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On the Mechanistic Basis of Killer Meiotic Drive in Fungi

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Abstract

Spore killers are specific genetic elements in fungi that kill sexual spores that do not contain them. A range of studies in the last few years have provided the long-awaited first insights into the molecular mechanistic aspects of spore killing in different fungal models, including both yeast-forming and filamentous Ascomycota. Here we describe these recent advances, focusing on the *wtf* system in the fission yeast *Schizosaccharomyces pombe*; the *Sk* spore killers of *Neurospora* species; and two spore-killer systems in *Podospora anserina*, *Spok* and [Het-s]. The spore killers appear thus far mechanistically unrelated. They can involve large genomic rearrangements but most often rely on the action of just a single gene. Data gathered so far show that the protein domains involved in the killing and resistance processes differ among the systems and are not homologous. The emerging picture sketched by these studies is thus one of great mechanistic and evolutionary diversity of elements that cheat during meiosis and are thereby preferentially inherited over sexual generations.

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INTRODUCTION

In sexually reproducing organisms, fair transmission of alleles in meiosis is necessary for natural selection to proceed efficiently. As a result, meiotic segregation of the alleles from both parents most generally operates with Swiss-clockwork precision (9). However not all nuclear genes segregate in a Mendelian fashion (29). Specific genetic elements are able to exert meiotic drive, that is, to skew their transmission to meiotic progeny over the expected Mendelian 1:1 ratio (38, 52). This phenomenon can thus uncouple allele frequency from organismal fitness because the drive element possesses an agenda of its own. As a result, the element itself or linked genetic variations can subsist in the sexual population even if detrimental. The selfish behavior of the drive element may thus instill a genomic conflict between the element and the rest of the genome, leading to emergence of resistance mechanisms. By causing genomic conflict, meiotic drive can have a profound impact on genome evolution (7). The very machinery ensuring faithful Mendelian segregation, the centromere/kinetochore ensemble, is itself shaped by an internal arms race between drive of centromeres in female meiosis and suppressors thereof (33). As the exception that proves the rule, meiotic drive provides a flip side of the entire neo-Darwinian edifice and leaves us marveling at how precisely allele transmission functions generally. Because it has the potential to dramatically alter the genetic structure of a population in just a few meiotic generations, the applied use of meiotic drive as a tool to control pests or improve crops is being considered (2, 19, 26, 69). However, major hazards and challenges must be overcome before drive systems can be applied in the field. Thorough comprehension of naturally occurring drivers seems a desirable preamble to future endeavors in that direction (46).

Meiotic drive has been described in various branches of the eukaryotic tree of life, in particular in mammals, insects, vascular plants, and fungi (7). Historically important and classical examples of meiotic drive are the sex-ratio bias observed as early as 1928 in *Drosophila* (20, 41), the *SD* locus also found in flies (34), the *t* haplotype in house mice (56), and chromosome knobs in maize (60). New instances of gene drive continue to be discovered in nonmodel species, for instance in the Alpine silver ant, *Formica selysi* (e.g., 1). For the classification of meiotic drive systems, two properties of the meiosis process in the considered species are to be taken into account (**Figure 1**). The first is whether meiosis is symmetric, as in fungi or typically in male organs of animals and plants, or asymmetric, as typical in female organs in which only one of four meiotic products is selected to become the egg. This first distinction relates directly to the mechanism of drive (32). In symmetric meiosis, drive relies on some form of interference, by which the meiotic products not carrying the drive element are inviable or otherwise functionally compromised (**Figure 1*a***). These are referred to as killer drivers. During asymmetric meiosis, on the other hand, so-called true drive elements can cheat by favoring their own position such that they are preferentially selected to end up in the egg (a process called gonotaxis) (7, 57). The second property is whether

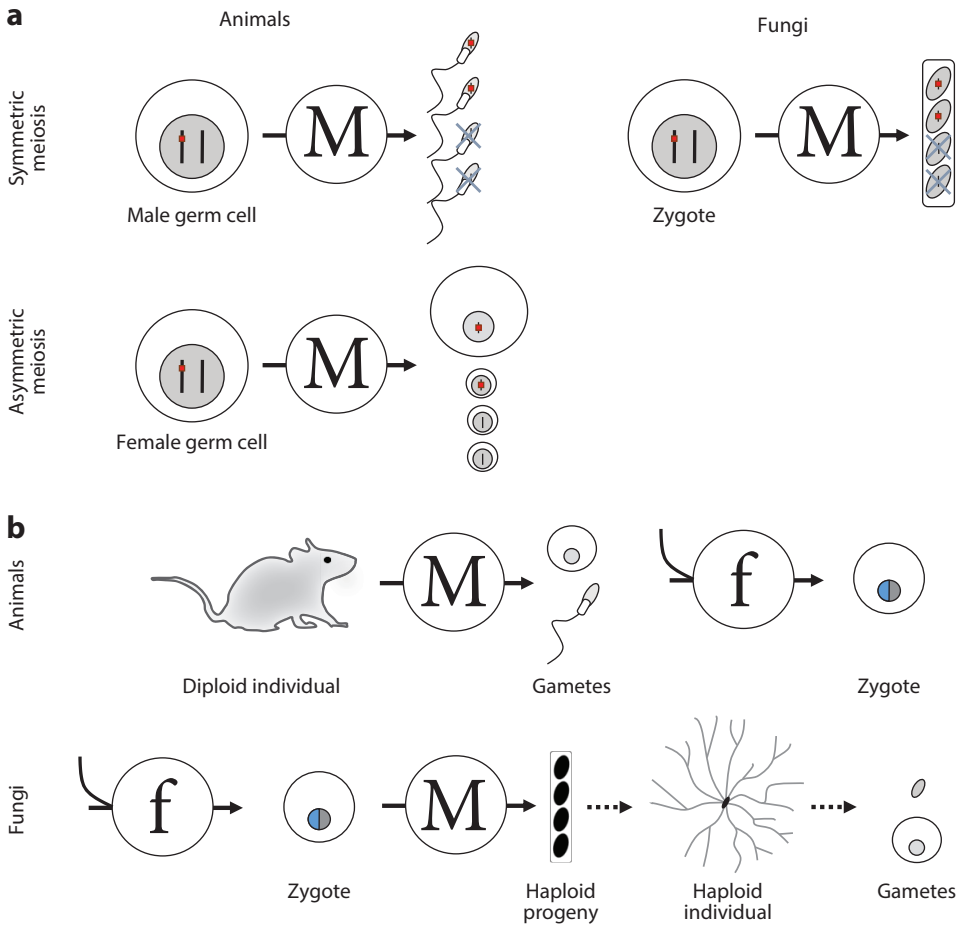

Figure 1

Properties of the meiotic process and their relation to meiotic drive phenomena. (*a*) Distinction of the drive mechanisms in symmetric and asymmetric meiosis. Meiosis is represented by the letter M, and the drive element by a red square. For symmetric meiosis in male animals, drive usually corresponds to the killing or alteration of the gametes not carrying the drive element (killer drive). For asymmetric meiosis in female animals, drive corresponds to the preferential accession of the nucleus or chromosome containing the drive element to the egg cell (gonotaxis, true drive). In fungi, meiosis is symmetric and, as with male drive in animals, leads to killing of the meiotic products not containing the drive element. (*b*) Gametic meiosis as typically found in animals and zygotic meiosis as seen in fungi. For animals, meiosis occurs in the germ cells of a diploid individual and results in the formation of gametes. During fertilization (*letter f*), two gametes fuse to become a zygote, which develops into a diploid individual. In fungi, meiosis occurs in the zygote after fertilization to produce haploid progeny (sexual spores) that germinate and develop into haploid individuals, which in turn differentiate gametes.

meiosis produces gametes, which is typical for animals and vascular plants, or haploid progeny, which is typical for fungi (**Figure 1*b***). This second distinction draws attention to the fact that the detectable manifestations of meiotic drive differ between plant and animal models and fungal models. Meiotic drive in animals and plants is generally hidden. Detection of drive requires genetic or genomic analysis of segregation patterns unless a detectable phenotype is associated to the drive element. In fungi, in contrast, killer drivers directly manifest by causing abortion of a

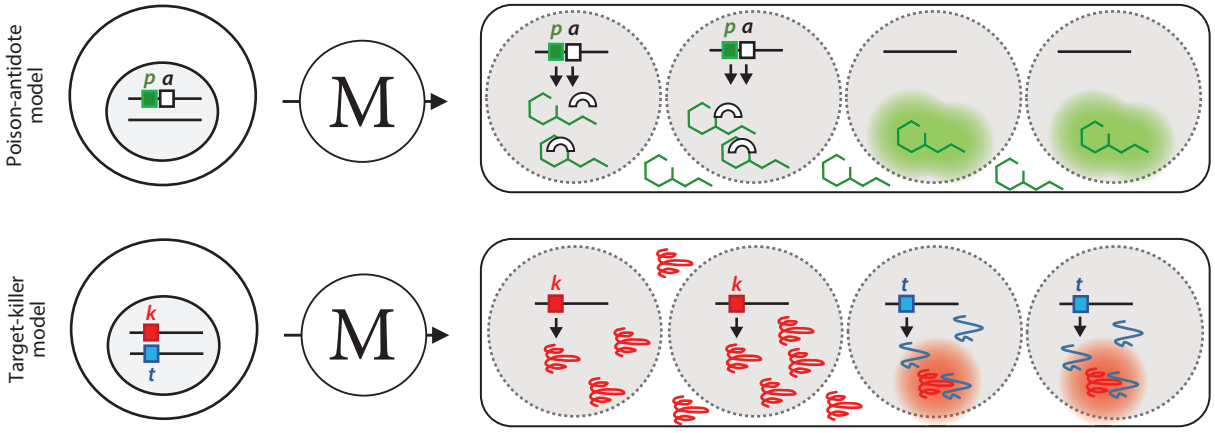

Figure 2

Mechanistic models for meiotic drive. In the poison-antidote model, the drive element expresses two activities: poison (*green*) and antidote (*white*). Genes encoding the poison (*p*) and the antidote (*a*) are linked, preventing recombination between the poison and the antidote genes that would lead to self-killing. Similarly, the mobility or stability of the poison is different from that of the antidote, with the antidote restricted to the resistant spore bearing the driving allele and the poison present in all spores. In the absence of the antidote, the toxin exerts its deleterious effect, symbolized as a green halo. In the target-killer model, the sensitive allele (*t*) expresses the target of a killer product expressed by the driving allele (*k*). This killer product is deleterious only in the presence of the target. The mobility or stability of the killer and target must be different, so that the target is restricted to the sensitive spores whereas the killer product is present in both the sensitive and resistant spores. Interaction of the target and the killer leads to an adverse effect, symbolized as a red halo.

fraction of the meiotic progeny. In Ascomycota, the meiotic spores are packaged into specialized structures termed asci, usually by four, or multiples of four. Drive is detected when spore numbers are abnormal and spore ghosts corresponding to the killed progeny are present (**Figure 1*a***). When comparing meiotic drive occurring in systems where meiosis produces gametes and progeny, it is also important to realize that the impact on fitness differs between these cases. As male gametes are produced in excess, drive in gametic meiosis generally does not impact the final amount of progeny, and as a result, the evolutionary dynamics of drive elements could differ between animal and plant models and fungal models. Hence, specific models need to be developed to predict evolutionary outcomes such as fixation, elimination of the driver, or emergence of suppressors (39, 42). Note that there are also additional meiotic drive phenomena in fungi that do not manifest as spore killing. For instance, supernumerary chromosomes in *Zymoseptoria tritici* exert meiotic drive and are transmitted to all progeny when carried by the female parent (23), but these are not covered in this review.

In fungi, spore killing occurs in crosses heterozygous for the drive element and corresponds to the specific abortion of all meiotic progeny that do not carry the driver (62). A spore-killer element thus has two activities, killing and resistance. Mechanistic studies on spore killers aim to identify the molecular basis of the killing and resistance functions. Put simply, what needs to be explained is how the naive spores die while the spores carrying the element survive. The characterized fungal spore-killer systems can be subdivided into two functional categories that have been referred to as poison-antidote and killer-target (or driver-responder) systems (32, 57) (**Figure 2**). In the poison-antidote model, the element expresses both a toxin and a product that allows resistance to this toxin (antidote). In the killer-target model, the killing activity is conditional and depends on the presence of a target present solely in the sensitive spore. The two models differ genetically. In the poison-antidote model, the sensitive allele is a null allele simply

defined by the absence of the element. In the killer-target model, the sensitive allele participates in the killing process, as it provides the target entity. The poison and antidote genes are linked on the same chromosome, whereas killer and target are located at allelic positions on homologous chromosomes (**Figure 2**). While these two models may cover a range of different mechanistic intricacies, both are based on a differential spatial distribution of the gene products between naive and surviving spores. This differential distribution could take place either before or after spore delineation. Differential distribution could, for instance, be achieved if the antidote is selectively retained in the surviving spores. An alternate model would be that both killer product and antidote diffuse freely in early development but not later on, and that the killer product has a longer half-life than the antidote. In the killer-target model, the killer product is present in all spores but the target is restricted to the sensitive spore. In analogous systems in animals, such as the Segregation Distorter (*SD*) system in *Drosophila*, differential spatial distribution of the target is readily accounted for since the target of the killer activity is the responder genomic locus itself. Taken together, key aspects of the study of spore killer molecular function are focused on the basis of the killing and resistance activities and of their differential spatial distribution.

Recent years have seen substantial progress in the genetic, genomic, and functional characterization of spore-killer elements in different ascomycete fungal species. This first bout of knowledge acquisition now allows comparison of the systems. We review here the study of spore killers in *Schizosaccharomyces pombe*, *Neurospora* species, and *Podospora anserina* with a particular focus on mechanistic aspects before comparing the systems and defining some pressing open questions.

THE *wtf* SPORE KILLERS IN *SCHIZOSACCHAROMYCES POMBE*

The fission yeast *S. pombe* is a single-celled ascomycete that has been widely used as a model system for genetics and cell cycle research alongside the relatively distantly related baker's yeast, *Saccharomyces cerevisiae*. Its natural habitat is not known, but most isolates have been recovered from fermented beverages (30). *S. pombe* shows mating-type switching during growth, and cells enter the sexual cycle upon starvation, conjugate, and form a zygote that generally immediately undergoes meiosis to form a single ascus with four haploid spores (27). The description of spore killers in *S. pombe* is relatively recent, which may be surprising considering the extensive use of this species as a genetic model system. As noted by Lopez Hernandez & Zanders (37), the phenomenon likely remained undetected for so long because development of *S. pombe* as a genetic model system was based on the use of highly isogenic strains whereas meiotic drive requires heterozygosity among mated strains. The discovery of spore killers in that species results from a strenuous effort to tackle the problem of the high level of sterility usually detected in crosses between wild *S. pombe* isolates (70). It was found that part of this hybrid sterility results from the presence of a family of genes that exert spore-killing meiotic drive. In *S. pombe*, spore-killing drive is monitored by measuring the biased transmission of genetic markers and by propidium iodine staining to detect the dead spores (43). Using recombination mapping approaches combined with next-generation sequencing in hybrid crosses, two distinct research groups at the same time reported genes of the *wtf* gene family as being responsible for meiotic drive (28, 43). This gene family of 25 members frequently associated with transposons (*wtf* stands for with Tf transposons) had previously been noted as the largest gene family specific to *S. pombe*. The gene family was poorly characterized, but several members were known to be highly upregulated during meiosis. Furthermore, its remarkable expansion and its nearly exclusive localization on chromosome 3 eluded explanation (4). The two research groups demonstrated that specific members of the *wtf* gene family act as autonomous spore-killing elements, independently from their genomic location or association with transposable elements. They do not require specific responder sequences in the naive genome to

drive (28, 43), pointing to a poison-antidote model for killing. In addition, the killing and resistance functions were found to be genetically separable, raising the question as to how these two functions could be carried out by a single gene (28). Due to its efficient killing, *wtf4* was used as model gene to investigate the mechanism of *wtf* drive. It turns out that the *wtf4* gene generates two alternate transcripts directing expression of two different proteins, a long product and a short product that differ by an N-terminal ~45–amino acid residue extension specific to the long product. After specifically mutating the start codons directing translation of the alternative constructs, researchers were able to attribute the poison and antidote functions to the short and long products, respectively (43). Consistent with the expectation of a poison-antidote killer, the short wtf4^{poison} product is found in the entire ascus while the longer wtf4^{antidote} protein appears to be restricted to the surviving spores. Using both *S. pombe* and the heterologous host *S. cerevisiae*, it was shown that both wtf4^{poison} and wtf4^{antidote} proteins are aggregation prone. The current mechanistic model for poison and antidote functions poses that wtf4^{antidote} coassembles with wtf4^{poison} aggregates and alleviates their toxicity by modifying their properties or cellular localization (44) (**Figure 3*a***). The relation of wtf4^{poison} and wtf4^{antidote} proteins could be recapitulated in vegetative cells of *S. cerevisiae*, suggesting a rather general toxicity mechanism. Nevertheless, the cellular localization of wtf4 aggregates differs between the species (vacuolar localization in *S. pombe* versus perivacuolar in *S. cerevisiae*). Therefore the *S. cerevisiae* model may not recapitulate all aspects of the process occurring during *S. pombe* meiosis. Researchers using high-throughput systematic protein localization approaches also report vacuole localization of wtf4 as well as the formation of cytoplasmic dots (40). The wtf4 protein is very hydrophobic and contains six predicted transmembrane helices. The N-terminal extension of wtf4^{antidote}, in turn, is rich in charged residues. Hence, it might be that this extension renders the aggregates less compact or reduces their overall hydrophobicity and therefore somehow alleviates their toxicity (44).

A striking feature of the *wtf* family, noted before its link to meiotic drive was discovered, is the high number of gene copies in the *S. pombe* genome—25 to 38 copies depending on the isolate. At the same time, the gene family is restricted to this genus (4, 18). Phylogenetic analyses indicate that members of this gene family frequently undergo nonallelic gene conversion. These events could be related to the fact that *wtf* genes show preferential association with double-strand break hot spots. *wtf* genes were also found to display an excess of nonsynonymous substitutions and repeat-number variation of a functionally important motif located within the coding sequence (18). *wtf* genes were classified based on gene architecture as predicted *wtf4*-like poison-antidote killers or antidote-only copies and systematically tested for drive and resistance function. These functional analyses indeed revealed that several other members of the gene family function as either bona fide killer drivers or antidote-only suppressors (6). The sequence homology between the driver and suppressor appears important to allow for suppression. Remarkably, at the same time, highly divergent *wtf* genes can similarly exert drive. The sequence identity between two separately active drivers can be as low as 30%. This apparently paradoxical observation could be explained by considering the current mechanistic model of *wtf* function and that high hydrophobicity and a related aggregative behavior, which can be achieved with a great variety of sequences, are central determinants for *wtf* poison function. It is interesting to note that the number of predicted helices varies in the different *wtf* homologs, and one could envision that the predicted transmembrane helices of *wtf* gene products are an ancestral remnant of a membrane-related function. In that hypothesis, a key event in the conversion of the *wtf* gene into a driver could be protein mislocalization. At any rate, the genomic landscape of the *wtf* gene family is such that many distinct (sometimes highly divergent) poison-antidote killer drivers coexist within the genome together with antidote-only suppressors. The killer drivers and suppressors in the many possible pairwise interactions may or may not display epistatic interactions leading to mutual killing or mutual

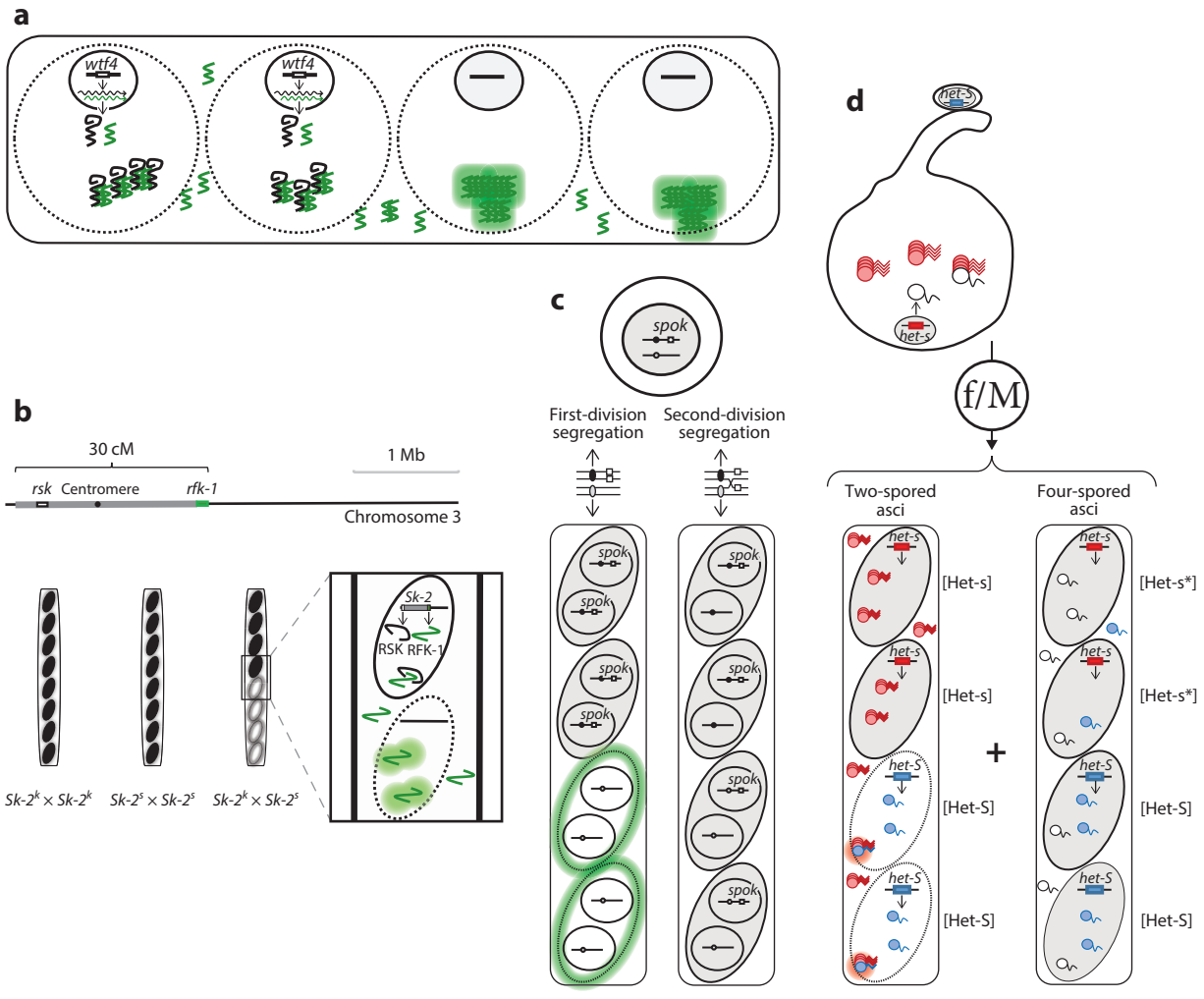

Figure 3

Spore-killer systems in different fungal models. (*a*) In *wtf4* spore-killing in *Schizosaccharomyces pombe*, the *wtf4* gene produces two different transcripts leading to the synthesis of two different proteins: a long product (*black*) and a short product (*green*), differing by an N-terminal extension. The short product is wtf4^{poison} and the long product wtf4^{antidote}. Both proteins are aggregation prone and are able to aggregate separately or together. wtf4^{poison} aggregates are toxic, but mixed wtf4^{poison}/wtf4^{antidote} aggregates are not. wtf4^{poison} is found in both sensitive and resistant spores, while wtf4^{antidote} is restricted to resistant spores. (*b*) The *Sk-2* locus in *Neurospora* spans a 30-cM recombination block region on chromosome 3 and is over 2 Mb long. The killer (*rfk-1*) and resistance (*rsk*) genes are found close to the borders of the recombination block. While an *Sk-2^s* × *Sk-2^s* cross and an *Sk-2^k* × *Sk-2^k* cross both yield normal eight-spored asci, in an *Sk-2^k* × *Sk-2^s* cross all *Sk-2^s* progeny are killed. The product of the resistance gene is proposed to act as an antidote of the toxic *rfk-1* product. The distribution for the poison and antidote has to be different, with the antidote *rsk* product restricted to the resistant spores. (*c*) Outcome of first- and second-division segregation of a *Spok* gene in *Podospora*. When the driver undergoes first-division segregation (no crossing over between the driver and the centromere), two-spored asci are produced. In the case of second-division segregation, all spores contain the *Spok* gene and no killing occurs. (*d*) Female drive caused by the [Het-s] prion in *Podospora*. In a cross performed at 18°, and when the maternal parent is [Het-s], a variable percentage of the asci (15–70%) are two-spored and show specific killing of the *het-S* progeny. The *het-s* progeny from two-spored asci yield [Het-s] (prion-infected) individuals. In the four-spored asci, the *het-s* genotype of the progeny yields [Het-s*] (prion-free) individuals. Killing occurs because expression of the HET-S protein in sensitive spores in the presence of [Het-s] prions leads to activation of the HeLo pore-forming domain. Abbreviations: f, fertilization; M, meiosis.

resistance, specific suppression of killing, or lack of suppression of killing. Within this exuberant genetic context, an additional oddity that calls for an explanation is the specific expansion of the *wtf* family on chromosome 3 (23 of 25 *wtf* copies in the reference strain are located on the smallest of the three *S. pombe* chromosomes) (4). Lopez Hernandez & Zanders (37) proposed that the burden of multiple drivers could be overcome specifically on that chromosome because it is the only one for which aneuploidy is tolerated, allowing for occasional formation of aneuploids that can escape the multiple-driver burden. An alternate explanation is that chromosome 3 has been initially introgressed from an ancestral strain that was replete with *wtf* gene copies on all three chromosomes (4).

One of the incentives to approach the study of meiotic drive in *S. pombe* was the perspective of making rapid progress by leveraging the tractability of this genetic system with a small genome, easy access to meiotic products, and very short generation time (37). This choice has paid off, with just a few years separating the initial genetic observation of meiotic drive in that species (70) and the characterization of an entire family of killers (5, 28, 43), description of some of its remarkable evolutionary dynamics (6, 28), and a plausible mechanistic model explaining killing and resistance (43, 44).

SPORE KILLERS IN *NEUROSPORA* SPECIES

Neurospora crassa is a filamentous ascomycete that has been extensively used as a genetic model for nearly a century. It is sexually self-incompatible at the haploid stage (heterothallic), meaning that two strains of opposite mating type must meet for the sexual cycle to be completed. Its common natural habitat is burnt vegetation, but some aspects of its life style in the wild remain to be clarified. As early as 1968, extensive sampling of the genus was carried out, and with a large collection of wild isolates available, *Neurospora* became a favorable system for population genetics and genomics (21, 64). Three distinct spore killers have been identified in species closely related to *N. crassa*, namely, *Sk-1* in *Neurospora sitophila* and *Sk-2* and *Sk-3* in *Neurospora intermedia* (62, 63). The title of the original paper reporting their existence synthesizes the essence of the drive process: "Spore Killer, a Chromosomal Factor in *Neurospora* That Kills Meiotic Products Not Containing It" (62). *Sk-2* and *Sk-3* are very rare and have been isolated in only four and one *N. intermedia* isolate, respectively. *Sk-1* is more prevalent and found in about 15% of the *N. sitophila* isolates. Early on, *Sk-2* and *Sk-3* were introgressed into *N. crassa*, facilitating their genetic dissection, while genetic analysis of *Sk-1* somewhat lagged behind. *Sk-2* and *Sk-3* reside in the same genetic location spanning a large, 30-cM recombination block surrounding the centromere of chromosome 3 (**Figure 3*b***). *Sk-2* and *Sk-3* are thus large, complex meiotic drive elements that in this aspect resemble meiotic drive elements found in animals and plants. *Sk-2* and *Sk-3* show mutual killing (an *Sk-2* × *Sk-3* cross produces no surviving spores) and have therefore been treated as separate entities. In addition to sensitive and *Sk-2* and *Sk-3* killer strains, neutral strains (that neither kill nor are killed) are found in the wild. While some display resistance to either *Sk-2* or *Sk-3*, others are resistant to both (62). *Sk-2* and *Sk-3* are thus distinct entities but show similarities such as genetic location and a shared resistance locus residing at one end of the recombination block. Molecular characterization of the *Sk-2* and *Sk-3* systems started with the identification of the *rsk* (resistance to spore killing) resistance gene rather than identification of the killers (24). This was made possible by the use of a resistant strain in which the *rsk* locus is not associated with the recombination block, allowing for its characterization using conventional mapping approaches. *rsk* is a single gene existing as different alleles that have distinct indel patterns and that confer either sensitivity or resistance. How the sequence difference in RSK proteins relates to resistance or lack thereof is currently unknown, and the RSK proteins contain no known domains and show

no homology to proteins of known function. Importantly, deletion of the *rsk* allele in a sensitive background does not suppress killing, indicating that sensitive *rsk* alleles do not represent an *Sk-2* (and *Sk-3*) killer target but rather that resistant *rsk* alleles function as antidotes. As a result, a cross between a sensitive Sk^S strain and an *Sk-2* Δ*rsk* strain is completely barren and produces no ascospores. This phenotype was used to select for mutations in the *Sk-2* killer element that restore ascospore formation in this context (25, 50). This approach led to the genetic identification of six alleles of the *rfk-1* (required for spore killing 1) gene. In an experimental setup involving recurrent three-point crosses and the assembly of short-read contigs from the *Sk-2* region, *rfk-1* could be mapped inside of the *Sk-2* recombination block near the end of the block opposite to *rsk*. A-to-I editing during sexual reproduction has been described in different filamentous fungi including *N. crassa* (35). The *rfk-1* transcript undergoes functionally relevant A-to-I editing during the sexual phase (50). A premature stop codon is edited in a fraction of the transcripts, and *rfk-1* is thus predicted to lead to the production of two polypeptides: a short, 102–amino acid version termed $RFK\text{-}1^A$ (translated from the unedited transcript) and a long, 130–amino acid version, $RFK\text{-}1^B$ (a product of the edited transcript). $RFK\text{-}1^B$ (but not $RFK\text{-}1^A$) is toxic to vegetative cells, and this toxicity is suppressed by coexpression with $RSK^{Sk\text{-}2}$ (49). Although specific spore-killer activity of $RFK\text{-}1^B$ is not yet demonstrated, the experiments performed in vegetative cells strongly suggest that the long version, $RFK\text{-}1^B$, is responsible for spore killing. In this model, stage-specific editing of the *rfk-1* transcript could be a way to prevent self-killing during the vegetative stage. Remarkably, it was reported recently that A-to-I editing of a transcript of the Sk^K spore-killer locus of *Fusarium verticillioides* is also required for spore-killing activity, pointing to a more general role of editing in fungal meiotic drive (36). *Rfk-1* shows some homology to another gene of *N. crassa* (gene ID NCU07086), and it is proposed that *rfk-1* evolved from a partial duplication of this gene. The two RFK-1 products share a hydrophobic N-terminal region homologous to the NCU07086 product. *Sk-2* spore-killing conforms to the poison-antidote model (**Figure 3*b***), but protein sequences of RSK and RFK-1 did not provide much of a hint on a possible killing/resistance mechanism. Both proteins seem to have a very narrow phylogenetic distribution and to be essentially absent outside of the *Neurospora* genus, although some distant *rsk* homologs are found scattered in a few additional Sordariomycetes, in particular in *Trichoderma* species.

The comparison of *Sk-2* and *Sk-3* required long-read sequencing approaches to allow for reconstruction and comparisons of the entire *Sk* regions. This approach revealed the existence of large inversions in the ∼2- to 3-Mb *Sk* regions, and importantly the set of inversions were totally different in *Sk-2* and *Sk-3* (58). In fact, the two non-recombining regions do not share any inversion or phylogenetic signal that would have suggested a common descent. In addition, inspection of the *Sk*-3 region revealed that killing does not rely on an *rfk-1* homolog (which means that the gene or genes responsible for killing in *Sk-3* remain to be identified). So remarkably, although they share a common resistance gene and a common genetic location, *Sk-2* and *Sk-3* meiotic drive elements have evolved convergently. Knowledge about *Sk-2* might aid in the identification of the killer in *Sk-3*. The genomic location of *rfk-1* close to noninverted sequences capable of pairing in meiosis appears critical. Indeed, it was reported that moving *rfk-1* by as little as 1.4 kb exposes it to meiotic silencing by unpaired DNA (MSUD) and suppresses meiotic drive. MSUD occurs in several fungi and leads to RNA interference–based silencing of sequences that are unpaired during meiosis (55). It might thus be expected that the killer element in *Sk-3* should be located relatively close to the introgression site unless some other mechanism allows for suppression of MSUD (48). The convergent evolution of *Sk-2* and *Sk-3* in the same genomic region might be explained if *rsk* is older than the killer elements, so that both killer elements might have relied on an already existing resistance gene (58). Consistent with this model is the narrower phylogenetic distribution of *rfk-1* as compared to *rsk*.

Molecular characterization of *Sk-1* progressed recently in one big leap made possible by implementation of next-generation sequencing approaches and the utilization of the large culture collection of *Neurospora* carried out by the community of fungal biologists. Long-read sequencing revealed that in contrast to *Sk-2* and *Sk-3*, *Sk-1* strains do not contain large chromosomal rearrangements when compared to sensitive strains (59). Genome-wide association studies combined with deletion and knock-in approaches indicate that *Sk-1* is a single-gene driver. At the locus in which sensitive strains contain a gene termed NCU09865, *Sk-1* has a truncated version of this gene and ~1 kb of a novel sequence showing a limited homology to another gene (NCU01957). This gene, termed *Spk-1* (spore killer 1), yields a 1,450-bp transcript displaying two open reading frames (ORFs), of which only the first (ORF1) is responsible for killing and resistance. ORF1 encodes a 134–amino acid polypeptide with no known domains but two predicted transmembrane regions. A role for the translation product of ORF2 was ruled out by showing that a mutant form lacking the ORF2-initiation codon was affected for neither killing nor resistance. However, the corresponding region in the transcript appears to play some functional role, as deleting this region affects killing. Phylogenetic and population genomics approaches revealed that *Sk-1* most likely has been introgressed into *N. sitophila* from another species (*Neurospora hispaniola*). The population structure of *N. sitophila* revealed three well-resolved clades in which *Sk-1* is unevenly distributed. While both *Sk-1* and the sensitive allele are found in clade 1, clade 2 appears fixed for the sensitive allele and clade 3 for *Sk-1*. Due to the described genomic structure of *Sk-1*, it is expected that *Spk-1* is exposed to silencing by MSUD, as the sequence difference between the killer and sensitive allele should lead to unpairing. Nonetheless, killing operates at a high efficiency. Svedberg et al. (59), however, noted that when backcrosses increased the overall relatedness between killer and sensitive allele, *Sk-1* killing efficiency receded. This decrease in killing efficiency is proposed to result from an increased efficacy of MSUD when strain relatedness increases. Consistent with this hypothesis is the observation that Δ*sad-2* mutants defective for MSUD restore killing in these inbred crosses. It is tempting to put this observation in parallel with the introgressed origin of *Sk-1*. It might be that some level of strain divergence is required to allow for *Sk-1* driving to occur, which in turn would mean that population structure and the genetic distances between strains from different clades play a role in the population dynamics of the driver.

Following their discovery by Turner & Perkins (62) as part of the effort to develop the population genetics of the *Neurospora* genus, the *Sk-1*, *Sk-2*, and *Sk-3* spore killers were long the prototypes for fungal spore killers. As such, they have intrigued and frustrated several generations of *Neurospora* geneticists. Classical mapping approaches, more recently aided by next-generation sequencing, have not only allowed for the identification of the molecular players at work but also uncovered unexpected evolutionary peculiarities such as the convergent evolution of *Sk-2* and *Sk-3* and the introgressed origin of *Sk-1*. The *Sk-2*/*Sk-3* and *Sk-1* systems appear in strong contrast to each other, with the former comprising a large, complex rearrangement region with separable killer and resistance components and the latter being a single-gene driver. Although the *Sk-3* killer element remains to be identified, in all three cases, a poison-antidote model is favored since a driver-target model is not consistent with available genetic information.

Spok AND [HET-S] SPORE KILLERS IN *PODOSPORA ANSERINA*

Podospora anserina is a filamentous ascomycete inhabiting herbivore dung and was developed as a genetic model mainly due to the work of Georges Rizet in the 1950s in France. Arguably, the first reported observations of spore killers in fungi occurred in *Podospora*, although they were at that time not designated so, nor conceptualized as meiotic drive elements (3, 45). *P. anserina* produces four-spored asci, and spore killing manifests typically as the formation of two-spored asci (**Figure 3*c***). This apparent simplicity conceals a more complex nuclear distribution process

during meiosis that is relevant to comprehending the genetics of spore killers in this species. After meiosis, a postmeiotic mitosis takes place and spindle orientation and nuclear packaging are such that in each half-tetrad, two nonsister nuclei are enclosed in each spore. Spores are thus initially binucleate and heterokaryotic for all markers showing second-division segregation in the cross but homokaryotic for markers that show first-division segregation (i.e., are centromere-linked) (**Figure 3*c***). Due to interference, there is an obligate crossing-over between the centromere of chromosome 1 and the mating-type locus. As a result, all ascospores are heterokaryotic for the mating-type locus and lead upon germination to a self-fertile mycelium, making *P. anserina* a secondary homothallic (or pseudohomothallic) species. If a spore-killer locus undergoes first-division segregation, two spores of the same half-tetrad will be homokaryotic for the killer and will survive while the two other spores containing only sensitive nuclei will succumb. If the drive element undergoes second-division segregation, binucleate spores will be heterokaryotic and carry a killer and a sensitive nucleus. In that case, the killer nucleus shields the sensitive nucleus and all four spores survive (**Figure 3*c***). Practically, this means that the frequency of first-division segregation of the killer determines killing percentage, so that a killer closely linked to the centromere will show close to 100% killing while killing percentage will decrease as the distance to the centromere and hence the frequency of second-division segregation increase.

A survey of a collection of wild *P. anserina* isolates from the Wageningen region in the Netherlands revealed the presence of at least six distinct spore-killer types (designated *Psks*) that could be distinguished based on genetic position and mutual killing (66). It was found that 23% of the wild isolates contained at least one spore killer. This pioneering study thus revealed the frequent occurrence and diversity of meiotic drive elements in this species. Grognet and coworkers (22) took up molecular identification of spore killers in this species. Mirroring the initial observation by Bernet and Padieu, they found that in a cross between *Podospora* strain T (now described as a different species, *Podospora comata*) and the S reference strain of *P. anserina*, a spore killer linked to the centromere of chromosome 5 segregates (45). This drive element was backcrossed into S and mapped, and the corresponding gene was identified and named *Spok1*. Gene disruption of *Spok1* abolishes killing and renders the strains sensitive to killing. *Spok1* can function as a spore killer when inserted ectopically (22). *Spok1* thus behaves as an autonomous single-gene killer. Again, a poison-antidote model had to be favored over a target-killer model. Grognet et al. (22) were careful to also analyze the effect of the inactivation of *Spok1* in the original T background, and in a remarkable twist, this allowed for the identification of a second *Spok* gene. Indeed, as a complete surprise, in a T Δ*Spok1* × S cross, 40% of asci were two-spored. This spore killing is in fact caused by a *Spok* homolog termed *Spok2* found in the S strain (*P. anserina*) but absent in T (*P. comata*). *Spok1* confers resistance to *Spok2*, so that *Spok2* killing could only be revealed in the absence of *Spok1* (as explained above, the 40% killing percentage is determined by the chromosomal position of *Spok2*). Informed by both the spore-killer type (*Psk*) population survey and the *Spok* gene characterization, Vogan and coworkers (68) used next-generation sequencing approaches and extensive crossing between wild isolates and single-gene knock-ins to provide a global view of the structure and distribution of spore-killer types and their relation to *Spok* genes in *Podospora*. Two additional *Spok* homologs behaving as autonomous single-gene killers (*Spok3* and *Spok4*) exist in that species. The three *Spok* genes found in *P. anserina* behave independently and show mutual killing dominance, whereas the interactions with *Spok1* (found only in *P. comata*) are more complex, with *Spok1* showing killing dominance over *Spok2* and *Spok3* but mutual resistance to *Spok4*. *Spok3* and *Spok4* should be considered the only actual drivers in *P. anserina* because *Spok1* is found only outside of *P. anserina* and *Spok2* is fixed in that species. *Spok3* and *Spok4* are found in a specific genome architecture, and they are always associated with a large region of 110 to 247 kb termed the *Spok* block. When present, the *Spok* block is found in only one of several locations on chromosome 3

or on either arm of chromosome 5 (67, 68). In these different locations, the *Spok* block can carry either *Spok3* or *Spok4* or both. It is the combinatorial association of *Spok3* and *Spok4* in different chromosomal sites that accounts for the diversity of the spore-killer types (*Psks*) in natural populations. A 6-bp RGGTAG motif is present at the end of all known *Spok* blocks, and it is proposed that the *Spok* block derives from a large transposable element termed *Enterprise* that is mobilized by a specific tyrosine recombinase (encoded by a gene termed *Kirc* residing in *Enterprise*) (67). Mobile *Enterprise*-like elements are also found in other fungal species. The association between *Spok* genes and *Enterprise* to form the *Spok* block could reflect that the capture of a driving *Spok* gene confers an advantage to the transposable element or alternatively that *Spok* genes benefit from the genomic mobility permitted by *Enterprise*. In particular, due to the peculiarities of spore formation in *P. anserina*, the voyage of a *Spok* gene closer to a centromeric location would increase killing percentage and thus driving. Similarly, regular mobility of the drive element could prevent the accumulation of deleterious mutations hitchhiking with the driver. It is difficult at present to decide whether *Enterprise* exploited *Spok* or if it is the other way around, and it is possible that both elements benefited from their association. This amounts to suggesting that a selfish genetic element has exploited the selfishness of another, or else—in a dialectic style—that two selfish elements interacted for their common good.

Different protein domains could be annotated in the SPOK proteins, in particular, with a predicted nuclease domain in the N-terminal part and a predicted kinase domain in the C-terminal region. Deletion constructs and point mutations allowed for a functional dissection of the protein into a toxicity domain encompassing the N-terminal nuclease domain and a resistance domain encompassing the C-terminal kinase domain (68). Specifically, a point mutation of the predicted nuclease active site inactivates the killing activity while a point mutation of the kinase predicted active site inactivates the resistance function. Thus, while the poison and antidote activities are apparently carried out by the same protein, they are separable and attributable to distinct domains and predicted enzymatic activities. A recent report supports the hypothesis that killing is related to a nuclease activity. When expressed in *E. coli* or *S. cerevisiae*, a *Spok* gene mutant affected for the resistance domain enacts killing apparently by targeting DNA (65). How sequence differences between the different *Spok* genes determine killing and resistance specificity is currently unknown.

[Het-s], the other (and first-described) spore-killing system in *Podospora*, results from the interaction during the sexual cycle of genes whose function had already been studied in the vegetative phase (10). *het-s* is the first heterokaryon incompatibility gene described in *Podospora*. *het* genes are polymorphic genes found in many filamentous fungi that lead to the regulated death of heterokaryotic cells formed by vegetative fusion of unlike strains (14). One adaptive role of these genes is to prevent the transmission of viruses between strains (mycoviruses are common in the wild and transmitted between strains during cell-cell fusion events). The *het-s* locus can be occupied by two alternate incompatible alleles, *het-s* and *het-S* (51). The HET-s protein self-assembles into an infectious amyloid form (in other words, it forms a prion). The soluble state of the protein leads to a phenotype designated [Het-s*], whereas the prion state is designated [Het-s]. Importantly, only (prion-infected) [Het-s] strains are incompatible with HET-S. This is because the amyloid-forming region of HET-s interacts with the homologous region from the HET-S protein and acts as a template to convert it to the amyloid fold. This amyloid conversion then induces a conformational change in the N-terminal globular domain of HET-S, termed HeLo, which expels an N-terminal hydrophobic transmembrane helix and turns into a pore-forming protein that relocates to the cell membrane and compromises membrane integrity (54). The HET-s protein also displays a HeLo domain, but a specific mutation in the N-terminal region forming the transmembrane helix abolishes its toxic activity. Therefore amyloid conversion of HET-s is innocuous. In this system, cell death is brought about because the [Het-s] prion acts as an inducer

of the toxic activity of HET-S. HET-S is in fact involved in a second distinct cell death induction system involving a NOD-like receptor (NLR) termed NWD2, which is encoded by the gene immediately adjacent to *het-S* (13). NLRs are intracellular immune receptors found in animals, plants, and fungi (17). A subclass of the fungal NLR genes occur as two-gene clusters associating a gene encoding an NLR (like NWD2) and a gene encoding its cognate cell death effector (like HET-S) (53). The signaling process between the NLR and the cell death execution protein similarly involves amyloid templating. NWD2 displays in its N-terminus a sequence homologous to the amyloid-forming region of HET-S/s. This region can adopt a HET-s-like fold and induce amyloid conversion and activation of HET-S (13).

While vegetative encounters between [Het-s] and [Het-S] are lethal, the strains are sexually compatible and usually show normal fertility, with asci typically containing two *het-s* and two *het-S* spores (because *het-s* is centromere-linked and shows high first-division segregation). However, Bernet (3) observed that when the cross is performed at 18°C and when the maternal parent in the cross is [Het-s], a variable fraction of the asci are two-spored, with the surviving spores invariably of the *het-s* genotype (**Figure 3*d***). Importantly, in two-spored asci the *het-s* spores are prion-infected ([Het-s]), while in four-spored asci the *het-s* spores are prion-free ([Het-s*]). The specific properties of this killing system can readily be explained based on knowledge acquired of [Het-s]/HET-S incompatibility in the vegetative phase to build a plausible model for [Het-s] spore killing. In that model, maternally inherited [Het-s] prions present in the maturating ascus are enclosed in prespores of the *het-S* genotype. When expression of HET-S occurs in these spores, the pore-forming toxin activity of HET-S is induced, resulting in death of the *het-S* spores (10, 11) (**Figure 3*d***). Consistent with this model, HET-s overexpression, which favors formation and maintenance of the [Het-s] prion form, increases killing efficiency and abolishes temperature dependence. [Het-s] aggregates can be detected in the ascus and spores being killed. A specific truncated version of HET-s [HET-s(157–289)] very proficient for fibril formation can lead to paternal transmission of [Het-s] prions, and in this case male [Het-s] drive is also observed (11). The relation of prion stability and spore-killing efficiency was exploited to quantify the effect of single–amino acid changes on the prion properties of [Het-s]. The effect on spore killing mirrored the effect on prion propagation in vegetative mycelium (12). The major differences between [Het-s] spore killing and other spore-killing systems in fungi are that in the former, (*a*) killing has a maternal effect and (*b*) in dikaryotic spores, nuclei containing the killer do not protect sensitive nuclei. [Het-s] spore killing is best described as a driver-responder model where the driver is the [Het-s] prion and the responder is the HET-S pore-forming toxin (the driver-responder designation seems better-suited here than killer-target). It should be noted that the situation in which drive occurs (outcross to *het-S*) also leads to [Het-s] loss (**Figure 3*d***). [Het-s*] strains are formed exclusively through outcrossing to *het-S*. In other words, the drive situation partially inactivates the driver. [Het-s*] strains resemble resistant strains in other drive systems (they neither kill nor are killed), but their status is epigenetically determined.

A survey of a natural population of 112 individuals revealed a 2:1 ratio of *het-s* to *het-S* genotype and a 92% [Het-s] infection rate among the *het-s*-isolates (15). The *het-s* to *het-S* ratio thus deviates from the equilibrium that is expected for alleles under balancing selection and documented for other *het* loci. Consistent with this higher representation, *het-s* strains also show higher rates of infection by a deleterious senescence plasmid. Based on the existence of the HET-S/NWD2 pathway, it is expected that *het-s* strains (deprived of that defense pathway) should suffer some fitness cost. It is thus plausible that the excess of *het-s* alleles in the population results from [Het-s] meiotic drive but that fixation of *het-s* is prevented because of the balancing selection acting on the incompatibility function and because of a potential fitness advantage associated to the *nwd2/het-S* haplotype (15). The epigenetics of [Het-s] could add another level of complexity here because

[Het-s] prevalence and *het-s*/*het-S* allele frequencies are expected to be interdependent. [Het-s*] strains (formed through outcrossing to *het-S*) can be reinfected by contact with other [Het-s] strains. Therefore, the rate of prion loss and reinfection is expected to be partially dependent on the prevalence of *het-s* and *het-S* in the population (with high prevalence of *het-S* favoring the [Het-s*] state). In turn, high prevalence of the [Het-s*] phenotype is expected to favors *het-S* because in the [Het-s*] state, drive and balancing selection are not operative.

Both initially described by Bernet, the *Spok* and [Het-s] meiotic drive systems in *Podospora* show marked differences, in that sense mirroring the sharp contrast between complex *Sk-2*/*Sk-3* drivers and the genomically simple *Sk-1* in *Neurospora* (3, 45, 58, 59). Given the relatively common occurrence of *Spok 3* and *Spok4* genes and [Het-s] wild strains, meiotic drive is to be considered common in that species. The high prevalence of drivers in *Podospora* is potentially connected with the secondary homothallic lifestyle with infrequent outcrossing. Drivers might be frequent in that species, but they might in fact rarely drive. The low measured prevalence of [Het-s*] in the wild might reflect this infrequent outcrossing.

A DIVERSITY OF KILLERS, COMPARISON OF THE DIFFERENT SYSTEMS

While the progress made in the characterization of spore killers in fungi these last few years is undeniable, a number, if not all, of these systems retain part their inherent mystery—which underpins the fascination they inspire in the fungal geneticist. Now that molecular details are at hand for five systems (*wtf*, *Sk-2*, *Sk-3*, *Sk-1*, *Spok*, and [Het-s]) from three genera (*Schizosaccharomyces*, *Neurospora*, and *Podospora*), the systems can be compared in terms of mechanisms, genomic architecture, and mode of emergence. While some common features emerge, none seems to be shared by all systems.

A first example of a commonality includes protein aggregation: At least two of the spore-killer systems, [Het-s] and *wtf*, involve protein aggregation as part of their killing mechanism. This similarity between the [Het-s] and *wtf* systems appears, however, only superficial. [Het-s] amyloid aggregates do not represent the toxic entity but rather a trigger of the pore-forming activity of HET-S, while in *wtf* killing, direct toxicity of protein aggregates is invoked. Considering this role of protein aggregates in *wtf* killing, it is of note that like *wtf* proteins, the *Spk-1* and *rfk-1* products also display highly hydrophobic regions. This meager resemblance might, of course, be of no relevance to the toxicity mechanism. Nevertheless, the *wtf* system illustrates that toxicity and resistance mechanisms based on modulation of protein aggregation can be achieved by a variety of protein sequences (a concept also underscored by the diversity of proteins or protein fragments causing proteinopathies in humans) (6, 8). One highly speculative model is that *Spk-1* and *rfk-1* toxicity is also mediated by protein aggregates. In this hypothesis, gene portions encoding hydrophobic segments, initially intended to be embedded in membranes, are a reservoir for aggregation-prone sequences when gene truncations lead to protein mislocalization. This protein aggregate toxicity model is, of course, not applicable to all systems. The *Spok* genes, for instance, appear to rely on a different mechanism altogether that involves the activity of a predicted nuclease domain (65, 68).

In several of the systems (*wtf*, *rfk-1*, [Het-s], and *Spok*), the killing activity can be transposed to vegetative cells and sometimes heterologous hosts. This observation probably reflects the fact the underlying toxicity mechanisms are rather general and apparently not directly connected with a specific spore maturation pathway. Combined with the common occurrence of single-gene killers, the existence of general killer toxicity mechanisms is probably good news regarding the prospect of using natural fungal drive systems for pest control in a heterologous context (19). Yet, these two

properties also call for caution in the development of such approaches, as the single-gene generalist drivers might more easily drive across species boundaries (as illustrated by *Sk-1*'s evolutionary history) (59).

With the caveat that there are a small number of examples at hand, it appears that poison-antidote single-gene drivers are relatively common, as they occur in three of the six systems considered here (*wtf*, *Spk-1*, and *Spok*). These single-gene poison-antidote drivers pose two central conundrums related to (*a*) the presence of two activities due to a single gene and (*b*) the differential spatial distribution of the antidote and poison. In this respect, the *wtf* model can serve as a guide to the other systems. The separable poison and antidote functions are carried out by different products of the same gene. The two products have different distributions in the ascus, with the antidote being restricted to the surviving spores (43, 44). These differences in distribution could be explained by differential stability or mobility of the two distinct wtf^{poison} and $wtf^{antidote}$ proteins. However, the problem gets really thorny when we consider single-gene drivers for which only one product is known (*Spok* and *Spk-1*). A possible solution would be that these other poison-antidote single-gene drivers also yield two different products by yet to be determined mechanisms that are distinct from formation of alternative transcripts.

Regarding the genomic architecture of drivers, of the described systems, only *Sk-2* and *Sk-3* show the type of large recombination blocks that are often associated with meiotic drive elements in animals and plants (58). In *P. anserina*, driving *Spok3* and *Spok4* genes are also embedded within a large genomic insert (*Spok* block), but as the drive element itself consists of a single gene, the presence of this block does not relate to the necessity of a suppressed recombination between a killer and resistance element (68). The presence of *Spok* drivers in this block might rather reflect their recruitment for the benefit of the *Enterprise* mobile element (67). In contrast, several other spore-killing systems are determined by discrete genetic differences such as the presence or absence of a single gene in *wtf* and *Sk-1* or in the extreme case of [Het-s] drive by a single relevant amino acid difference (16).

The phylogenetic distribution of the killers also varies greatly among systems. Homologs of the killer have an extremely limited distribution, often restricted to a single or very closely related species in the case of *wtf*, *Sk-1*, *Sk-2*, and *Sk-3*, while *Spok* and *het-s* homologs have a patchy but much wider representation in fungal genomes. The fact that the *het-S/het-s* polymorphism has a role outside of the sexual cycle raises the question of whether some of the other identified drivers have or have had additional functions—especially considering that toxicity of the killer could also be observed in the vegetative stage. Two contrasting hypotheses can be made here. The first relies on a polymorphism conferring an advantage that is ancestral or conserved alongside the spore-killing activity (as in [Het-s]), in which case the initial head start of the driver in the population might be aided by a distinct selective pressure. Alternatively, a killer emerges totally de novo, from scratch, which would explain cases in which the phylogenetic distribution of the element is extremely narrow. This latter case could be applicable to *wtf* (and possibly also *rfk-1* and *Spk-1* if the tentative protein mislocalization and aggregation model applies).

Finally, some emerging trends suggest that poison-antidote single-gene drivers might be the more common structure of fungal spore killers, yet the idiosyncrasies of the different systems make each one interesting in its own right. Many pressing basic mechanistic questions remain, for instance the nature of the killer in *Sk-3*, the targets of the predicted nuclease and kinase activities of the *Spok* gene products, and the mode of emergence of the killers. Clearly, similar to what has been described in animal and plant models, meiotic drive is in no way a mechanistically uniform phenomenon and can probably only be understood by comparative exploration of multiple systems. The differences between the fungal spore-killer systems might be taken as an indication that exploration of spore killing in further genera would most certainly lead to the discovery of

new drive mechanisms. The early description of spore killing in *Fusarium* and *Bipolaris* could be an incentive to either tackle the molecular basis of drive in these species or look for additional killers in established fungal models and economically and ecologically important species (31, 36, 47, 61).

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LITERATURE CITED

1. Avril A, Purcell J, Beniguel S, Chapuisat M. 2020. Maternal effect killing by a supergene controlling ant social organization. *PNAS* 117:17130–34
2. Barrett LG, Legros M, Kumaran N, Glassop D, Raghu S, Gardiner DM. 2019. Gene drives in plants: opportunities and challenges for weed control and engineered resilience. *Proc. Biol. Sci.* 286:20191515
3. Bernet J. 1965. Mode d'action des gènes de barrage et relation entre l'incompatibilité cellulaire et l'incompatibilité sexuelle chez le *Podospora anserina*. *Ann. Sci. Natl. Bot.* 6:611–768
4. Bowen NJ, Jordan IK, Epstein JA, Wood V, Levin HL. 2003. Retrotransposons and their recognition of pol II promoters: a comprehensive survey of the transposable elements from the complete genome sequence of *Schizosaccharomyces pombe*. *Genome Res.* 13:1984–97
5. Bravo Nunez MA, Lange JJ, Zanders SE. 2018. A suppressor of a *wtf* poison-antidote meiotic driver acts via mimicry of the driver's antidote. *PLOS Genet.* 14:e1007836
6. Bravo Nunez MA, Sabbarini IM, Eickbush MT, Liang Y, Lange JJ, et al. 2020. Dramatically diverse *Schizosaccharomyces pombe wtf* meiotic drivers all display high gamete-killing efficiency. *PLOS Genet.* 16:e1008350
7. Burt A, Trivers R. 2006. *Genes in Conflict: The Biology of Selfish Genetic Elements*. Cambridge, MA: Harvard Univ. Press
8. Chiti F, Dobson CM. 2017. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. *Annu. Rev. Biochem.* 86:27–68
9. Crow JF. 1991. Why is Mendelian segregation so exact? *BioEssays* 13:305–12
10. Dalstra HJ, Swart K, Debets AJ, Saupe SJ, Hoekstra RF. 2003. Sexual transmission of the [Het-S] prion leads to meiotic drive in *Podospora anserina*. *PNAS* 100:6616–21
11. Dalstra HJ, van der Zee R, Swart K, Hoekstra RF, Saupe SJ, Debets AJ. 2005. Non-Mendelian inheritance of the HET-s prion or HET-s prion domains determines the *het-S* spore killing system in *Podospora anserina*. *Fungal Genet. Biol.* 42:836–47
12. Daskalov A, Gantner M, Walti MA, Schmidlin T, Chi CN, et al. 2014. Contribution of specific residues of the beta-solenoid fold to HET-s prion function, amyloid structure and stability. *PLOS Pathog.* 10:e1004158
13. Daskalov A, Habenstein B, Martinez D, Debets AJ, Sabate R, et al. 2015. Signal transduction by a fungal NOD-like receptor based on propagation of a prion amyloid fold. *PLOS Biol.* 13:e1002059
14. Daskalov A, Heller J, Herzog S, Fleissner A, Glass NL. 2017. Molecular mechanisms regulating cell fusion and heterokaryon formation in filamentous fungi. *Microbiol. Spectr.* 5. **https://doi.org/10.1128/microbiolspec.FUNK-0015-2016**

15. Debets AJ, Dalstra HJ, Slakhorst M, Koopmanschap B, Hoekstra RF, Saupe SJ. 2012. High natural prevalence of a fungal prion. *PNAS* 109:10432–37
16. Deleu C, Clave C, Begueret J. 1993. A single amino acid difference is sufficient to elicit vegetative incompatibility in the fungus *Podospora anserina*. *Genetics* 135:45–52
17. Dyrka W, Lamacchia M, Durrens P, Kobe B, Daskalov A, et al. 2014. Diversity and variability of NOD-like receptors in fungi. *Genome Biol. Evol.* 6:3137–58
18. Eickbush MT, Young JM, Zanders SE. 2019. Killer meiotic drive and dynamic evolution of the *wtf* gene family. *Mol. Biol. Evol.* 36:1201–14
19. Gardiner DM, Rusu A, Barrett L, Hunter GC, Kazan K. 2020. Can natural gene drives be part of future fungal pathogen control strategies in plants? *New Phytol*. 228:1431–39
20. Gershenson S. 1928. A new sex-ratio abnormality in *DROSOPHILA OBSCURA*. *Genetics* 13:488–507
21. Gladieux P, De Bellis F, Hann-Soden C, Svedberg J, Johannesson H, Taylor JW. 2020. *Neurospora* from natural populations: population genomics insights into the life history of a model microbial eukaryote. *Methods Mol. Biol.* 2090:313–36
22. Grognet P, Lalucque H, Malagnac F, Silar P. 2014. Genes that bias Mendelian segregation. *PLOS Genet*. 10:e1004387
23. Habig M, Kema GHJ, Stukenbrock EH. 2018. Meiotic drive of female-inherited supernumerary chromosomes in a pathogenic fungus. *eLife* 7:e40251
24. Hammond TM, Rehard DG, Xiao H, Shiu PK. 2012. Molecular dissection of *Neurospora* Spore killer meiotic drive elements. *PNAS* 109:12093–98
25. Harvey AM, Rehard DG, Groskreutz KM, Kuntz DR, Sharp KJ, et al. 2014. A critical component of meiotic drive in *Neurospora* is located near a chromosome rearrangement. *Genetics* 197:1165–74
26. Hay BA, Oberhofer G, Guo M. 2021. Engineering the composition and fate of wild populations with gene drive. *Annu. Rev. Entomol.* 66:407–34
27. Hoffman CS, Wood V, Fantes PA. 2015. An ancient yeast for young geneticists: a primer on the *Schizosaccharomyces pombe* model system. *Genetics* 201:403–23
28. Hu W, Jiang ZD, Suo F, Zheng JX, He WZ, Du LL. 2017. A large gene family in fission yeast encodes spore killers that subvert Mendel's law. *eLife* 6:e26057
29. Hurst LD. 2019. A century of bias in genetics and evolution. *Heredity* 123:33–43
30. Jeffares DC, Rallis C, Rieux A, Speed D, Prevorovsky M, et al. 2015. The genomic and phenotypic diversity of *Schizosaccharomyces pombe*. *Nat. Genet.* 47:235–41
31. Kathariou S, Spieth PT. 1982. Spore killer polymorphism in *FUSARIUM MONILIFORME*. *Genetics* 102:19–24
32. Kruger AN, Mueller JL. 2021. Mechanisms of meiotic drive in symmetric and asymmetric meiosis. *Cell Mol. Life Sci.* 78:3205–18
33. Kumon T, Ma J, Akins RB, Stefanik D, Nordgren CE, et al. 2021. Parallel pathways for recruiting effector proteins determine centromere drive and suppression. *Cell* 184(19):4904–18.e11
34. Larracuente AM, Presgraves DC. 2012. The selfish *Segregation Distorter* gene complex of *Drosophila melanogaster*. *Genetics* 192:33–53
35. Liu H, Li Y, Chen D, Qi Z, Wang Q, et al. 2017. A-to-I RNA editing is developmentally regulated and generally adaptive for sexual reproduction in *Neurospora crassa*. *PNAS* 114:E7756–65
36. Lohmar JM, Rhoades NA, Patel TN, Proctor RH, Hammond TM, Brown DW. 2022. A-to-I mRNA editing controls spore death induced by a fungal meiotic drive gene in homologous and heterologous expression systems. *Genetics* 15:iyac029
37. Lopez Hernandez JF, Zanders SE. 2018. *Veni*, *vidi*, *vici*: the success of *wtf* meiotic drivers in fission yeast. *Yeast* 35:447–53
38. Lyttle TW. 1993. Cheaters sometimes prosper: distortion of mendelian segregation by meiotic drive. *Trends Genet*. 9:205–10
39. Martinossi-Allibert I, Veller C, Ament-Velasquez SL, Vogan AA, Rueffler C, Johannesson H. 2021. Invasion and maintenance of meiotic drivers in populations of ascomycete fungi. *Evolution* 75:1150–69
40. Matsuyama A, Arai R, Yashiroda Y, Shirai A, Kamata A, et al. 2006. ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. *Nat. Biotechnol.* 24:841–47

41. Muirhead CA, Presgraves DC. 2021. Satellite DNA-mediated diversification of a sex-ratio meiotic drive gene family in *Drosophila*. *Nat. Ecol. Evol.* 5(12):1604–12
42. Nauta MJ, Hoekstra RF. 1993. Evolutionary dynamics of spore killers. *Genetics* 135:923–30
43. Nuckolls NL, Bravo Nunez MA, Eickbush MT, Young JM, Lange JJ, et al. 2017. *wtf* genes are prolific dual poison-antidote meiotic drivers. *eLife* 6:e26033
44. Nuckolls NL, Mok AC, Lange JJ, Yi K, Kandola TS, et al. 2020. The *wtf4* meiotic driver utilizes controlled protein aggregation to generate selective cell death. *eLife* 9:e55694
45. Padieu E, Bernet J. 1967. Mode d'action des gènes responsables de l'avortement de certains produits de la méiose chez l'Ascomycète *Podospora anserina* [Mode of action of the genes responsible for abortion of certain products of meiosis in the Ascomycete *Podospora anserina*]. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D* 264:2300–3
46. Price TAR, Windbichler N, Unckless RL, Sutter A, Runge JN, et al. 2020. Resistance to natural and synthetic gene drive systems. *J. Evol. Biol.* 33:1345–60
47. Pyle J, Patel T, Merrill B, Nsokoshi C, McCall M, et al. 2016. A meiotic drive element in the maize pathogen *Fusarium verticillioides* is located within a 102 kb region of chromosome V. *G3* 6:2543–52
48. Raju NB, Metzenberg RL, Shiu PK. 2007. *Neurospora* spore killers *Sk-2* and *Sk-3* suppress meiotic silencing by unpaired DNA. *Genetics* 176:43–52
49. Rhoades NA, Hammond TM. 2021. RNA editing controls meiotic drive by a *Neurospora* Spore killer. bioRxiv 2020.12.30.424869, Jan. 1
50. Rhoades NA, Harvey AM, Samarajeewa DA, Svedberg J, Yusifov A, et al. 2019. Identification of *rfk-1*, a meiotic driver undergoing RNA editing in *Neurospora*. *Genetics* 212:93–110
51. Riek R, Saupe SJ. 2016. The HET-S/s prion motif in the control of programmed cell death. *Cold Spring Harb. Perspect. Biol.* 8:a023515
52. Sander L, Novitski E. 1957. Meiotic drive as an evolutionary force. *Am. Nat.* 91:105–10
53. Saupe SJ. 2020. Amyloid signaling in filamentous fungi and bacteria. *Annu. Rev. Microbiol.* 74:673–91
54. Seuring C, Greenwald J, Wasmer C, Wepf R, Saupe SJ, et al. 2012. The mechanism of toxicity in HET-S/HET-s prion incompatibility. *PLOS Biol.* 10:e1001451
55. Shiu PK, Raju NB, Zickler D, Metzenberg RL. 2001. Meiotic silencing by unpaired DNA. *Cell* 107:905–16
56. Silver LM. 1985. Mouse *t* haplotypes. *Annu. Rev. Genet.* 19:179–208
57. Srinivasa AN, Zanders SE. 2020. Meiotic drive. *Curr. Biol.* 30:R627–29
58. Svedberg J, Hosseini S, Chen J, Vogan AA, Mozgova I, et al. 2018. Convergent evolution of complex genomic rearrangements in two fungal meiotic drive elements. *Nat. Commun.* 9:4242
59. Svedberg J, Vogan AA, Rhoades NA, Sarmarajeewa D, Jacobson DJ, et al. 2021. An introgressed gene causes meiotic drive in *Neurospora sitophila*. *PNAS* 118:e2026605118
60. Swentowsky KW, Gent JI, Lowry EG, Schubert V, Ran X, et al. 2020. Distinct kinesin motors drive two types of maize neocentromeres. *Genes Dev.* 34:1239–51
61. Taga M, Bronson CR, Yoder OC. 1985. Nonrandom abortion of ascospores containing alternate alleles at the *Tox-1* locus of the fungal plant pathogen *Cochliobolus heterostrophus*. *Can. J. Genet. Cytol.* 27:450–56
62. Turner BC, Perkins DD. 1979. Spore killer, a chromosomal factor in *Neurospora* that kills meiotic products not containing it. *Genetics* 93:587–606
63. Turner BC, Perkins DD. 1991. Meiotic drive in *Neurospora* and other fungi. *Am. Nat.* 137:416–29
64. Turner BC, Perkins DD, Fairfield A. 2001. *Neurospora* from natural populations: a global study. *Fungal Genet. Biol.* 32:67–92
65. Urquhart AS, Gardiner DM. 2022. A natural fungal gene drive enacts killing through targeting DNA. bioRxiv 2022.01.19.477016, Jan. 21
66. van der Gaag M, Debets AJ, Oosterhof J, Slakhorst M, Thijssen JA, Hoekstra RF. 2000. Spore-killing meiotic drive factors in a natural population of the fungus *Podospora anserina*. *Genetics* 156:593–605
67. Vogan AA, Ament-Velasquez SL, Bastiaans E, Wallerman O, Saupe SJ, et al. 2021. The *Enterprise*, a massive transposon carrying *Spok* meiotic drive genes. *Genome Res.* 31:789–98

68. Vogan AA, Ament-Velasquez SL, Granger-Farbos A, Svedberg J, Bastiaans E, et al. 2019. Combinations of *Spok* genes create multiple meiotic drivers in *Podospora*. *eLife* 8:e46454
69. Wedell N, Price TAR, Lindholm AK. 2019. Gene drive: progress and prospects. *Proc. Biol. Sci.* 286:20192709
70. Zanders SE, Eickbush MT, Yu JS, Kang JW, Fowler KR, et al. 2014. Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast. *eLife* 3:e02630