than the dogma of drive evolution. Association of *wtf* genes with repetitive elements may allow *wtf* genes to expand in genomes by duplication. Contrary to other drive system for which the age is difficult to predict, the shared ancestry of *wtf* genes indicates that poison and antidote meiotic driver can persist for 119 million years.
### Table 3.1: Yeast strains used in this Chapter.

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<td>this study</td>
</tr>
<tr>
<td>Code</td>
<td>Strain</td>
<td>Genotype</td>
<td>Study</td>
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<td>DY47919</td>
<td><em>Schizosaccharomyces octosporus</em></td>
<td>$h^- mat2\Delta &amp; mat3\Delta::bleMX6 \text{wtf68(SOCG_01240)}\Delta::kanSVEM</td>
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<tr>
<td>DY47920</td>
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<td><em>Schizosaccharomyces octosporus</em></td>
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<td>DY47908</td>
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<td>this study</td>
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<td>DY47921</td>
<td><em>Schizosaccharomyces octosporus</em></td>
<td>$h^- mat2\Delta &amp; mat3\Delta::bleMX6 \text{wtf60(SOCG_04742)}\Delta::kanSVEM</td>
<td>this study</td>
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<td>DY47922</td>
<td><em>Schizosaccharomyces octosporus</em></td>
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<td>this study</td>
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<td>DY47923</td>
<td><em>Schizosaccharomyces octosporus</em></td>
<td>$h^- mat2\Delta &amp; mat3\Delta::bleMX6 \text{wtf62(SOCG_04077)}\Delta::kanSVEM</td>
<td>this study</td>
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<td>DY47924</td>
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<td>this study</td>
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<td>DY47925</td>
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<td>this study</td>
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<td>DY47926</td>
<td><em>Schizosaccharomyces octosporus</em></td>
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<td>this study</td>
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<tr>
<td>DY47931</td>
<td><em>Schizosaccharomyces octosporus</em></td>
<td>$h^+ mat2\Delta &amp; mat3\Delta::hphMX6 \text{wtf25(SOCG_04480)}\Delta::kanSVEM</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{leu1(SOCG_02003)}\Delta::wtf25(SOCG_04480)\Delta::natMX$</td>
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<td>DY47932</td>
<td><em>Schizosaccharomyces octosporus</em></td>
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<td>this study</td>
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<td>DY47933</td>
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<td>this study</td>
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<td>SZY4611</td>
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<td>this study</td>
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<td>Oligos</td>
<td>Sequence</td>
<td>Description</td>
<td>Reference</td>
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<tr>
<td>oGS-1</td>
<td>TTTTGAAATTTAAGTGGATTTGATTCTGTCA</td>
<td>Mating type PCR primer for <em>S. octosporus</em> from Niki’s 2017 paper (Universal primer)</td>
<td>(T. Seike and H. Niki, 2017)</td>
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<td>oGS-2</td>
<td>CTTGACACACTTCGACAGGTATCT</td>
<td>Mating type PCR primer for <em>S. octosporus</em> from Niki’s 2017 paper (Specific primer for h+)</td>
<td>(T. Seike and H. Niki, 2017)</td>
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<tr>
<td>oGS-3</td>
<td>TCCTCTTCCAAGTTGACCAACCAG</td>
<td>Mating type PCR primer for <em>S. octosporus</em> from Niki’s 2017 paper (Specific primer for h-)</td>
<td>(T. Seike and H. Niki, 2017)</td>
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<td>oGS-24</td>
<td>CCTGTAAGTGATTGAAACTCTTCAGT</td>
<td>wrf25(SOCG_04480) knockout cassette upstream-arm forward primer</td>
<td>This study</td>
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<tr>
<td>oGS-26</td>
<td>aggggtgagttggtttcagCCAGCTAAGCCGATGCTTTTCAAT</td>
<td>wrf25(SOCG_04480) knockout cassette upstream-arm reverse primer (overlap with P495)</td>
<td>This study</td>
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<tr>
<td>oGS-27</td>
<td>CCGATTACGAGTGGATAATCTT</td>
<td>wrf25(SOCG_04480) knockout downstream-arm reverse primer</td>
<td>This study</td>
</tr>
<tr>
<td>oGS-29</td>
<td>gggtaatttgcagttgcccGAGGCTCTCTCGAGCGTAGG</td>
<td>wrf25(SOCG_04480) knockout</td>
<td>This study</td>
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Table 3.2: Oligos used in this Chapter.
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<tr>
<th>Primer Code</th>
<th>Oligo Sequence</th>
<th>Description</th>
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<tr>
<td>oGS-30</td>
<td>GTCTGTGCAAGACAGAAATAGACG</td>
<td>downstream-arm forward primer (overlap with P496)</td>
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<tr>
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<td>wrf46(SOCG_00084) knockout upstream-arm forward primer</td>
<td>This study</td>
</tr>
<tr>
<td>oGS-31</td>
<td>agggttgagtgttccagCCGTTTAGAAGCTTACACTCATATG</td>
<td>knockout upstream-arm reverse primer (overlap with P495)</td>
</tr>
<tr>
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<td>wrf46(SOCG_00084) knockout downstream-arm forward primer (overlap with P495)</td>
<td>This study</td>
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<tr>
<td>oGS-32</td>
<td>ggttaatttgcctggccgCCAGCCTCTTTGATCATTTCAAG</td>
<td>knockout downstream-arm forward primer (overlap with P495)</td>
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<tr>
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<td>wrf46(SOCG_00084) knockout downstream-arm reverse primer</td>
<td>This study</td>
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<tr>
<td>oGS-33</td>
<td>CTCTGTGATGAATGATGAACTGAGGT</td>
<td>knockout downstream-arm reverse primer</td>
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<td>wrf46(SOCG_00084) knockout downstream-arm reverse primer</td>
<td>This study</td>
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<tr>
<td>oGS-39</td>
<td>GCCATGTTCAGAACAACACTCT</td>
<td>Verification primer F on 5’ end of kan-marker CDS region</td>
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<tr>
<td>oGS-40</td>
<td>GCAGTTTCATTTGATGCTCGAT</td>
<td>Verification primer R on 3’ end of kan-marker CDS region</td>
</tr>
<tr>
<td>oGS-69</td>
<td>TGCCCCCGAATATGCAAAGT</td>
<td>Forward primer for amplifying wrf25(SOCG_044 80) gene locus (including flanking 5S rDNA) from genomic DNA</td>
</tr>
<tr>
<td>oGS-70</td>
<td>CCTCGGAAAAGTCTGACGCT</td>
<td>Reverse primer for amplifying wrf25(SOCG_044 80) gene locus (including flanking 5S rDNA) from genomic DNA</td>
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<tr>
<td>OGS-109</td>
<td>TGCCATTGCAAAAgcGAAGAATAAATAC</td>
<td>In order to introduce MtoA mutation at the start codon of wtf25(SOCG_04480) antidote protein, this is the forward primer to amplify the target sequence. Mutation is designed on the primer.</td>
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<tr>
<td>OGS-110</td>
<td>GTATTTATCTgctTTTGCAATGGCA</td>
<td>In order to introduce MtoA mutation at the start codon of wtf25(SOCG_04480) antidote protein, this is the reverse primer to amplify the target sequence. Mutation is designed on the primer.</td>
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<tr>
<td>OGS-111</td>
<td>TGATTTAGAAgcGCTGATGGATC</td>
<td>In order to introduce MtoA mutation at the start codon into wtf25(SOCG_04480) poison protein, this is the forward primer to amplify the target sequence. Mutation is designed on the primer.</td>
</tr>
<tr>
<td>OGS-112</td>
<td>GATCCATCAGGgcTTCTAAATCA</td>
<td>In order to introduce MtoA mutation at the start codon into wtf25(SOCG_04480) poison protein, this is the reverse primer to amplify the target sequence.</td>
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<td>oGS</td>
<td>Sequence</td>
<td>Knockout Cassette</td>
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<td>gaatcagcaatagagtttgtg</td>
<td>wrf21(SOCG_02322) knockout cassette</td>
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<td>upstream-arm forward primer</td>
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<td>124</td>
<td>aggg tgtgagttggtg</td>
<td>wrf21(SOCG_02322) knockout cassette</td>
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<td>upstream-arm reverse primer (overlap with P495)</td>
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<td>125</td>
<td>ggttaatttcgagctttgctg</td>
<td>wrf21(SOCG_02322) knockout downstream-arm</td>
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<td>forward primer (overlap with P496)</td>
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<td>126</td>
<td>cattgtataatcaaggtgtgca</td>
<td>wrf21(SOCG_02322) knockout downstream-arm</td>
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<td>141</td>
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<td>wrf60(SOCG_04742) knockout cassette</td>
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<td>upstream-arm forward primer</td>
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<td>aggg tgtgagttggtg</td>
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<td>upstream-arm reverse primer (overlap with P495)</td>
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<td>143</td>
<td>ggttaatttcgagctttgctg</td>
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<td>forward primer (overlap with P496)</td>
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<td>144</td>
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<td>wrf60(SOCG_04742) knockout downstream-arm</td>
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<td>oGS-147</td>
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<td>oGS-148</td>
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<td>oGS-149</td>
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<td>oGS-150</td>
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<td>oGS-192</td>
<td><code>CGAATAAGAGTTGcggccgcGTAGTGTAACTTTTGCTAGATGTT</code></td>
<td>forward primers for amplification of leu1 locus 5’ fragment sequence, with NotI enzyme cutting site and 15bp overlapping sequence with oGS-195 added</td>
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<td>oGS-193</td>
<td><code>cgggtatgcaaaTTATGTTCTTGttACTCGAAGTTT</code></td>
<td>reverse primers for amplification of leu1 locus 5’ fragment sequence, with 15bp overlapping with backbone-1 from pAV0854</td>
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<td>oGS-195</td>
<td><code>gcggccgaACTTCCATTTTCGCGTAAATA</code></td>
<td>reverse primers for amplification of leu1 locus 3’ fragment sequence, with NotI enzyme</td>
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<tr>
<td>oGS-197</td>
<td>t cacacgccccagCAAGTAACAGTTTACGCTATG</td>
<td>cutting site added</td>
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<td>forward primers for amplification of leu1 locus 3' fragment sequence, with 15bp overlapping with backbone-2 from pAV0854</td>
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<td>oGS-201</td>
<td>gtaagaagctctctctgtgatcg</td>
<td>wt<a href="SOCG_01240">68</a> knockout cassette upstream-arm forward primer</td>
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<td>oGS-202</td>
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<td>wt<a href="SOCG_01240">68</a> knockout cassette upstream-arm reverse primer (overlap with P495)</td>
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<td>oGS-203</td>
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<td>wt<a href="SOCG_01240">68</a> knockout downstream-arm forward primer (overlap with P496)</td>
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<td>oGS-204</td>
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<td>wt<a href="SOCG_01240">68</a> knockout downstream-arm reverse primer</td>
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<td>oGS-219</td>
<td>ggcattcttagctcattgac</td>
<td>wt[33] knockout cassette upstream-arm forward primer</td>
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<td>oGS-220</td>
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<td>wt[33] knockout cassette upstream-arm reverse primer (overlap with P495)</td>
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<td>oGS-221</td>
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<td>wt[33] knockout downstream-arm forward primer (overlap with P496)</td>
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<td>oGS-222</td>
<td>ctaactaaatgactactctgttc</td>
<td>wt[33] knockout downstream-arm reverse primer</td>
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<td>Forward Primer for Amplifying kanSVEM Targeting Cassette</td>
<td>Reverse Primer for Amplifying kanSVEM Targeting Cassette</td>
<td>Reverse Oligo to Clone Octosporus S. octosporus wtf61 (SOCG_044114)</td>
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<td>oGS-271 ctggaaacacactaaccctatctcgg</td>
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<td>This study</td>
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<td>oGS-272 cgccaagctgaaattacccctcac</td>
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<td>This study</td>
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<td>1442 ATTATGGGCCGCTCGGCC TCA AAC TTC AGC GCT TTG CCC AGC AAA AG</td>
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<td>This study</td>
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<td>1432 ATTATTCCTGAG ATGGCTGATGGATCTGCTCAGCATTC</td>
<td>Forward oligo to amplify SOCG_04114 wtf61</td>
<td>This study</td>
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<tr>
<td>1195 tatatgtaccGCGCCCATCCAGTTTAAC</td>
<td>Forward primer to amplify lexA boxes with KpnI Site</td>
<td>(Nucholls et al., 2020)</td>
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<tr>
<td>1240 tatatctcagTATCGAATTCTGCAGCCCG</td>
<td>Reverse primer to amplify lexA boxes with XhoI Site</td>
<td>(Nucholls et al., 2020)</td>
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<td>2011 ATTATTCCTGAG ATGAAGATAATTACACTCCAGTTTCAG</td>
<td>Forward to amplify exon 1 of SOCG_04114 with XhoI Tails</td>
<td>This study</td>
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<td>2170 AAATAGGATCC CAAATTAAGCCCTCGAGCG</td>
<td>Reverse oligo cycT with BamHI Tails</td>
<td>This study</td>
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<tr>
<td>2194 AGCTACTTACCATGGGGCCGCTAAACCTCACGCCTTTTGCC</td>
<td>Reverse primer of S. octosporus wtf61 antidote with cycT Tails</td>
<td>This study</td>
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<tr>
<td>2195 GGCAAAGGGCTGAAGTTTGAAGGCCCCTGAGTAAGTAGCT</td>
<td>Forward primer of cycT with S. octosporus wtf61 Antidote Tails</td>
<td>This study</td>
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<tr>
<td>2277 aaatctTCGAG ATGGCTCCTGAACAGAAAGAAAGT</td>
<td>Forward to amplify gBlock Poison SPOG_03611 and SPOG_01632 with XhoI Tails</td>
<td>This study</td>
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<tr>
<td>2278 AGCTACTTACCATGGGGCCGCTAAACATTAGGCCCCCTTTTGCT</td>
<td>Reverse to amplify gBlock SPOG_03611 wtf1 with cycT Tails</td>
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<td>2279 AGCAAGGGCTAATGTTTGAGGCCCCATGGTAAAGTAGCT</td>
<td>Forward to amplify gBlock cycT Tails</td>
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<td>2276</td>
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<td>Forward to amplify gblock SPOG_03611 with XhoI tails</td>
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<td>2278</td>
<td>TGAAATAGGAGTGTATTTTTCATGTATCGAATTCCTGCAGCCCCGGGGGA</td>
<td>Reverse amplify LexA with S. osmophilus wtf42 971g antidote tails</td>
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<tr>
<td>2279</td>
<td>TCCCCGGGGCTGCAAGGAATTCATGAGAAAAATAATACACTCTCCTATTTCA</td>
<td>Forward S. osmophilus wtf42 971g antidote with LexA tails</td>
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<tr>
<td>2280</td>
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<td>Reverse amplify S. osmophilus wtf41 with cycT tails</td>
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<tr>
<td>2281</td>
<td>TGCTGAGCAAGCGCTGAATTACCGCCCCCATGGTAAAGTAGCT</td>
<td>Forward amplify cycT with S. osmophilus wtf41 tails</td>
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<tr>
<td>2282</td>
<td>AATTCTGAGCGGATCCATCAGCATATCGAATTCCTGCAGCCCCGGGGGA</td>
<td>Reverse amplify LexA with S. osmophilus wtf41 971g poison tails</td>
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<tr>
<td>2283</td>
<td>TCCCCGGGGCTGCAAGGAATTCATGACATGGCAGATCGCCTGCAGCA</td>
<td>Forward S. osmophilus wtf41 971g poison with LexA tails</td>
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<tr>
<td>2668</td>
<td>TGCTGAGGGATCCATCAGCATATCGAATTCCTGCAGCCCCGGGGGA</td>
<td>Rererse amplify LexA with SOCG_04480 S. octosporus wtf25 poison tails</td>
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<tr>
<td>2669</td>
<td>TCCCCGGGGCTGCAAGGAATTCATGAGATGGCATGCGCTCGTCGA</td>
<td>Forward amplify SOCG_04480 S. octosporus wtf25 poison with LexA tails</td>
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<td>2830</td>
<td>AATTCTCAGCTTTAGACATACCCACCCACCAACCAACTTCAGCCCCCTTGTTCAGCA</td>
<td>Rererse amplify SOCG_04480 S. octosporus wtf25 poison with linker and yeGFP tails</td>
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<tr>
<td>Sequence</td>
<td>Description</td>
<td>References</td>
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<td>TGCTGAGCAAGGGGCTGAAGTTGTCGTCGGTGCATTGCTGCTGCTAAGGGTGAAGCTGAAGTTGGTGGTGGTGGTGGTGGTGGTATGGTGAGCAAGGGCGAGGA</td>
<td>Forward amplify linker and yeGFP with SOCG_04480 S. octosporus wtf25 poison tails</td>
<td>This study</td>
</tr>
<tr>
<td>CGGTAGCTACTTACCATTGGGCCGGCTTATTGTACAATTCAATTAACCACCA</td>
<td>Reverse amplify yeGFP with cycT tails</td>
<td>This study</td>
</tr>
<tr>
<td>TGCTGAGCAAGGGGCTGAAGTTGGTGGTGGTGGTGGTGGTATGGTGAGCAAGGGCGAGGA</td>
<td>Forward amplify cycT with yeGFP tails</td>
<td>This study</td>
</tr>
<tr>
<td>AACAGGACT GTATTTATTCTCATGTATCGAATTTCTCGACGCGCCGGGGGA</td>
<td>Reverse to amplify LexA boxes with SOCG_04480 S. octosporus wtf25 tails</td>
<td>This study</td>
</tr>
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4 Chapter 4: Discussion and future directions

4 CHAPTER 4: DISCUSSION AND FUTURE DIRECTIONS .................................................................121

4.1 List of Figures ..........................................................................................................................122

4.2 Key Conclusions .....................................................................................................................123

4.2.1 wtf genes found outside of S. pombe ..............................................................................123

4.2.2 wtf active meiotic drivers over 119 Mya ......................................................................126

4.3 Future Directions ..................................................................................................................127

4.3.1 New meiotic drivers in fission yeast .............................................................................127

4.3.2 Investigating meiotic drive mechanism .......................................................................128

4.3.3 Measuring fitness cost of wtf genes and inbreeding in other fission yeast species .........129

4.3.4 Repeat facilitated expansion of the wtf gene family ....................................................130

4.4 Outlook: Model for the long-term persistence of wtf drivers ............................................135

5.1 Summary ..............................................................................................................................138
4.1 List of Figures

Figure 5.1: Model for long-term persistence of \textit{wtf} meiotic drivers. ........................................ 125

Figure 5.2: \textit{wtf} genes are unlikely to form extrachromosomal circles. ................................. 132
4.2 Key conclusions

Meiotic drivers favor their own transmission to the progeny at the detriment of the host genome (Sandler and Novitski, 1957; Zimmering et al., 1970). On the contrary, in genomes selection will favor genes that will suppress drivers (Jaenike, 2001). This tug of war between genomes and meiotic drivers is known as genetic conflict, a conflict that involves an ongoing battle. Meiotic drivers are thought to exist for a short evolutionary period because of this genetic conflict (Burt and Trivers, 2006). The difficulty to identify meiotic drivers and the lineage specificity of most known drivers contribute to the evolutionary dogma of meiotic drivers. The examples of meiotic drivers found in different species seems to be more and more common with the new sequencing era. Sequencing of different isolates or different species in different taxa gives insight to the evolution of meiotic drivers under suppression, decay, and fixation, often causing driver extinction.

My thesis work demonstrates that meiotic drivers can exist for longer evolutionary timespan (>119 Mya). Although wtf genes have been identified in *S. pombe*, I found wtf genes homologs in three other species: *S. octosporus*, *S. osmophilus*, and *S. cryophilus*. Guo-Song Jia and I show that wtf genes in *S. octosporus* are actively driving when heterozygous.

4.2.1 wtf genes found outside of *S. pombe*

*S. pombe* wtf genes are fission yeast meiotic drivers that kill spores that do not inherit them. *wtf4*, one of the first meiotic driver identified in fission yeast encode a poison, killing the spores that do not inherit the *wtf4* allele, and an antidote that protect the spores that inherit the *wtf4* allele. Both poison and antidote proteins are encoded by one gene with two start sites. The long transcript allows to encode for an antidote, the short one encodes the poison protein. I
have identified \textit{wtf} genes, present in \textit{S. pombe}, in three other \textit{Schizosaccharomyces} species. We identified 83 copies of \textit{wtf} genes in \textit{S. octosporus}, 42 copies in \textit{S. osmophilus} and 5 copies in \textit{S. cryophilus}. The newly identified \textit{S. octosporus}, \textit{S. osmophilus}, and \textit{S. cryophilus} \textit{wtf} genes share similar features with \textit{S. pombe} \textit{wtf} genes. Amongst those features we found \textit{wtf} genes have 5 exons, two start sites, and the presence of a FLEX motif, which, like \textit{S. pombe}, allow the binding of Mei4 and activation of poison transcription (Nuckolls, et al. unpublished).

Transcription during meiosis occurs for \textit{S. octosporus} \textit{wtf} genes with detection of a short and long isoform (poison and antidote). \textit{wtf} (\textit{with Transposon Fission yeast}) genes are associated with LTR in \textit{S. pombe}, in the other species \textit{wtf} genes are also associated with another type of dispersed repetitive elements.

Gene conversion within \textit{S. pombe} \textit{wtf} genes occurs (Eickbush et al., 2019). Our analysis shows that gene conversion is also present in \textit{S. octosporus}, \textit{S. osmophilus}, and \textit{S. cryophilus} \textit{wtf} genes too, which is a hallmark of rapid evolution. We found two possible breakages of sequences within the coding region of \textit{S. octosporus} and \textit{S. osmophilus} \textit{wtf} genes. Those breakages were determined by phylogenetic analysis where each part of the gene have a different phylogeny than the full-length gene. This phenomenon called incongruence support the idea of gene conversion amongst \textit{wtf} genes (Figure 2.13). Gene conversion may allow spread of mutations between \textit{wtf} genes that may increase or decrease the drive activity (i.e. increase allelic transmission).
The presence of dispersed repetitive elements like LTRs (yellow) or 5S rDNA (red) associated with \textit{wtf} genes (orange) may facilitate duplication of the genes to novel sites in the genome by recombination mechanisms schematized in Figure 6. Non-allelic gene conversion and expansion/contraction of repeat elements can also fuel the birth and rejuvenation of \textit{wtf} meiotic drivers allowing them to avoid fixation, suppression, mutational decay and, ultimately, extinction.

Like for \textit{S. pombe} \textit{wtf} genes, we observe a repeat number variation of \textit{S. octosporus} and \textit{S. osmophilus} \textit{wtf} exon 4 (figure 2.14). This extension and contraction of repeats is another hallmark of rapid evolution and has been shown to be important in the \textit{S. pombe} \textit{wtf} genes suppression. The same number of repeats and high identity of the C-terminal region between the poison or the antidote is necessary to rescue the poison toxicity. The identification and characterization of new \textit{wtf} genes homologs outside of \textit{S. pombe} show signs of rapid evolution. This rapid evolution is in concordance with genes in conflict that will be under positive selection.
4.2.2  *wtf* active meiotic drivers over 119 Mya

*wtf* genes encode both poison and antidote proteins. Because we found limitations working in *S. cryophilus*, *S. octosporus* and *S. osmophilus*, using budding yeast allowed to test the function of *wtf* genes in a more tractable organism. *Saccharomyces cerevisiae* and *S. pombe* share a common ancestor 400 million years ago (Hoffman et al., 2015). Expression of *S. pombe* Wtf4\textsuperscript{Poison} in budding yeast kills the cells (Nuckolls et al., 2020). Using budding yeast, we show other fission yeasts species *wtf* genes encoding poison and antidote proteins in an ectopic species. Furthermore, Wtf\textsuperscript{Antidote} can only rescue the cognate Wtf\textsuperscript{Poison} because very high identity in the C-terminal region. It is not unlikely that a Wtf\textsuperscript{Antidote} from non-cognate could rescue the poison toxicity but that could be only for *wtf* suppression (Bravo Núñez et al., 2018a). For instance, *S. pombe* wtf18-2 is an antidote only (does not express a poison protein) that suppresses the activity of *S. pombe* wtf13 drive. Between *S. pombe* wtf18-2 and wtf13 changing two amino acid causes the antidote only (*S. pombe* wtf18-2) to not rescue the drive of *S. pombe* wtf13 (Bravo Núñez et al., 2018a). In the case of *S. pombe*, antidote only *wtf* genes have lost the second start site which allows them to produce a poison protein (Eickbush et al., 2019). Interestingly *S. octosporus* RNA-seq data shows expression of poison proteins for a handful of drivers, whereas other *wtf* genes considered meiotic drivers do not seem to encode a poison (Figure 3.2). Variation of poison expression may indicate a suppression mechanism of drive by transcriptional repression. Epigenetics mutations, controlled expression, and dosage sensitivity as already been shown important in other meiotic drive systems (Brand et al., 2015; Dawe et al., 2018; Kruger et al., 2019; Shen et al., 2017). Finally, Guo-song Jia and my analysis shows *S. octosporus* wtf25+ to cause meiotic drivers when heterozygous (*wtf25+/wtf25Δ*). The transmission bias by *S. octosporus* wtf25+ was lower than the drive observed for *S. pombe* wtf4. Furthermore, the separation of function performed by Guo-Song Jia show that *S. octosporus*
wtf25 encode a poison and antidote proteins, which confirms the mechanism of poison and antidote to bias allelic transmission in the *S. octosporus* genome. (Figure 3.1 and 3.6).

4.3 Future directions

The identification of new meiotic drivers in fission yeast brings new genes to study in future research. More than 80 possible meiotic drivers are predicted to encode both poison and antidote proteins. The long timespan in which wtf genes have been evolving in four different species is an opportunity to study the fitness effects of drive in genomes, the expansion of wtf genes, and the mechanism of poison and antidote.

4.3.1 New meiotic drivers in fission yeast

The identification of *S. octosporus*, *S. osmophilus*, and *S. cryophilus* wtf genes are an opportunity to study a large variety of poison and antidote proteins. Isolates of *S. pombe* have a variable number of wtf genes between 25 and 38 genes, we expect to observe this variability in the isolates of *S. octosporus* and *S. osmophilus*. Between the different wtf syntenic loci in *S. octosporus* and *S. osmophilus*, we observed different numbers of wtf genes. Sequencing other *S. octosporus* and *S. osmophilus* isolates may show gains and losses of wtf genes. Varied number of wtf genes is a hallmark of rapid evolution. Furthermore, phylogeny of all intact wtf genes (Figure 2.12) show the rapid evolution of wtf genes with *S. pombe* wtf and *S. octosporus* wtf genes that are in lineage-specific clusters.
4.3.2 Investigating meiotic drive mechanism

The 83 predicted meiotic drivers amongst all species offers a new possibility of studying a multitude of poison and antidote proteins. For now, we know that wtf genes in *S. octosporus*, *S. osmophilus*, and *S. cryophilus* encode poison and antidote proteins, but the interactions between the different proteins are not well known. *wtf* genes produce a variety of the same type of proteins (poison and antidote), but they show low amino acid identity when aligned. Studying *wtf* genes sequence variability in the context of drive could identify important features of drive (i.e., increase allelic transmission). Many factors can change the drive between *wtf* genes. Level of expression and poison toxicity are two of them. *wtf* genes do not drive at the same level (figure 3.6 D). Some like, *S. pombe* *wtf4*, drive at 92.8% whereas *S. octosporus* *wtf25* drive at ~70% ((Nuckolls et al., 2017), Chapter 3). The difference of the allelic transmission bias between *S. octosporus* *wtf25* and *S. pombe* *wtf4* indicate that the meiotic process may be different between *S. pombe* and *S. octosporus*. *S. octosporus* produces 8 spores instead of 4; more spores could decrease the amount of poison toxicity within an ascus, allowing an increased survival rate of *S. octosporus* spores. To produce 8 spores, *S. octosporus* meiosis includes a post meiotic mitosis. The expression timing is unknown, so having tag proteins of *S. octosporus* *wtf* genes will help understanding of when and how poison and antidote proteins are expressed. Additionally, the level expression seems to be an important factor of drive where the transmission bias seems to correlate with the poison expression (Figure 3.5). Many *S. octosporus* *wtf* genes predicted meiotic drivers do not show expression of the second transcript isoform (poison). Regulation at the expression level could indicate a mechanism of drive suppression in *S. octosporus* that will be further studied by switching different promoters and expressing *S. octosporus* *wtf* genes in *S. pombe*. I tried to express different *S. octosporus* *wtf* genes with their endogenous promoters by introduction in *S. pombe*. However, no drive was observed, which could indicate that the poison is not express nor the
antidote. Mei4 seems to be implicated in the expression of both poison proteins in *S. octosporus* and *S. pombe*. Mei4 between *S. octosporus* and *S. pombe* could be different because the two species have diverged 119 Mya ago. Switching *S. octosporus* *wtf* genes poison promoters and *S. pombe* *wtf* promoter and testing drive in *S. pombe* could show that *S. octosporus* *wtf* genes are still meiotic driver in *S. pombe* only the poison regulation has change. A budding yeast expression screen could identify the amino acid that makes a better driver (increase of poison toxicity). Even though the sequence identity between different *wtf* genes is low (Figure 2.2), the presence of disordered and transmembrane domains could identify critical features for poison toxicity; antidote rescue; or even poison-antidote, poison-poison, or antidote-antidote interaction.

### Measuring fitness cost of *wtf* genes and inbreeding in other fission yeast species

Meiotic drivers are expected to have a fitness cost associated with their detrimental effects in genomes (Zanders and Unckless, 2019). Meiotic drivers favor selection in meiotic genes that will increase the positive evolution of meiosis (Bravo Núñez et al., 2020b), and more importantly, they decrease fertility. In heterozygous crosses, we observe a decrease of spore viability indicating a reduction of viable spores in the gamete population. To study the fitness effects of meiotic drive in fission yeast, we could delete the only *S. cryophilus* *wtf1* meiotic driver. The experimental evolution under competition (with a strain with *wtf* gene) could measure the cost of having a driver in a population. The issue is induction of *S. cryophilus* meiosis has been challenging, and that could indicate, if a species does not undergo meiosis, the number of *wtf* genes could be lower than a species that mates, which allows the spread of *wtf* genes.
The fitness cost of \textit{wtf} genes in fission yeast may favor alleles that will increase inbreeding to reduce the spread of \textit{wtf} genes in a population (López Hernández et al., 2021). The capacity of inbreeding within wild isolates varied. \textit{S. pombe} lab strain seems to inbreed more than wild isolate \textit{S. kambucha} that favor outcrossing. In \textit{S. pombe}, inbreeding slows down the increase of \textit{wtf} gene frequency in the population but \textit{wtf} genes act when heterozygote and will have an advantage if it outcrosses. We do not know if \textit{S. octosporus} or/and \textit{S. osmophilus} outcross. Further evolutionary experiment will be needed to measure inbreeding in those species to determine if the evolution of \textit{wtf} genes in \textit{S. octosporus} is like \textit{S. pombe} \textit{wtf} genes.

4.3.4 Repeat facilitated expansion of the \textit{wtf} gene family

Our results indicate that the ancestor of the four species contained at least 2 \textit{wtf} genes, and the extant species all carry between 5-67 \textit{wtf} loci, including pseudogenes. Our analyses are consistent with novel gene duplications occurring in the lineages leading to all four species. The \textit{wtf} genes are compact and autonomously cause drive. These features likely facilitated their spread within genomes. We previously proposed that the expansion of \textit{wtf} genes on the lineage leading to \textit{S. pombe} was promoted by non-allelic gene conversion between Tf transposon sequences (Eickbush et al., 2019; Hu et al., 2017). LTR sequences in \textit{S. pombe} are found in one case of extrachromosomal DNA with a duplication of the gene \textit{sod2} (Albrecht et al., 2000). \textit{Sod2} is implicated in the transport of \textit{Na}^+(\textit{Li}^+). Increase \textit{LiCl} concentration will select for \textit{S. pombe} cells that have duplicated the \textit{sod2} gene. The newly duplicated gene is found near a LTR which may have favorized the duplication event.

Specifically, DNA double-strand breaks near a Tf LTR could be repaired using a non-allelic \textit{wtf}-associated LTR as a template and generate a copy the \textit{wtf} gene in the process. This
template could be chromosomal or present as an extrachromosomal circle. The later pathway was recently implicated in the spread of Rsp-like meiotic drive associated sequences in Drosophila species (Sproul et al., 2020).

In Chapter 3, we show that gene conversion may be the predominant mechanism by which wtf genes duplicate in genomes. We still investigate the possibility of extrachromosomal circle formation. For that we use different pairs of oligos upstream and downstream of a wtf81-wag20 unit (Figure 5.1A) and wtf72 (Figure 5.1 B). None of the PCR reaction shows a product when we use reverse oligos. Even though this does not disprove that wtf genes may be able to form extrachromosomal DNA, we think that this may be unlikely. The newly formed wtf gene duplicates could be maintained at a high rate by selection given their potential to cause drive or to suppress drive of other wtf genes with a similar sequence. Dispersed repetitive elements like LTR, 5S rDNA or satellite DNA seem to have an important role in gene duplication in many organisms (Eickbush and Eickbush, 2007; Krasileva, 2019). Interestingly 359bp repeats from the satellite repeat family 1.688 has been shown to be associated with driver genes in D. simulans (Muirhead and Presgraves, 2021; Vedanayagam et al., 2021). The association of repeats with the Dox (Distorter on the X chromosome) could be responsible for the duplication of driver within the simulans clade (D. mauritiana, D. sechellia and, D. simulans).
Figure 4.2: *wtf* genes are unlikely to form extrachromosomal circles.

PCR to verify the hypothesis that *wtf* genes and 5S rDNA or *wtf*-wag units with 5S rDNA could form extrachromosomal DNA. We tested (A) *S. octosporus* wtf81-wag20 (B) *S. octosporus* wtf72 to form extrachromosomal chromosomes with 5S rDNA been associated in a circular chromosome. We use oligos pair 2982-2983 that should only amplify DNA with there is re circularization of wtf81-wag20, and oligo pair 2988-2987 for wtf72. (C) PCR gel shows no amplification for oligos pairs 292-2983 and 2988-2987.

In the lineages leading *S. cryophilus*, *S. osmophilus* and *S. octosporus*, we again observed an intimate association with *wtf* genes and small, multicopy DNA sequences
distributed throughout the genome. But in this case, the repeats were 5S rDNA genes instead of Tf LTRs. We therefore propose that a similar non-allelic recombination mechanism facilitated the spread of wtf genes in the lineages leading to *S. octosporus*, *S. osmophilus*, and *S. cryophilus*. In these lineages, the gene duplication events sometimes included the wag genes and led to their expansion into a gene family as well. Indeed, the 5S rDNA genes are known hotspots for non-allelic recombination in these species as 12 out of 19 breaks in synteny identified between *S. octosporus* and *S. cryophilus* have 5S rDNA genes at the breakpoints (Ács-Szabó et al., 2018). Notably the linear distribution of 5S rDNA in the genome of fission yeast may allow non-allelic gene conversion to occur between different copies (Mao et al., 1982).

It may be relevant that both the Tf LTRs and the 5S rDNA sequences cluster in a restricted region of the nucleus. The Tf LTR transposons are bound by members of the CENP-B gene family and are then clustered to a nuclear domain known as the Tf body in a process that requires CENP-B homolog *cbpl*, histone lysine H3-K4 methyltransferase *setl* and Ku domain protein *pku80* genes (Johansen and Cam, 2015). The 5S rDNA genes are transcribed by RNA polymerase III (pol III) and cluster with all other pol III transcripts within the nucleus (Daulny et al., 2016; Mao et al., 1982). This clustering could promote the duplication of wtf genes to novel repeat-associated sites in the genome. The clusters could potentially affect recombination outcomes. Factors found in the clusters could limit crossover recombination events between non-allelic sites that would generate costly chromosome rearrangements. The clusters could also facilitate non-allelic gene conversion that helps enable the rapid evolution of the wtf genes (Eickbush et al., 2019; Hu et al., 2017). It is also interesting to note that Rsp-like sequences of *Drosophila* mentioned above also spread to distributed repetitive sequences that cluster within nuclei (Herbette et al., 2021). In *S. pombe*, increase concentration of lithium
chloride select for an extrachromosomal DNA containing the *sod2* gene (rescue lithium chloride toxicity). The extrachromosomal DNA contains a LTR sequence in the “telomeres” of this sequences, which may indicate that the duplication could be LTR-mediated (Albrecht et al., 2000; Jia et al., 1992; Patterson et al., 1999).

5S rDNA, implicated in the formation of ribosomes structures, are usually not considered as selfish repetitive DNA. However, some examples in plants show that 5S rDNA sequences can form B chromosomes in *Plantago lagopus* (Dhar et al., 2019). B chromosome are a type of selfish elements that favor their own transmission (Houben, 2017). The fission yeast genomes shows a linear distribution of the 5S rDNA genes, whereas budding yeast and other organisms have clusters of 5S rDNA (Mao et al., 1982). The linear distribution of the 5S rDNA in fission yeast corroborate our hypothesis of 5S rDNA to be a hotspot of gene conversion. If a double strands break happens near the 5S rDNA, repair will involve a search for homology that will be in most cases the homologous chromosome. Because 5S rDNA are found distributed throughout the genome the repair process may involve a non-allelic 5S rDNA. This could duplicate the gene associated with the 5S rDNA like *wtf* or *wag* genes.

Our hypothesis is that LTR and/or 5S rDNA are hotspots of non-allelic gene conversion. To prove that dispersed repetitive elements increase the rate of non-allelic gene conversion we need to use a reporter of duplication in *S. pombe*. *Saccharomyces cerevisiae CUP1* is a master regulator of copper metabolism in budding yeast (Shi et al., 2020). An increase number of *CUP1* genes increased the tolerance of copper toxicity (Zhang et al., 2013). An increase of copper concentration favors duplication of the *CUP1* gene in *S. cerevisiae* (Hull et al., 2017; Zhang et al., 2013). Even though the mechanism of copper tolerance in *S. pombe* is different,
CUP1 still rescues copper toxicity in S. pombe (Rustici et al., 2007). To detect recombination events in S. pombe, we propose to introduce in fission yeast S. cerevisiae CUP1 as a selective tool to detect duplication events. We planned to induce CUP1 associated with LTR/5S DNA or without dispersed repetitive elements to test the hypothesis of duplication events been favor by dispersed repetitive elements. We expect to observe more frequency of duplication events with CUP1 associated with LTR/5S rDNA than without those dispersed repetitive elements. This result will show that 5S rDNA and/or LTR sequences are hotspots of duplication events.

4.4 Outlook: Model for the long-term persistence of wtf drivers

Theoretical models of drive generally consider a single, stationary drive locus (Crow, 1991). The reality of the long-term evolution of the wtf drivers is a great deal more complex. The wtf drivers are part of a large, rapidly evolving gene family that also includes drive suppressors (Bravo Núñez et al., 2018a). We propose that this complexity creates a cycle of driver death and rebirth analogous to the mythological phoenix (Figure 5.1). wtf drivers are perpetually reborn anew via gene duplication and rapid evolution of existing genes. This rebirth allows the genes to evade extinction by short-circuiting the two main paths to extinction mentioned above: extinction following suppression and extinction following fixation. We discuss both paths in more detail below.

We propose that the number and diversity of the wtf drivers create a significant challenge for the evolution of suppressors. The mapped genic suppressors of wtf drivers are other wtf genes. Importantly, however, the wtf-mediated suppression in all known cases is highly specific in that the antidotes only neutralize poison proteins with amino acid sequences that are highly like the antidote (Figure 3.1). Because of this, even changing two amino acids is sufficient to
disrupt the ability of a Wtf\textsuperscript{antidote} to neutralize a Wtf\textsuperscript{poison} (2018 Bravo Nunez). In addition, the fitness benefits of suppressing one driver are minimized if there are still several active drivers (Bravo Núñez et al., 2020b). It is not yet clear if other variants could act more broadly against multiple \textit{wtf} drivers. Sequence-directed transcriptional silencing via installation of heterochromatin, similar to that used to control transposons, could be a potential route for widespread silencing of \textit{wtf} drivers (Mizuguchi et al., 2017). However, silencing of \textit{wtf} drivers would not be trivial to evolve for several reasons (Nuckolls et al., 2021). In particular, the regions linked to \textit{wtf} genes benefit from their drive. Because of this, variants that resisted heterochromatin installation and \textit{wtf} silencing would have an advantage over those that were permissive to heterochromatin installation.

Driver fixation is a second route to driver extinction. Population surveys of \textit{wtf} gene diversity are currently available only in \textit{S. pombe} where the entire complement of \textit{wtf} genes has been assembled for 4 isolates (Eickbush et al., 2019; Hu et al., 2017). This limited analysis suggested that the rapid evolution of \textit{wtf} genes make fixation of any given sequence unlikely. Within this group, there is only one locus (\textit{wtf4}) where all four isolates contain a driver. The sequence of the \textit{wtf4} driver, however, is not fixed. Even the two most similar \textit{wtf4} drivers, from the reference genome (\textit{Sp}) and the \textit{S. kambucha} isolate (\textit{Sk}), are distinct drivers in that the antidote from \textit{Sp} \textit{wtf4} does not neutralize the poison from \textit{Sk} \textit{wtf4} and \textit{vice versa} (Nuckolls et al., 2017). The non-allelic gene conversion of \textit{wtf} genes and the expansion and contraction of repetitive coding sequence that largely drive the evolution of the \textit{wtf} genes in \textit{S. pombe} also occurred in the \textit{S. octosporus} and \textit{S. osmophilus} lineages. Those lineages also have many (>20) \textit{wtf} genes, so fixation may also be unlikely within those species. \textit{S. cryophilus}, however, has fewer \textit{wtf} genes and has lost the repetitive coding sequence shared by the other drivers, so fixation may be more likely in that species.
An alternate hypothesis to explain the long-term persistence of the *wtf* drivers is that the genes are not merely selfish parasites. It is possible that *wtf* drivers promote fitness in some way that we have yet to discover. This theoretical additional function of the *wtf* genes could have promoted their long-term maintenance in fission yeast genomes. It is important to note, however, that genes do not need to promote fitness to be maintained in genomes and there is currently no evidence supporting a role of *wtf* drivers in promoting fitness, except in cases where they suppress other *wtf* drivers.

Most drivers identified are not mapped and the gene responsible is unknown. Many meiotic drive systems are cryptic or only identified when mating with wild isolates that did not develop the suppressors for the meiotic driver. This was the case for *S. pombe* *wtf* genes (Hu et al., 2017; Nuckolls et al., 2017; Zanders et al., 2014). By using prediction tools in other species, we were able to identify homologs of *wtf* genes in other fission yeast species. The identification of meiotic driver homologs is difficult because they are expected to evolve under positive selection which favors accumulation of mutation to escape suppression. Investigating different species allows to study the evolution of the driver in a larger scale than the one species specific.

The favored model for persistent driver in genomes is a cycle of dead and reborn meiotic drivers. Different *SD* haplotype in *D. melanogaster* is found in the wild with different number of inversions. To avoid fixations, inversions within *SD* will impede to break the system. The important cost of the *SD* haplotype for homozygotes male results in low frequency in *D. melanogaster* populations. The different *SD* haplotypes are found in excess and do not exert
the same cost for homozygous males. This shows that there is a turnover of $SD$ chromosomes between different population that maintain the $SD$ (Presgraves et al., 2009).

When a meiotic driver with a fitness cost, could benefits the genome. The driver allele could persist in the genome because its loss will be more costly for the genome than the driver cost. In a particular environment, meiotic driver may favor rapid adaptation than without drive. Genes that will increase the survival of the organism in a peculiar environment will be favorable if linked with the driver because they will spread quickly in the population. In this hypothesis the driver could still be lost by fixation.

Persistence of meiotic drivers is puzzling. How can a meiotic driver with so many defects for more than 119 million years? The answer seems to be that the $wtf$ meiotic drive system could favor duplication events. Many driver systems like $SD$ or the $t$-haplotype are in regions of high inversions and with almost no recombination (Kelemen and Vicoso, 2018; Larracuente and Presgraves, 2012). Absence of recombination is beneficial for the $SD$ system that cannot be broken by recombination. With time no recombination will favor regions with recessive lethal mutations that otherwise would have been purge by recombination. The presence of turnover between different haplotypes in the case of the SD may allowed those system to rejuvenated even though the mechanism is not well understood. In the case of $wtf$ genes, recombination is a key element of the spread of $wtf$ genes. Recombination is beneficial in the persistence of $wtf$ genes in fission yeast genomes.

4.5 Summary

My thesis work shows that $wtf$ genes are not lineage specific. $wtf$ genes have been in fission yeast genomes for at least 110 million years. We found more than 130 new $wtf$ genes in the genomes of $S. octosporus$, $S. osmophilus$, and $S. cryophilus$. I demonstrate that $S.$
octosporus, S. osmophilus and S. cryophilus wtf genes encode both poison and antidotes proteins. The poison and antidote mechanism in S. octosporus wtf genes seem to be like S. pombe wtf. We show that wtf genes act as meiotic driver when heterozygous in S. octosporus, a distantly species of S. pombe These finding show how meiotic drivers can persist in genome, for a longer period than the predicted evolution for meiotic drivers. Evolution may now be seen not only in the order of species but in the order of an entire genus. Meiotic drivers are so detrimental that extinction by suppression, fixation, or decay are the only roads thought for driver evolution. Here, we show that drivers are found in many copies in the genomes underlying a possible mechanism of non-allelic gene conversion and mutation to create new drivers. In the era of sequencing, more and more genomes are available to study, and we expect that more meiotic driver families will be detected in different species. Meiotic drivers like criminal suspect left behind clues that can be identified. The clues in genomes seem to be non-allelic gene conversion amongst different copies and contraction and extension of repeats. Duplication seems to be key of meiotic drivers evolution, and with new experiments to detect those duplication events, we could show a different way for meiotic drivers to escape suppression, decay, or fixation.
5 Chapter 5: Material and Methods
5 CHAPTER 5: MATERIAL AND METHODS ................................................................. 140

TABLE OF CONTENT CHAPTER 5 ........................................................................... 141

5.1 INTRODUCTION .............................................................................................. 142
5.2 NANOPORE SEQUENCING AND ASSEMBLY OF S. OSMOPHILUS ...................... 143
5.3 RNA SEQUENCING AND NANOPORE cDNA SEQUENCING (GUO-SONG JIA) .......... 143
   5.3.1 Sample preparation .................................................................................... 143
   5.3.2 RNA extraction ........................................................................................ 144
   5.3.3 RNA sequencing ...................................................................................... 144
   5.3.4 ONT cDNA sequencing .......................................................................... 145
   5.3.5 RNA-seq data processing ...................................................................... 145
   5.3.6 ONT cDNA data processing .................................................................. 145
5.4 ANNOTATION .................................................................................................. 146
   5.4.1 S. osmophilus genome annotation ........................................................... 146
   5.4.2 Calculating amino acid identity between Schizosaccharomyces species ........ 146
   5.4.3 Sequence homology search ..................................................................... 147
   5.4.4 S. octosporus wtf and wag genes ............................................................. 147
   5.4.5 S. osmophilus wtf and wag genes annotations ......................................... 148
   5.4.6 5S rDNA annotation .............................................................................. 148
   5.4.7 LTR annotation ...................................................................................... 149
   5.4.8 Identifying Mei4 binding sites (FLEX motif) outside of S. pombe ................ 149
5.5 DNA SEQUENCE ALIGNMENTS AND PHYLOGENIC TREE CONSTRUCTION .... 149
5.6 ANALYSES OF REPETITIVE REGIONS WITHIN WTF GENES ......................... 150
5.7 GARD ANALYSES OF RECOMBINATION WITHIN WTF GENE FAMILY .......... 151
5.8 SYNTENIC ANALYSIS .................................................................................... 151
5.9 MOLECULAR BIOLOGY ................................................................................... 151
   5.9.1 S. cerevisiae LExA-ER-AD 8-estradiol inducible system .......................... 151
5.9.2 Cloning S. octosporus, S. osmophilus and S. cryophilus \( \text{wtf}^{\text{poison}} \) and \( \text{wtf}^{\text{antidote}} \) alleles for expression in \( S. \text{cerevisiae} \) .......................................................... 152

5.9.2.1 Cloning S. octosporus \( \text{wtf}^{\text{61poison}} \) (SOCG_04114) under the control of a \( \beta \)-estradiol inducible promoter. 152

5.9.2.2 Cloning S. octosporus \( \text{wtf}^{\text{61antidote}} \) (SOCG_04114) under the control of a \( \beta \)-estradiol inducible promoter. 153

5.9.2.3 Cloning S. cryophilus \( \text{wtf}^{\text{1poison}} \) (SPOG_03611) under the control of a \( \beta \)-estradiol inducible promoter. 153

5.9.2.4 Cloning S. cryophilus \( \text{wtf}^{\text{1antidote}} \) (SPOG_03611) under the control of a \( \beta \)-estradiol inducible promoter. 154

5.9.2.5 Cloning S. osmophilus \( \text{wtf}^{\text{41poison}} \) under the control of a \( \beta \)-estradiol inducible promoter. ............ 154

5.9.2.6 Cloning S. osmophilus \( \text{wtf}^{\text{41antidote}} \) under the control of a \( \beta \)-estradiol inducible promoter. ........ 155

5.9.2.7 Cloning S. octosporus \( \text{wtf}^{\text{25poison}} \) (SOCG_04480)-GFP under the control of a \( \beta \)-estradiol inducible promoter. 155

5.9.2.8 Cloning S. octosporus \( \text{wtf}^{\text{25antidote}} \) (SOCG_04480) mCherry under the control of a \( \beta \)-estradiol inducible promoter. .................................................................................................................. 155

5.9.3 Plasmid transformation in \( S. \text{cerevisiae} \) .......................................................................................................................... 156

5.9.4 Spot assays in \( S. \text{cerevisiae} \) ......................................................................................................................................... 156

5.9.5 Imaging Wtf proteins expressed in \( S. \text{cerevisiae} \) ................................................................................................................................... 157

5.9.6 S. octosporus strains (Guo-Song Jia) .................................................................................................................. 157

5.9.7 Plasmids integrated into S. octosporus (Guo-Song Jia) ..................................................................................... 158

5.9.8 Spore viability analysis (Guo-Song Jia) .............................................................................................................. 159

5.9.9 Crosses and random spore assay ......................................................................................................................... 159

5.1 Introduction

This chapter is a material and method chapter about experiments done in chapter 2 and 3
5.2 Nanopore sequencing and assembly of *S. osmophilus*

To sequence *S. osmophilus* we extracted DNA with the QIAGEN Genomic-tip kit. We then used a standard ligation sequencing prep and kit (SQK-LSK109), including DNA end repair using the NEB End Prep Enzyme, FFPE prep with the NEB FFPE DNA repair mix, and ligated using NEB Quick Ligase. We sequenced using two Flongle Sequencers and performed base calling with guppy version 2.1.3. This generated approximately 521 megabases of sequence or approximately 40x coverage. We then performed de novo assembly pathway using canu v1.8 and the ovl overlapper with a predicted genome size of 13 mb and a corrected error rate of 0.12. We corrected our assembly using pilon with paired end illumina data generated with the same DNA. We assembled 11 nuclear contigs with a total length of 11.3 mb and one mitochondrial contig that was 68 kb in length. Assembly statistics were generated using an existing perl script [(https://github.com/SchwarzEM/ems_perl/blob/master/fasta/count_fasta_residues.pl)](https://github.com/SchwarzEM/ems_perl/blob/master/fasta/count_fasta_residues.pl). The assembled genome scored at 89% complete with busco which is comparable to the score for the closely related species *S. octosporus*.

5.3 RNA sequencing and Nanopore cDNA sequencing (Guo-Song Jia)

5.3.1 Sample preparation

For RNA sequencing and ONT (Oxford Nanopore Technologies) cDNA sequencing of *S. octosporus* diploid cells undergoing azygotic meiosis, we crossed DY44617 and DY44598 on a SPASK plate for about 12 hours. Cells were subsequently scraped from SPASK when we observed conjugated cells under the microscope. Afterward, we spread the cells on YES plates with nourseothricin (NAT) and G418 (YES + NAT&G418) for diploid cell selection. After 3 days, we collected all clones that appeared on YES + NAT&G418 and spread them on YES plates again for 24 hours of re-growth. We then washed diploid cells from the plate and placed...
them on SPASK plates for azygotic meiosis induction. Next, approximately 5 OD\textsubscript{600} units of cells were harvested and snap frozen using liquid nitrogen at 0, 6, 10, 19, and 24 hours after the start of induction.

5.3.2 RNA extraction

All cells collected at each time point were thawed on ice for about 5 minutes then washed once with chilled DEPC water. The cell pellets were resuspended with TES buffer (10 mM Tris pH 7.5, 10 mM EDTA pH 8.0, 0.5% SDS), and mixed with acidic phenol-chloroform (1:1) immediately. The samples were firstly mixed completely by vortexing and then incubated in a 65°C-heat block for 1 hour. Then the samples were centrifuged at 4°C, and the aqueous phase was collected. The aqueous phase was then treated with phenol-chloroform (1:1) and chloroform:isoamyl alcohol (24:1) successively. 3 M NaAc (pH 5.2) and isopropanol were added to the aqueous phase and mixed thoroughly by inverting. The mixture was stored at -20°C overnight then centrifuged at top speed at 4°C. After centrifuging, the supernatants were removed, and the RNA pellets were washed with 70% ethanol. RNA samples were dissolved in DEPC water after air-drying.

5.3.3 RNA sequencing

The total RNA samples at all 5 time points (0, 6, 10, 19 and 24 hours after start of induction) were submitted to a commercial sequencing service provider for RNA-seq library preparation and sequencing.
5.3.4 ONT cDNA sequencing

The total RNA sample at 19 hours after azygotic meiosis induction was submitted to commercial service provider for ONT cDNA-seq library preparation and sequencing.

5.3.5 RNA-seq data processing

Sequencing reads were firstly conducted using fastp (version:0.20.0), with default parameters. Sequencing adaptor and low-quality bases were trimmed from the raw reads. The cleaned pair-end reads were mapped to a high-quality *S. octosporus* reference genome (http://bifx-core.bio.ed.ac.uk/~ptong/genome_assembly/oct Genome.fa) published in Tong et al 2019 using STAR (version: 2.6.0a) with the following settings: ‘--alignIntronMin 29 --alignIntronMax 819 --outFilterMultimapNmax 1 --outFilterMismatchNmax 0 --alignEndsType EndToEnd’ (Dobin et al., 2016).

5.3.6 ONT cDNA data processing

Raw ONT cDNA sequencing reads were firstly processed using pychopper (version: 2.3.1) from ONT with default parameters to trim and orient full-length Nanopore cDNA reads. In this step, full-length reads (with sequencing primers at both ends) can be identified from raw reads and the sequencing primers were removed. Full-length reads were then analyzed using an integrated software: FLAIR (Full-Length Alternative Isoform analysis of RNA) (Tang et al., 2020) which helped identify coding sequences. Full length reads were firstly mapped to a same *S. octosporus* reference genome described above using ‘flair.py align’ with default parameters. The splicing junction information generated in read mapping were extracted using a FLAIR script “junctions_from_sam.py” and be used subsequently in “flair.py correct” step. Finally, the high-quality FLAIR transcripts were generated using “flair.py collapse” with default
parameters (Tang et al., 2020). The reads were then observed in the Integrative Genomics Viewer (IGV) as in Figure 2.4B (Robinson et al., 2011).

5.4 Annotation

5.4.1 *S. osmophilus* genome annotation.

For *S. osmophilus*, we annotated all the coding sequences with the Augustus gene prediction software webpage (Stanke et al., 2006) (http://bioinf.uni-greifswald.de/webaugustus/). First, we train Augustus software with *S. octosporus* genome from Tong et al., 2019, and we upload the cDNA sequences of *S. octosporus* genes from (Rhind et al., 2011). This training set allowed Augustus to construct a model to then predict *S. osmophilus* genes. Augustus annotated the predicted exons and introns of all the genes in *S. osmophilus* genome. To match *S. osmophilus* genes with orthologous gens within *S. octosporus, S. cryophilus* and *S. pombe*, we extracted all the predict translations of *S. osmophilus* genes and used OrthoVenn2 to find orthologs for each genes (Xu et al., 2019).

5.4.2 Calculating amino acid identity between *Schizosaccharomyces* species.

To calculate the percentage amino acid identity shared between proteins of the different *Schizosaccharomyces* species, we used BLASTp (default parameters) to compare each protein sequence to a protein database created for each genome (Altschul et al., 1990). For example, we compared all the genes of *S. osmophilus* with the *S. octosporus* database. We then compared all the genes of *S. octosporus* with the *S. osmophilus* database. The best hit was saved for each gene from the reciprocal BLASTp to calculate the percentage of identity between two orthologs. We then calculate the mean of all the percentage identity (all the genes) between the two genomes. The percentage of identity for each paired comparisons between genomes can be found in Table 2.1. This percentage of identity was used to verified and construct the
Schizosaccharomyces phylogeny in Figure 2.1 in concordance with previously published results (Brysch-Herzberg et al., 2019; Rhind et al., 2011).

5.4.3 Sequence homology search

To find wtf genes outside of S. pombe, we performed a PSI-BLAST search within the Schizosaccharomyces species with the S. pombe wtf4 gene as a query (E-value threshold 0.05, Word size=3, matrix=BLOSUM62, existence=11, extension=1, PSI-BLAST threshold=0.005) (Altschul et al., 1997). We repeated the search until no new significant hits were found (E-value threshold <0.05). Then we perform a BLASTn search using novel wtf genes from S. octosporus, S. osmophilus and S. cryophilus as queries to find additional wtf genes and pseudogenes within each genome (E-value threshold <0.05) (Altschul et al., 1990). To search for S. japonicus wtf genes, we used sequences of S. octosporus, S. cryophilus, and S. pombe wtf genes as query for BLAST with S. japonicus (Altschul et al., 1990; Rhind et al., 2011). This yielded no hits. We also carried out a MEME motif search of all the available wtf genes sequences and then perform PSI-BLAST to find genes with wtf genes motifs in S. japonicus (parameters: expect threshold: 10, word size: 3, matrix: BLOSUM62, gap costs = existence: 11, extension: 1, PSI-BLAST threshold: 0.005) (Altschul et al., 1997). This also yielded no conclusive hits. Finally, we manually inspected S. japonicus genes defined as lineage-specific by OrthoVenn2 to search for multi-exon (5-6) candidate genes with a potential alternate translational start site in intron 1 or exon 2, similar to the wtf drivers (Xu et al., 2019). This search also yielded no promising hits.

5.4.4 S. octosporus wtf and wag genes

To annotate wtf genes in S. octosporus we used two different approaches listed below.
First, we aligned the short-read RNA-sequencing data described to the *S. octosporus* genome using Geneious Prime® 2021.1.1. For each *wtf* and *wag* gene identified, we manually viewed RNA-sequencing data and used it to annotate the exons and introns. For genes and pseudogenes with insufficient sequence coverage, we determined coding sequences using homology to other *wtf* or *wag* genes that we were able to annotate with RNA-sequencing data. Specifically, we first aligned the unannotated genes with annotated genes using MAFFT with parameters L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123) (Katoh, 2002; Katoh and Standley, 2013). We then used the alignment to manually inspect genes to annotate splicing sites and predict coding sequences. Genes with incomplete coding sequences, including those determined to have lost splice sites, were considered pseudogenes.

All the annotation of *S. octosporus* wtf genes in Appendix A1.

### 5.4.5 *S. osmophilus* wtf and wag genes annotations

We first annotated *S. osmophilus* wtf and wag genes using Augustus prediction (trained with *S. octosporus* data). We then manually inspected the annotations using alignments of all the *S. osmophilus* wtf or wag genes generated by MAFFT (L-INS-I; 200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123). Genes with 4 exons were annotated as pseudogenes when a 5th exon was not predicted by Augustus and was found to be absent after inspection of the alignment. In many of these pseudogenes, the 5th exons were degenerate with accumulated stop codons.

### 5.4.6 5S rDNA annotation

To annotate 5S rDNA in the genomes of *S. octosporus* and *S. cryophilus* we used BLASTn using annotated 5S rDNA sequences in each genome as a query. For *S. osmophilus*, we used
an *S. octosporus* 5S rDNA gene as a query. In all genomes, hits with 70-100% DNA sequences identity were considered 5S rDNA genes.

### 5.4.7 LTR annotation

To annotate Tf transposon LTRs in *S. osmophilus*, we used BLASTn to search for sequences similar to the already annotated LTRs found in *S. cryophilus* and *S. pombe* (Rhind et al., 2011). We found many hits in *S. osmophilus* (E-value less than 0.05). In addition, we also used the LTR_retriever program which identified additional LTRs in *S. osmophilus* (Ou and Jiang, 2018). All the LTR identified are reported in Appendix A4.

### 5.4.8 Identifying Mei4 binding sites (FLEX motif) outside of *S. pombe*.

To identify likely Mei4 binding sites in *S. octosporus*, *S. osmophilus*, and *S. cryophilus* *wtf* genes we first used the emboss fuzznuc tool in Geneious Prime® 2021.1.1 to search for the core FLEX motif (GTAAACA, GTAAATA, ATAAATA, or ATAAACA) and also for sequences that had the 3’ flanking nucleotide AACA in addition to the core sequence (Alves-Rodrigues et al., 2016; Horie et al., 1998)(Rice et al., 2000). Results are reported in Appendix A1, A2 and A3 for each *wtf* gene.

### 5.5 DNA Sequence alignments and phylogenetic tree construction.

All DNA or amino acid sequence alignments were constructed using the MAFFT (Katoh, 2002; Katoh and Standley, 2013) plugin in Geneious Prime® 2021.1.1 with parameters L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123). We generated trees using the PhyML 3.0 (Guindon et al., 2010) in the webpage [http://www.atgc-
montpellier.fr/phyml/. The substitution model used was selected by Smart model Selection which calculates an AIC (Akaike Information Criterion) for each substitution model and then selects the best model for the dataset (Akaike, 1998; Lefort et al., 2017). The starting tree for each phylogeny was generated by BIONJ, an improved version of neighbor-joining (Gascuel, 1997). The trees were then improved with NNI (nearest neighbor interchange) (Joseph Felsenstein, 2004). The branch support was calculated by aLRT SH-like, a likelihood ratio test (Anisimova and Gascuel, 2006). Then the trees were routed by midpoint using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree). We use the cladogram tree construction to help visualization.

5.6 Analyses of repetitive regions within wtf genes

We aligned the full length of all *S. octosporus*, *S. osmophilus* wtf genes within each species using MAFFT with parameters L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123) using Geneious Prime® 2021.1.1. We then manually identified the repeat region within the alignments and manually quantified the number of bases within the repeat. The results can be found in Appendix A12 for *S. octosporus* wtf genes and for *S. osmophilus* wtf genes (Figure 2.14). The results from *S. pombe* repeats are from Eickbush et al., 2019. To obtain sequence logos of *S. octosporus* and *S. osmophilus* repeats in exon 4, we extracted the first complete repeat for all wtf genes containing a repeat. We then separately aligned all the *S. octosporus* and *S. osmophilus* repeats to produce fasta files (which we uploaded to the Weblogo3 interface (http://weblogo.threeplusone.com/) (Crooks, 2004). The output generated the logos displayed in Figure 2.14.
5.7 GARD analyses of recombination within wtf gene family.

To study the recombination within wtf gene family within a species, we first produced an alignment of the coding sequence of wtf genes with Translation align in Geneious Prime® 2021.1.1 with MAFFT alignment L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123). We then used our alignments to find recombination events within the wtf gene family by using GARD (Kosakovsky Pond et al., 2006a) with general discrete model of site-to-site variation with three class rates executed within the Datamonkey website (https://www.datamonkey.org/) (Weaver et al., 2018).

5.8 Syntenic analysis

To find wtf loci shared by Schizosaccharomyces species (Figure 2.10 and 2.11) and to assay the relationship between wtf loci and ancestral 5S rDNA sites (Figure 2.15), we manually inspected synteny of loci in S. octosporus, S. osmophilus, S. cryophilus, and S. pombe. In order to study the synteny between different wtf loci we used OrthoVenn2 file generated previously (see S. osmophilus genome annotation section of Material and Methods) and the Ensembl fungi database to identify the orthologous genes (Howe et al., 2019; Xu et al., 2019). For each wtf loci we identified the immediately upstream and downstream gene and then the correspond orthologs in each species. An analogous approach was used with the analysis of 5S rDNA sites.

5.9 Molecular biology

5.9.1 S. cerevisiae LExA-ER-AD β-estradiol inducible system

The LExA-ER-AD system (Ottoz et al., 2014) uses a heterologous transcription factor containing LexA DNA-binding protein, the human estrogen receptor (ER) and an activation
domain (AD). β-estradiol binds the human estrogen receptor (ER) and tightly regulates the activity of the LexA-ER-AD transcription factor. The LexA DNA-binding domain recognizes lexA boxes in the target promoter.

5.9.2 Cloning S. octosporus, S. osmophilus and S. cryophilus wtfoison and wtfantidote alleles for expression in S. cerevisiae

All plasmids used in this study are listed in Table 3.3. All oligos used in this study are listed in Table 3.2.

5.9.2.1 Cloning S. octosporus wtfoison61 (SOCG_04114) under the control of a β-estradiol inducible promoter.

We amplified the predicted coding sequence of the S. octosporus wtfoison61 from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 1432 and 1442. The CYC1 terminator was digested from pSZB395 (Nuckolls et al. 2020) using SfiI and XhoI. We then cloned S. octosporus wtfoison61 CDS and the CYC1 terminator into XhoI and BamHI site of pSZB385 to generate SZB985. We then digested pSZB985 with XhoI and BamHI to extract wtfoison61 CDS with the CYC1 terminator. We next PCR amplified the LexA promoter (LexApr) using oligos 1195 and 1240 from FRP1642 (Addgene #58442, Ottoz et al. 2014). We then cloned both the promoter and the wtfoison61 CDS fragment into pRS316 (Sikorski and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1040.
5.9.2.2 Cloning *S. octosporus wtf61antidote* (SOCG_04114) under the control of a β-estradiol inducible promoter.

We amplified the predicted *S. octosporus wtf61antidote* from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2011 and 2170. We PCR amplified LexApr using oligos with 1195 and 1240 from FRP1642 (Addgene #58442, Ottoz et al. 2014). We PCR amplified CYC1 terminator from pSZB1040 using oligos 2194 and 2195. We then used overlap PCR to stitch together *S. octosporus wtf61antidote* and CYC1 terminator using PCR using oligos 2011 and 2195. We digested LexApr with KpnI and XhoI. We digested the fragment *S. octosporus wtf61antidote-CYC1* with XhoI and BamHI. Finally we cloned LexApr and *S. octosporus wtf61antidote-CYC1* fragments into pRS314 (Sikorski and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1108.

5.9.2.3 Cloning *S. cryophilus wtf1poison* (SPOG_03611) under the control of a β-estradiol inducible promoter.

We amplified the predicted coding sequence of the *S. cryophilus wtf1poison* (SPOG_03611) from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2277 and 2278. We amplified CYC1 terminator from pSZB1040 using oligos with 2279 and 2170. We used overlap PCR to stitch together *S. cryophilus wtf1poison* with CYC1 terminator using oligos 2277 and 2170. We digested that PCR product with XhoI and BamHI. We also amplified the LexApr was amplified from pSZB1040 using oligos 1195 and 1240 and then digested with KpnI and XhoI. We then cloned the *S. cryophilus wtf1poison-CYC1* and LexApr cassettes into of pRS316 (Sikorski and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1122.
5.9.2.4 Cloning S. cryophilus wtf1antidote (SPOG_03611) under the control of a β-estradiol inducible promoter.

We amplified the predicted coding sequence of *S. cryophilus* *wtf1*\textsuperscript{antidote} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2276 and 2278. We amplified *CYC1* terminator from pSZB1040 via PCR using oligos 2279 and 2170. We used overlap PCR to stitch together *S. cryophilus* *wtf1*\textsuperscript{antidote} and *CYC1* terminator using oligos 2276 and 2170. We then digested the resulting PCR product with XhoI and BamHI. The LexApr was amplified from pSZB1040 via PCR using oligos 1195 and 2778 and then digested with KpnI and XhoI. We then cloned both the promoter and *S. cryophilus* *wtf1*\textsuperscript{antidote}-*CYC1* fragments into pRS314 (Sikorski and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1192.

5.9.2.5 Cloning *S. osmophilus* wtf41poison under the control of a β-estradiol inducible promoter.

We amplified the predicted coding sequence of *S. osmophilus* *wtf41*\textsuperscript{poison} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2783 and 2780. We amplified the *CYC1* terminator from pSZB1040 via PCR using oligos 2781 and 2771. We amplified the LexApr from pSZB1040 via PCR using oligos 1195 and 2778. We use overlap PCR to stitch together LexApr, *S. osmophilus* *wtf41*\textsuperscript{poison} and the *CYC1* terminator using oligos 1195 and 2771. We then cloned the resulting digested product with KPNI into pRS316 (Sikorski and Hieter, 1989) digested with KpnI to generate pSZB1327.
5.9.2.6 Cloning *S. osmophilus* *wtf41*antidote under the control of a β-estradiol inducible promoter.

We amplified the predicted coding sequence of *S. osmophilus* *wtf41*antidote from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2779 and 2780. We amplified the CYC1 terminator from pSZB1040 via PCR using oligos 2781 and 2771. We amplified LexApr from pSZB1040 via PCR using oligos 1195 and 2782. We use overlap PCR to stitch together the three fragments using oligos 1195 and 2771. We then cloned the digested product with KPNI into pRS314 (Sikorski and Hieter, 1989) digested with KPNI to generate pSZB1325.

5.9.2.7 Cloning *S. octosporus* *wtf25poison* (SOCG_04480)-GFP under the control of a β-estradiol inducible promoter.

We amplified the predicted coding sequence of *S. octosporus* *wtf25poison* from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2669 and 2830. We amplified LexApr from SZB1040 via PCR using oligos 1195 and 2668. We amplified GFP from pKT0127 (Sheff and Thorn, 2004) via PCR using oligos 2831 and 2832. We amplified the CYC1 terminator from SZB1040 using oligos 2833 and 2771. We use overlap PCR to stitch together LexApr-*S. octosporus* *wtf25poison*-GFP-CYC1 terminator using oligos 1195 and 2771. We then cloned the digested product with KPNI into pRS316 (Sikorski and Hieter, 1989) digested with KpnI generate SZB1353.

5.9.2.8 Cloning *S. octosporus* *wtf25*antidote (SOCG_04480) mCherry under the control of a β-estradiol inducible promoter.

We amplified the predicted coding sequence of *S. octosporus* *wtf25*antidote from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2662 and 2663. We amplified LexApr
from pSZB1040 via PCR using oligos 1195 and 2661. We amplified mCherry from pSZB457 via PCR using oligos 2664 and 2665. We amplified CYC1 terminator from pSZB1040 via PCR using oligos 2666 and 2771. We use overlap PCR to stitch together the three products. We then cloned the resulting digested PCR product with KPNI into pRS314 (Sikorski and Hieter, 1989) digested with KpnI site to generate pSZB1347.

5.9.3  Plasmid transformation in S. cerevisiae

All yeast strains used in this study are listed in Table 3.1 with detailed genotype and citation information. Plasmids used in this study are listed in Table 3.3. We transformed plasmids into S. cerevisiae SZY1637 (Nuckolls et al., 2020) using a protocol modified from (Elble, 1992). Specifically, we incubated a yeast colony in a mix of 240 μL 50% PEG3500, 36 μL 1M Lithium Acetate, 50 μL boiled salmon sperm (10 mg/ml), and 10 μL plasmid for 4-6 hours at 30°C before selecting transformants. We selected transformants on Synthetic Complete (SC) media (6.7 g/L yeast nitrogen base without amino acids and with ammonium sulfate, 2% agar, 1X amino acid mix, 2% glucose) lacking histidine, uracil, and tryptophane (SC -His -Ura -Trp).

5.9.4  Spot assays in S. cerevisiae

For The spot assay we grew 5 mL overnight cultures in SC -His -Ura -Trp of each strain. We then diluted each culture to an OD₆₀₀ of 1 and performed a serial dilution. We then plated 10 μL of each dilution on a solid SC -His -Ura -Trp petri plate with or without 500 nM β-estradiol.
5.9.5 Imaging Wtf proteins expressed in *S. cerevisiae*

For imaging of Wtf proteins expressed in *S. cerevisiae* (Figure 3.1), we first grew 5 mL saturated overnight cultures in SC -His -Ura -Trp media. The next day, we diluted 1 mL of each saturated culture into 4 mLs of fresh SC -His -Ura -Trp media. We then added β-estradiol to a final concentration of 500 nM to induce *wtf* expression and shook the cultures at 30°C for 4 hours prior to imaging.

Cells (2 µL concentrated culture) were then imaged on an LSM-780 (Zeiss) with a 40x LD C-Apochromat (NA = 1.1) objective. A physical zoom of 8 was used which yielded an XY pixel size of 0.052µm. The fluorescence of GFP was excited with the 488 nm laser and filtered through a 491-553 nm bandpass filter before being collected onto a GaAsP detector running in photon counting mode. The fluorescence of mCherry was excited with the 561 nm laser and filtered through a 562-624 nm bandpass filter before being collected onto the same detector. When z stacks were acquired, the z spacing was 0.56µm.

5.9.6 *S. octosporus* strains (Guo-Song Jia)

The two wild-types heterothallic *S. octosporus* strains (DY44286=NIG10005 and DY44284=NIG10006) were a kind gift from Dr. Hironori Niki and all other *S. octosporus* strains were constructed based on these two heterothallic strains. *S. octosporus*-related genetic methods are performed according to or adapted from *S. pombe* (Forsburg and Rhind, 2006; Seike and Niki, 2017). The construction of *wtf* gene deletion strains was carried out by PCR-based gene targeting using an SV40-EM7 (SVE) promoter-containing G418-resistance marker referred to here as *kanSVE* (Erler et al., 2006). According to our previous bioinformatic analysis, sequences between the *wtf*-flanking 5S rDNA genes share high
similarity among different *wtf* gene loci. To ensure the specificity of gene deletion, we used homologous arm sequences outside of 5S rDNA genes and the length of at least one homologous arm was above 1 kb. All *wtf* gene deletion strains were verified using PCR. PCR primer sequences are listed in Table 3.2.

To analyze the spore killing activity of *wtf25* at an ectopic genomic locus, we constructed integrating plasmids based on the pDB4978 vector described below. A pDB4978-based plasmid was linearized with NotI digestion and integrated at the *leu1* (*SOCG_02003*) locus. Transformants were selected by the resistance to clonNAT conferred by the natMX marker on pDB4978. Successful integration resulted in the deletion of the ORF sequence of the *leu1* (*SOCG_02003*) gene and leucine auxotrophic phenotype (Figure 3.14).

### 5.9.7 Plasmids integrated into *S. octosporus* (Guo-Song Jia)

All *S. octosporus* plasmids were generated by recombination cloning using the ClonExpressII One Step Cloning Kit (Vazyme, Nanjing, China). For the construction of the pDB4978 vector, the plasmid pAV0584 (Vještica et al., 2019) was firstly digested using NotI and HindIII, and the largest resulting fragment (about 4.5-kb) was purified and then digested using SpeI to obtain an approximately 3.7-kb fragment containing AmpR, ori, and the natMX marker. A sequence containing the f1ori and multiple cloning sites was PCR amplified from pAV0584 using primers oGS-177 and oGS-178 (oligo sequences are listed in Table 3.3). The sequences upstream and downstream of the *leu1* (*SOCG_02003*) ORF were amplified from *S. octosporus* genomic DNA using primers oGS-192 and oGS-193, and primers oGS-195 and oGS-197, respectively. Finally, all four fragments were combined by recombination cloning to generate the pDB4978 vector.
5.9.8 Spore viability analysis (Guo-Song Jia)

Spore viability was assessed by octad dissection using a TDM50 tetrad dissection microscope (Micro Video Instruments, Avon, USA). The method of octad dissection was adapted from (Seike and Niki, 2017) and a detailed description of the experiment procedure follows. First, to maximize mating efficiency, before mating, all parental strains were streaked on YES plates for overnight growth. Then, parental strains were mixed at a one-to-one ratio and dropped on PMG plate (or PMG plates with the leucine supplement for leucine auxotrophic strains) and incubated at 30°C. After 2 days, about 1 OD$_{600}$ unit of cells were resuspended in 200 μl of 1 mg/ml solution of snailase (Beijing Solarbio Science & Technology Co.). The mixture was incubated without agitation at 25°C for 1 day and then the supernatant was aspirated. Snailase-treated cells were diluted in sterile water and then dropped on a YES plate for octad dissection. After dissection, plates were incubated at 30°C for about 5 days, and then plates were scanned, and the genotypes of colonies were determined by replica plating.

For data analysis, we excluded spores dissected from asci with fewer than 8 spores (asci with fewer than 8 spores are rare when sporulation was conducted on PMG plates) and octads containing >4 spores harboring one allele of a heterozygous locus (excluded octads represent < 2% of the octads analyzed). Numeric data of octad dissection analysis are in Appendix A13-A22. For statistical analysis of the spore viability data, Fisher’s exact test was performed using the web page https://www.langsrud.com/fisher.htm and exact binomial test were performed using an Excel spreadsheet downloaded from http://www.biostathandbook.com/exactgof.html (McDonald, 2009).

5.9.9 Crosses and random spore assay
Briefly, we mixed patch yeast on 100 uL spun down and plated in PMG media (2% glucose, 14.7 mM KH₂PO₄, 15.5 mM Na₂HPO₄, vitamins, 3.75 g/L L-glutamic acid, agar) for 3 days at 30 degrees. We scraped off the mated cells and put them in 500 uL sterile water into an Eppendorf tube. I add 10uL of glusulase to the tube for 4 hours at 30 degrees. Then I add 500 uL of 60% ethanol, vortex and incubate at room temperature for 10 minutes to kill the diploid cells and haploid that did not mate. After spinning down we dump the supernatant and wash twice with 1mL of water and resuspend in 500 uL of H2O. After we re process with 10 uL of glusulase overnight. The next day we add 500 uL of 60% ethanol, vortex and incubate at room temperature for 10 minutes to kill the diploid cells and haploid that did not mate. After spinning down we dump the supernatant and wash twice with 1mL of water and resuspend in 500 uL of H2O. Then we spread the cells on YEA plate for 5 days and then pick colonies and patch onto a YEA plate. Then each colony with via replica plating to diagnostic media to verify that the culture was the corresponding genotype. We then phenotype the spore colonies using standard approaches.


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Appendix

Appendix 1: Location and features of *S. octosporus* wtf genes. ...........................................181

Appendix 2: *Location and features of S. osmophilus wtf genes* ...........................................185

Appendix 3: Location and features of *S. cryophilus* wtf genes ...........................................190

Appendix 4: Locations of LTR sequences in *S. osmophilus* ..............................................190
Appendix 5: Maximum likelihood phylogeny of the regions between *S. octosporus* *wtf* genes and a downstream flanking 5S rDNA gene.

Appendix 6: Maximum likelihood phylogeny of the regions between *S. octosporus* *wtf* genes and an upstream flanking 5S rDNA gene.

Appendix 7: Maximum likelihood phylogeny of *S. octosporus* *wtf* genes.

Appendix 8: *wtf* genes in synteny between *S. kambucha*, *S. cryophilus*, *S. octosporus*, and *S. osmophilus*.

Appendix 9: *S. octosporus* lineage specific *wtf* genes and presence of a 5S rDNA.

Appendix 10: GARD analysis of *S. octosporus* *wtf* genes.

Appendix 11: GARD analysis of *S. osmophilus* *wtf* genes.

Appendix 12: Repeat count from Figure 2.X.

Appendix 13: Total viability numerical data summary.

Appendix 14: *wtf25*(SOCG_04480) deletion related numerical data of the octad spore dissection analysis.

Appendix 15: *wtf68*(SOCG_01240) deletion related numerical data of the octad spore dissection analysis.

Appendix 16: *wtf33* deletion related numerical data of the octad spore dissection analysis.

Appendix 17: *wtf33* deletion related numerical data of the octad spore dissection analysis.

Appendix 18: *wtf46*(SOCG_00084) deletion related numerical data of the octad spore dissection analysis.

Appendix 19: *wtf60*(SOCG_04742) deletion related numerical data of the octad spore dissection analysis.

Appendix 20: *wtf62*(SOCG_04077) deletion related numerical data of the octad spore dissection analysis.
Appendix 21: \textit{wtf21}(SOCG\_02322) deletion related numerical data of the octad spore dissection analysis.................................................................216

Appendix 22: \textit{octo-pSIV-leu1-1D} plasmid related numerical data of the octad spore dissection analysis........................................................................................................217
**Appendix 1: Location and features of *S. octosporus* wtf genes.**

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181
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| wtf26 | SOCG_04 636 | oct_chrI | 40 | 940 | 42 | 040 | 1 | 101 | forward | C | C | YES | pass | DRIVER | 221 | YES | 3 | YES | 1 |
| wtf27 | SOCG_04 638 | oct_chrI | 35 | 814 | 36 | 833 | 1 | 017 | forward | C | wag 7 | W | C | pass | divergencen | DRIVER | 12 | No | 0 | NO | 1 |
| wtf28 | SOCG_03 605 | oct_chrII | 3 570 | 504 | 3 687 | 455 | 1 | 035 | reverse | W | NO | fail | PSEUDOG | ENE | 18 | No | 0 | YES | 0 |
| wtf29 | SOCG_03 724 | oct_chrII | 3 615 | 349 | 3 616 | 388 | 1 | 040 | reverse | W | wag 8 | C | W | W | pass | divergencen | DRIVER | 246 | YES | 1 | NO | 1 |
| wtf30 | SOCG_02 691 | oct_chrII | 4 356 | 692 | 4 357 | 809 | 1 | 118 | reverse | W | NO | fail | PSEUDOG | ENE | 11 | No | 0 | YES | 0 |
| wtf31 | SOCG_03 727 | oct_chrII | 3 608 | 400 | 3 609 | 518 | 1 | 119 | reverse | W | NO | fail | PSEUDOG | ENE | 0 | No | 0 | YES | 0 |
| wtf32 | SOCG_06 182 | oct_chrII | 3 121 | 432 | 3 122 | 605 | 1 | 174 | reverse | W | W | W | YES | pass | B | DRIVER | 271 | YES | 10 | YES | 0 |
| wtf33 | SOCG_06 181 | oct_chrII | 2 964 | 295 | 2 965 | 487 | 1 | 193 | reverse | W | W | YES | pass | D | DRIVER | 361 | YES | 22 | NA | 0 |
| wtf34 | SOCG_04 808 | oct_chrII | 2 896 | 740 | 2 897 | 832 | 1 | 093 | reverse | W | W | W | YES | pass | B | PSEUDOG | ENE | 16 | YES | 1 | NA | 0 |
| wtf35 | oct_chrII | 2 633 | 395 | 2 634 | 093 | 699 | reverse | W | W | W | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |
| wtf36 | oct_chrII | 2 630 | 918 | 2 631 | 592 | 675 | reverse | W | W | W | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |
| wtf37 | oct_chrII | 2 599 | 444 | 2 600 | 115 | 672 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |
| wtf38 | oct_chrII | 2 596 | 898 | 2 597 | 611 | 714 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |
| wtf39 | oct_chrII | 2 594 | 315 | 2 595 | 025 | 711 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | YES | 0 |
| wtf40 | SOCG_02 780 | oct_chrII | 4 1678 | 78 | 4 1689 | 28 | 1 | 052 | reverse | W | wag 1 | C | W | pass | divergencen | PSEUDOG | ENE | 3 | No | 0 | NA | 0 |
| wtf41 | oct_chrII | 2 591 | 725 | 2 592 | 436 | 712 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |
| wtf42 | oct_chrII | 2 589 | 215 | 2 589 | 892 | 678 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |
| wtf43 | oct_chrII | 2 586 | 654 | 2 587 | 373 | 720 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |
| wtf44 | oct_chrII | 2 584 | 071 | 2 584 | 81 | 316 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA | 0 |

182
<p>| wtf45 | oct_chrI | 2578 | 2579 | 732 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | YES |
| wtf46 | oct_chrI | 2401 | 2402 | 106 | reverse | W | W | W | YES | pass | B | DRIVER | 263 | YES | 10 | YES |
| wtf47 | oct_chrI | 2250 | 2251 | 937 | reverse | W | W | W | YES | pass | B | PSEUDOG | ENE | 180 | No | 0 | YES |
| wtf48 | oct_chrI | 3997 | 3998 | 109 | reverse | C | C | C | YES | pass | B | DRIVER | 18 | No | 0 | YES |
| wtf49 | oct_chrI | 1957 | 1958 | 990 | reverse | W | W | W | YES | pass | B | DRIVER | 68 | No | 0 | YES |
| wtf50 | oct_chrI | 1569 | 1570 | 110 | reverse | C | C | C | YES | pass | D | PSEUDOG | ENE | 41 | No | 0 | YES |
| wtf51 | oct_chrI | 843 | 844 | 590 | reverse | W | W | W | YES | pass | D | DRIVER | 249 | YES | 1 | YES |
| wtf52 | oct_chrI | 673 | 674 | 105 | reverse | W | W | W | YES | pass | D | DRIVER | 409 | No | 0 | YES |
| wtf53 | oct_chrI | 395 | 396 | 101 | forword | C | wag | 9 | W | C | C | pass | divergence | DRIVER | 14 | No | 0 | NO |
| wtf54 | oct_chrI | 97 | 98 | 102 | forward | W | wag | 11 | C | W | pass | divergence | DRIVER | 14 | No | 0 | NO |
| wtf55 | oct_chrI | 94 | 95 | 992 | reverse | W | W | W | YES | pass | D | DRIVER | 247 | YES | 3 | YES |
| wtf56 | oct_chrI | 25 | 26 | 107 | reverse | W | wag | 12 | C | W | W | pass | divergence | DRIVER | 244 | No | 0 | YES |
| wtf57 | oct_chrI | 15 | 16 | 113 | forward | C | wag | 13 | W | C | C | pass | divergence | DRIVER | 224 | No | 0 | YES |
| wtf58 | oct_chrI | 253 | 254 | 109 | forward | C | C | C | YES | pass | U | DRIVER | 41 | No | 0 | NO |
| wtf59 | oct_chrI | 253 | 254 | 109 | forward | C | C | C | YES | pass | B | DRIVER | 127 | No | 0 | YES |
| wtf60 | oct_chrI | 2160 | 2162 | 146 | reverse | W | wag | 2 | C | W | W | pass | divergence | DRIVER | 19 | No | 0 | YES |
| wtf61 | oct_chrI | 2027 | 2028 | 104 | forward | C | wag | 14 | W | C | C | pass | divergence | DRIVER | 18 | No | 0 | YES |
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| wtf63 | oct_chrI | 1383 | 1384 | 690 | forward | C | C | C | YES | pass | B | PSEUDOG | ENE | 0 | No | 0 | NA |
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Appendix 3: Location and features of *S. cryophilus* wtf genes

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<th>Predict function</th>
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Appendix 4: Locations of LTR sequences in *S. osmophilus*

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Appendix 5: Maximum likelihood phylogeny of the regions between *S. octosporus* wtf genes and a downstream flanking 5S rDNA gene.

The region downstream of 67 *S. octosporus* wtf genes with a downstream 5S rDNA gene were aligned with MAFFT and a maximum likelihood phylogeny was built with PhyML. Branch support values shown at the nodes (0-1) were calculated using aLRT SH-like, a fast likelihood-based method. The shaded clades and letter designations correspond to the colors and letters shown in Figure 3-figure supplement 3.
Appendix 6: Maximum likelihood phylogeny of the regions between *S. octosporus wtf* genes and an upstream flanking 5S rDNA gene.

The region upstream of 40 *S. octosporus wtf* genes with an upstream 5S rDNA gene were aligned with MAFFT and a maximum likelihood phylogeny was built with PhyML. Branch support values shown at the nodes (0-1) were calculated using aLRT SH-like, a fast likelihood-based method. The shaded clades and letter designations correspond to the colors and letters shown in Figure 3-figure supplement 3.
Appendix 7: Maximum likelihood phylogeny of *S. octosporus* wtf genes.

The sequences of 83 *S. octosporus* wtf genes were aligned using MAFFT and a maximum likelihood phylogeny was constructed using PhyML. Branch support values shown at the nodes (0-1) were calculated using aLRT SH-like. The color-coded letter designations to the right of the gene names indicate the phylogenetic groupings of the sequences flanking the wtf genes from Figure 3-figure supplement 3.
Appendix 8: *wtf* genes in synteny between *S. kambucha*, *S. cryophilus*, *S. octosporus*, and *S. osmophilus*.

| *S. kambucha* *wtf* in synteny | *S. cryophilus* *wtf* in synteny | Type of synteny with *S. cryophilus* *wtf* in synteny | Type of synteny with *S. octosporus* *wtf* in synteny | Type of synteny with *S. osmophilus* *wtf* in synteny | Gene upstream of *S. kambucha* *wtf* | Gene downstream of *S. kambucha* *wtf* | Gene upstream of *S. cryophilus* *wtf* | Gene downstream of *S. cryophilus* *wtf* | Gene upstream of *S. octosporus* *wtf* | Gene downstream of *S. octosporus* *wtf* | Gene upstream of *S. osmophilus* *wtf* | Gene downstream of *S. osmophilus* *wtf* | Comment |
|---------------------------------|---------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-----------|
| *wtf1*                          | *wtf2*                          | *wtf3*                                              | *wtf4*                                              | *wtf5*                                              | SPAC2E12.03c                  | SPAC1006.01                  | SOCG_010.06                  | SOCG_010.07                  | SOCG_010.06                  | SOCG_010.07                  | SOCG_010.19                  | SOCG_010.07                  | *wtf5* was lost in the *S. cryophilus* lineage and then 5S rDNA acquire and *wtf* went to that locus in *S.* |

This synteny seem to be more complexes than the other it is possible that the *wtf5* was lost in the *S. cryophilus* lineage and then 5S rDNA acquire and *wtf* went to that locus in *S.*
|   | wtf6 and wtf28 | wtf4 upstream | COMPLE | no | No synten | No ortholog | No synten | No synten | No synten | No synten | No synten | No synten | No synten | g2675 | g2688 | SOCG_037 | SOCG_037 | SPOG_03 | SPOG_034 | See Figure 2.11 |
| wtf7 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC533.0 4 | SPCC533.0 6 | g2675 | g2688 | SOCG_037 58 | SOCG_037 46 | SPOG_03 480 | SPOG_034 92 | | | | | |
| wtf8 and wtf29 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC736.0 4c | SPCC736.0 6 | g2681 | g2682 | SOCG_037 52 | SOCG_037 51 | SPOG_034 86 | SPOG_034 87 | | | | | |
| wtf9 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC306.0 9c | SPCC306.1 1 | g2649 | g2651 | SOCG_037 77 | SOCG_037 76 | SPOG_034 60 | SPOG_034 61 | | | | | |
| wtf11 and wtf12 and wtf13 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC1281.07c | SPCC1282.0 1c | g2350 | No ortho | SOCG_040 27 | No ortho | SPOG_034 20 | SPOG_034 18 | | | | | |
| wtf14 and wtf15 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC663.0 1c | SPCC663.0 3 | g756 | g736 | SOCG_029 12 | SOCG_029 3 | SPOG_025 11 | SPOG_025 11 | | | | | |
| wtf16 and wtf17 and wtf18 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC1450.09c | SPCC1450.07c | g2243 | g1210 | SOCG_021 68 | SOCG_041 07 | SPOG_006 55 | SPOG_004 34 | | | | | |
| wtf19 and wtf20 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC285.0 5 | SPCC285.1 0c | g1188 | g731 | SOCG_040 87 | SOCG_029 32 | SPOG_025 34 | SPOG_025 34 | | | | | |
| wtf21 and wtf26 | No ortholog | No synten | No ortholog | No synten | No ortholog | No synten | SPCC1739.14 | SPCC1739.01 | g932 | g865 | SOCG_027 68 | SOCG_027 19 | SPOG_024 65 | SPOG_024 18 | | | | | |

osmophilus and S. octosporus
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**See Figure 2.10**
Appendix 9: *S. octosporus* lineage specific *wtf* genes and presence of a 5S rDNA

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</tr>
<tr>
<td>wtf49</td>
<td>SOCG_00294</td>
<td>SOCG_0029</td>
<td>6</td>
<td>g4061</td>
<td>g4875</td>
<td>2</td>
<td>SPOG_0439</td>
<td>SPOG_0220</td>
<td>9</td>
</tr>
<tr>
<td>wtf50</td>
<td>SOCG_00464</td>
<td>SOCG_0046</td>
<td>2</td>
<td>g5077</td>
<td>g5076</td>
<td>9</td>
<td>SPOG_0203</td>
<td>SPOG_0204</td>
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<tr>
<td>wtf54 wtf55</td>
<td>SOCG_01106</td>
<td>SOCG_0111</td>
<td>1</td>
<td>g5857</td>
<td>g5859</td>
<td>2</td>
<td>SPOG_0091</td>
<td>SPOG_0091</td>
<td>1</td>
</tr>
<tr>
<td>wtf59</td>
<td>SOCG_04759</td>
<td>SOCG_0476</td>
<td>1</td>
<td>hsp-16</td>
<td>g1701</td>
<td>ND</td>
<td>SPOG_0003</td>
<td>SPBC3E7.02c</td>
<td>SPCC330.02</td>
</tr>
<tr>
<td>wtf60</td>
<td>SOCG_04743</td>
<td>SOCG_0474</td>
<td>1</td>
<td>g1672</td>
<td>g1670</td>
<td>6</td>
<td>SPOG_0004</td>
<td>SPOG_0004</td>
<td>7</td>
</tr>
<tr>
<td>wtf62</td>
<td>SOCG_04078</td>
<td>SOCG_0407</td>
<td>6</td>
<td>g1175</td>
<td>g1173</td>
<td>3</td>
<td>SPOG_0479</td>
<td>SPOG_0323</td>
<td>6</td>
</tr>
<tr>
<td>wtf63-wtf64</td>
<td>CENTROMERE</td>
<td>CENTROMERE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wtf65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wtf66</td>
<td>SOCG_01214</td>
<td>SOCG_0594</td>
<td>0</td>
<td>g406</td>
<td>ND</td>
<td>SPOG_0081</td>
<td>ND</td>
<td>SPCC132.04c</td>
<td>ND</td>
</tr>
<tr>
<td>wtf68</td>
<td>SOCG_01239</td>
<td>SOCG_0124</td>
<td>1</td>
<td>ND</td>
<td>g571</td>
<td>SPOG_0362</td>
<td>SPOG_0298</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>wtf72</td>
<td>SOCG_01597</td>
<td>SOCG_0159</td>
<td>5</td>
<td>g5333</td>
<td>g82</td>
<td>SPOG_0355</td>
<td>SPOG_0447</td>
<td>9</td>
<td>SPBC2A9.02</td>
</tr>
<tr>
<td>wtf75</td>
<td>SOCG_01755</td>
<td>SOCG_0175</td>
<td>g4509</td>
<td>g4507</td>
<td>SPOG_0129</td>
<td>SPOG_0377</td>
<td>SPBC354.09c</td>
<td>SPAC3F10.06c</td>
<td>X</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
<td>-----------</td>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------------</td>
<td>-------------</td>
<td>---</td>
</tr>
<tr>
<td>wtf76</td>
<td>SOCG_01856</td>
<td>SOCG_0185</td>
<td>g4629</td>
<td>g4688</td>
<td>SPOG_0119</td>
<td>SPOG_0118</td>
<td>SPAC869.07c</td>
<td>SPAC869.11</td>
<td>X</td>
</tr>
<tr>
<td>wtf78</td>
<td>SOCG_01873</td>
<td>SOCG_0187</td>
<td>g85</td>
<td>SPOG_0570</td>
<td>0</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>wtf79</td>
<td>SOCG_01892</td>
<td>SOCG_0189</td>
<td>g4652</td>
<td>g4655</td>
<td>SPOG_0088</td>
<td>SPOG_0161</td>
<td>SPBC1271.09</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>wtf80</td>
<td>SOCG_01906</td>
<td>SOCG_0190</td>
<td>g4670</td>
<td>ND</td>
<td>SPOG_0162</td>
<td>ND</td>
<td>SPCC1739.04c</td>
<td>ND</td>
<td>X</td>
</tr>
</tbody>
</table>
Appendix 10: GARD analysis of *S. octosporus wtf* genes.

GARD analysis of *S. octosporus wtf* predicted meiotic drivers and suppressors. This analysis found that a hypothesis allowing multiple trees for different segments of the alignment is >100 times more likely than a hypothesis allowing only a single tree, supporting that nonallelic recombination has occurred within the gene family. The analysis identified two likely breakpoints corresponding to positions 204 and 355 in the alignment, yielding three segments as depicted by the colored rectangles at the top of the figure. Both breakpoints have strong statistical support (***; p<0.0004). The trees generated for each segment (below) are distinct. The yellow highlighting is to help illustrate the incongruence between the trees.
Appendix 11: GARD analysis of *S. osmophilus* wtf genes.

*** p-value= 0.0004
GARD analysis of *S. osmophilus* wtf predicted meiotic drivers and suppressors. This analysis found that a hypothesis allowing multiple trees for different segments of the alignment is >100 times more likely than a hypothesis allowing only a single tree, supporting that nonallelic recombination has occurred within the gene family. The analysis identified two likely breakpoints corresponding to positions 159 and 298 in the alignment, yielding three segments as depicted by the colored rectangles at the top of the figure. Both breakpoints have strong statistical support (***, p<0.0004). The trees generated for each segment (below) are distinct. The yellow highlighting is to help illustrate the incongruence between the trees.

Appendix 12: Repeat count from Figure 2.14

<table>
<thead>
<tr>
<th>Size bp repeat region in <em>S. octosporus</em> wtf genes</th>
<th>Number of <em>S. octosporus</em> wtf genes</th>
<th>Size bp repeat region <em>S. osmophilus</em> wtf genes</th>
<th>Number of <em>S. osmophilus</em> wtf genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>39</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>0-21</td>
<td>2</td>
<td>0-21</td>
<td>3</td>
</tr>
<tr>
<td>22-42</td>
<td>7</td>
<td>22-42</td>
<td>3</td>
</tr>
<tr>
<td>43-63</td>
<td>17</td>
<td>43-63</td>
<td>6</td>
</tr>
<tr>
<td>64-84</td>
<td>12</td>
<td>64-84</td>
<td>9</td>
</tr>
<tr>
<td>85-120</td>
<td>6</td>
<td>85-105</td>
<td>4</td>
</tr>
</tbody>
</table>

Appendix 13: Total viability numerical data summary

<table>
<thead>
<tr>
<th>Diploid genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>151</td>
<td>1101</td>
<td>107</td>
<td>1208</td>
<td>91.14%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------------</td>
</tr>
<tr>
<td>wtf25Δ/wtf25Δ</td>
<td>85</td>
<td>615</td>
<td>65</td>
<td>680</td>
<td>90.44%</td>
</tr>
<tr>
<td>wtf68Δ/wtf68Δ</td>
<td>66</td>
<td>482</td>
<td>46</td>
<td>528</td>
<td>91.29%</td>
</tr>
<tr>
<td>wtf33Δ/wtf33Δ</td>
<td>65</td>
<td>469</td>
<td>51</td>
<td>520</td>
<td>90.19%</td>
</tr>
<tr>
<td>wtf46Δ/wtf46Δ</td>
<td>58</td>
<td>439</td>
<td>25</td>
<td>464</td>
<td>94.61%</td>
</tr>
<tr>
<td>wtf60Δ/wtf60Δ</td>
<td>54</td>
<td>391</td>
<td>41</td>
<td>432</td>
<td>90.51%</td>
</tr>
<tr>
<td>wtf62Δ/wtf62Δ</td>
<td>55</td>
<td>414</td>
<td>26</td>
<td>440</td>
<td>94.09%</td>
</tr>
<tr>
<td>wtf21Δ/wtf21Δ</td>
<td>39</td>
<td>273</td>
<td>39</td>
<td>312</td>
<td>87.50%</td>
</tr>
<tr>
<td>wtf25Δ/wtf25+</td>
<td>151</td>
<td>830</td>
<td>378</td>
<td>1208</td>
<td>68.71%</td>
</tr>
<tr>
<td>wtf68Δ/wtf68+</td>
<td>106</td>
<td>576</td>
<td>272</td>
<td>848</td>
<td>67.92%</td>
</tr>
<tr>
<td>wtf33Δ/wtf33+</td>
<td>108</td>
<td>638</td>
<td>226</td>
<td>864</td>
<td>73.84%</td>
</tr>
<tr>
<td>wtf46Δ/wtf46+</td>
<td>85</td>
<td>606</td>
<td>74</td>
<td>680</td>
<td>89.12%</td>
</tr>
<tr>
<td>wtf60Δ/wtf60+</td>
<td>131</td>
<td>917</td>
<td>131</td>
<td>1048</td>
<td>87.50%</td>
</tr>
<tr>
<td>wtf62Δ/wtf62+</td>
<td>89</td>
<td>644</td>
<td>68</td>
<td>712</td>
<td>90.45%</td>
</tr>
<tr>
<td>wtf21Δ/wtf21+</td>
<td>102</td>
<td>704</td>
<td>112</td>
<td>816</td>
<td>86.27%</td>
</tr>
</tbody>
</table>
Appendix 14: \( \textit{wtf25(SOCG\_04480)} \) deletion related numerical data of the octad spore dissection analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \textit{DY47906} \times \textit{DY44286} )</td>
<td>( \textit{wtf25\Delta/wtf25})</td>
<td>151</td>
<td>830</td>
<td>378</td>
<td>1208</td>
<td>68.71%</td>
<td>278</td>
<td>552</td>
<td>23.01% 45.70% 0.67</td>
</tr>
<tr>
<td>( \textit{DY47905} \times \textit{DY44287} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Type of octad according to the numbers and genotypes of viable spores |
|--------------------------|---------------------|-----------------|-------------------------|-----------------------------|-------------------------|-----------------|---------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------|
| 4R4S                     | 4R3S                | 3R4S            | 4R2S                    | 2R4S                        | 3R3S                    | 4R1S            | 1R4S                                                            | 3R2S                                                             | 2R3S                          |
| Number of octads         |                      |                 |                         |                             |                         |                  |                                                                |                                   |                                |
|                         | 16                   | 1               | 20                      | 0                           | 29                      | 0               | 37                                                             | 1                                 | 8                             |

| <= 3 viable spore        |
|--------------------------|---------------------|-----------------|-------------------------|-----------------------------|-------------------------|-----------------|---------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------|
|                         |                      |                 |                         |                             |                         |                  |                                                                |                                   |                                |
|                         | 16                   | 1               | 20                      | 0                           | 29                      | 0               | 37                                                             | 1                                 | 8                             |

209
Appendix 15: *wtf68*(SOCG_01240) deletion related numerical data of the octad spore dissection analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY47920 ×</td>
<td>*wtf68Δ/wtf68</td>
<td>106</td>
<td>576</td>
<td>272</td>
<td>848</td>
<td>67.92 %</td>
<td>212</td>
<td>25.00 %</td>
<td>42.92 %</td>
</tr>
<tr>
<td>DY44286</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>364</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DY47919</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42.92 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DY44287</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Type of octad according to the numbers and genotypes of viable spores**

<table>
<thead>
<tr>
<th>4R4S</th>
<th>4R3S</th>
<th>3R4S</th>
<th>4R2S</th>
<th>2R4S</th>
<th>3R3S</th>
<th>4R1S</th>
<th>1R4S</th>
<th>3R2S</th>
<th>2R3S</th>
<th>3R1S</th>
<th>1R3S</th>
<th>2R2S</th>
<th>4R0S</th>
<th>0R4S</th>
<th>&lt;= 3 viable spore</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>6</td>
<td>17</td>
<td>0</td>
<td>26</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>


Appendix 16: *wtf33* deletion related numerical data of the octad spore dissection analysis.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>wtf33Δ</em></td>
<td><em>wtf33</em></td>
<td><em>wtf33Δ</em></td>
</tr>
<tr>
<td>DY47903 × DY44286</td>
<td><em>wtf33Δ/wtf33</em></td>
<td>108</td>
<td>638</td>
<td>226</td>
<td>864</td>
<td>73.84%</td>
<td>267</td>
<td>371</td>
<td>30.90%</td>
</tr>
<tr>
<td>DY47904 × DY44287</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Type of octad according to the numbers and genotypes of viable spores | 4R4S | 4R3S | 3R4S | 4R2S | 2R4S | 3R3S | 4R1S | 1R4S | 3R2S | 2R3S | 3R1S | 1R3S | 2R2S | 4R0S | 0R4S | <= 3 viable spore |
|----------------------------------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----------------|---|
| Number of octads | 20   | 3    | 18   | 2    | 15   | 10   | 1    | 11   | 5    | 5    | 0    | 2    | 3    | 0    | 4    | 9 |
Appendix 17: wtf33 deletion related numerical data of the octad spore dissection analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY47903 ×</td>
<td>wtf33Δ/wtf33Δ'</td>
<td>108</td>
<td>638</td>
<td>226</td>
<td>864</td>
<td>73.84%</td>
<td>wtf33Δ</td>
<td>30.90%</td>
<td>0.58</td>
</tr>
<tr>
<td>DY44286 ×</td>
<td>wtf33Δ/wtf33Δ'</td>
<td>267</td>
<td>371</td>
<td>30.90%</td>
<td>42.94%</td>
<td>0.58</td>
<td>wtf33Δ</td>
<td>30.90%</td>
<td>0.58</td>
</tr>
<tr>
<td>DY47904 ×</td>
<td>wtf33Δ/wtf33Δ'</td>
<td>42.94%</td>
<td>30.90%</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DY44287 ×</td>
<td>wtf33Δ/wtf33Δ'</td>
<td>42.94%</td>
<td>30.90%</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Type of octad according to the numbers and genotypes of viable spores

| Number of octads | 20 | 3  | 18 | 2  | 15 | 10 | 1  | 11 | 5  | 5  | 0  | 2  | 3  | 0  | 4  | 9  |

<= 3 viable spore
Appendix 18: \textit{wtf46}(SOCG\_00084) deletion related numerical data of the octad spore dissection analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY47908 × DY44286</td>
<td>\textit{wtf46Δ/wtf46} \textsuperscript{a}</td>
<td>85</td>
<td>606</td>
<td>74</td>
<td>680</td>
<td>89.12%</td>
<td>wtf46\textsuperscript{a}</td>
<td>wtf46\textsuperscript{a}</td>
<td>wtf46\textsuperscript{a}</td>
</tr>
<tr>
<td>DY47907 × DY44287</td>
<td>\textit{wtf46Δ/wtf46} \textsuperscript{a}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>291</td>
<td>315</td>
<td>42.79%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of octad according to the numbers and genotypes of viable spores</th>
<th>4R4S</th>
<th>4R3S</th>
<th>3R4S</th>
<th>4R2S</th>
<th>2R4S</th>
<th>3R3S</th>
<th>4R1S</th>
<th>4R1S</th>
<th>3R2S</th>
<th>2R3S</th>
<th>3R1S</th>
<th>1R3S</th>
<th>2R2S</th>
<th>4R0S</th>
<th>0R4S</th>
<th>&lt;= 3 viable spore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of octads</td>
<td>41</td>
<td>6</td>
<td>18</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>


Appendix 19: \textit{wtf60}(SOCG\_04742) deletion related numerical data of the octad spore dissection analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>Type of octad according to the numbers and genotypes of viable spores</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{wtf60}Δ/wtf60\textsuperscript{+}</td>
<td>4R4S</td>
<td>4R3S</td>
<td>4R2S</td>
<td>2R4S</td>
<td>3R3S</td>
<td>4R1S</td>
<td>4R4S</td>
<td>3R2S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of octads</td>
<td>65</td>
<td>10</td>
<td>21</td>
<td>0</td>
<td>6</td>
<td>11</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{wtf60}Δ/wtf60\textsuperscript{+}</td>
<td>131</td>
<td>917</td>
<td>131</td>
<td>1048</td>
<td>87.50%</td>
<td>440</td>
<td>477</td>
</tr>
</tbody>
</table>

Note: The table indicates the number of viable and inviable spores for each cross type, along with the viability percentage and transmission distortion ratio for the\textit{wtf60}Δ/wtf60\textsuperscript{+} genotype. The table also categorizes the type of octad based on the numbers and genotypes of viable spores, with a specific emphasis on the number of octads that have <= 3 viable spores.
Appendix 20: *wtf62*(SOCG_04077) deletion related numerical data of the octad spore dissection analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability %</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DY47923 × DY44286</strong></td>
<td><em>wtf62Δ/wtf62Δ</em></td>
<td>89</td>
<td>644</td>
<td>68</td>
<td>712</td>
<td>90.45</td>
<td>317</td>
<td>44.52%</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>DY47924 × DY44287</strong></td>
<td><em>wtf62Δ/wtf62Δ</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>327</td>
<td>45.93%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of octad according to the numbers and genotypes of viable spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>4R4S</td>
</tr>
<tr>
<td>Number of octads</td>
</tr>
</tbody>
</table>
Appendix 21: *wtf21*(SOCG_02322) deletion related numerical data of the octad spore dissection analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>Number of spores that are viable and with the indicated genotype</th>
<th>% Spores that are viable and with the indicated genotype</th>
<th>Transmission distortion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY47925 × DY44286</td>
<td>wtf21Δ/wtf21′</td>
<td>102</td>
<td>704</td>
<td>112</td>
<td>816</td>
<td>86.27%</td>
<td>357</td>
<td>43.75%</td>
<td>42.52%</td>
</tr>
<tr>
<td>DY47926 × DY44287</td>
<td>wtf21Δ/wtf21′</td>
<td>202</td>
<td>704</td>
<td>112</td>
<td>816</td>
<td>86.27%</td>
<td>357</td>
<td>43.75%</td>
<td>42.52%</td>
</tr>
</tbody>
</table>

Type of octad according to the numbers and genotypes of viable spores:

- 4R4S
- 4R3S
- 3R4S
- 4R2S
- 2R4S
- 3R3S
- 4R1S
- 1R4S
- 3R2S
- 2R3S
- 3R1S
- 1R3S
- 2R2S
- 4R0S
- 0R4S

Number of octads:

- 47
- 13
- 16
- 1
- 3
- 9
- 0
- 0
- 3
- 0
- 3
- 0
- 3
- 1
- 0
- 3
Appendix 22: octo-pSIV-leu1-1D plasmid related numerical data of the octad spore dissection analysis.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Diploid Genotype</th>
<th>Number of octad</th>
<th>Number of viable spores</th>
<th>Number of inviable spores</th>
<th>Total number of spores</th>
<th>Viability</th>
<th>% Spores that are viable and with wt25</th>
<th>% Spores that are viable and without wt25</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY47931 × DY47905</td>
<td>leu1Δ::wt25::leu1+ wt25Δ::kanSVE/twt25Δ::kanSVE</td>
<td>56</td>
<td>329</td>
<td>119</td>
<td>448</td>
<td>73.44%</td>
<td>47.32%</td>
<td>26.12%</td>
</tr>
<tr>
<td>DY47934 × DY47905</td>
<td>leu1Δ::wt25::leu1+ wt25Δ::kanSVE/wt25Δ::kanSVE</td>
<td>37</td>
<td>263</td>
<td>33</td>
<td>296</td>
<td>88.85%</td>
<td>45.61%</td>
<td>43.24%</td>
</tr>
<tr>
<td>DY47932 × DY47906</td>
<td>leu1Δ::wt25::leu1+ wt25Δ::kanSVE</td>
<td>40</td>
<td>146</td>
<td>174</td>
<td>320</td>
<td>45.63%</td>
<td>20.63%</td>
<td>3.44%</td>
</tr>
<tr>
<td>DY47933 × DY47905</td>
<td>leu1Δ::wt25::leu1+ wt25Δ::kanSVE</td>
<td>44</td>
<td>252</td>
<td>100</td>
<td>352</td>
<td>71.59%</td>
<td>24.72%</td>
<td>13.92%</td>
</tr>
</tbody>
</table>