Spore-Killing Meiotic Drive Factors in a Natural Population of the Fungus *Podospora anserina*

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ABSTRACT

In fungi, meiotic drive is observed as spore killing. In the secondarily homothallic ascomycete *Podospora anserina* it is characterized by the abortion of two of the four spores in the ascus. We have identified seven different types of meiotic drive elements (Spore killers). Among 99 isolates from nature, six of these meiotic drive elements occurred in a local population. Spore killers comprise 23% of the natural population of *P. anserina* in Wageningen, The Netherlands, sampled from 1991 to 1997. One Spore-killer type was also found in a French strain dating from 1937. All other isolates found so far are sensitive to spore killing. All seven Spore killer types differ in the percentage of asci that show killing and in their mutual interactions. Interactions among Spore killer types showed either mutual resistance or dominant epistasis. Most killer elements could be assigned to linkage group III but are not tightly linked to the centromere.

SEGREGATION distorters are genetic elements that show meiotic drive, a phenomenon in which one member of a pair of heterozygous alleles is transmitted in excess of the expected Mendelian ratio of 50% (Sandler and Novitski 1957; Lyttle 1991). Well-known examples of segregation distorters are the *sex-ratio* chromosomes (*SR*) in Drosophila, a male sex chromosomal drive system, and the *t*-haplotype in mice and *segregation distorter* (*SD*) in Drosophila, both male autosomal drive systems (Lyttle 1991). In Drosophila and mouse, the meiotic drive systems minimally involve two closely linked loci, a distorter and its *cis*-acting target. All distorters are associated with polymorphic chromosomal structures, such as inversions. Their ratio of distortion in these examples can exceed 90% and they are closely linked to the centromere. It is not known for most drive systems whether they involve two closely linked loci. Likewise, distortion ratios for Drosophila and mice in nature may vary greatly. Meiotic drive systems in these organisms showing <90% distortion are harder to detect. Furthermore, classes of insensitive target or suppressor alleles have accumulated to counter these selfish elements (Lyttle 1991).

Meiotic drive allows deleterious alleles to spread through populations if the frequency gain from their segregation advantage more than compensates the frequency loss due to elimination by natural selection. Thus it threatens adaptive evolution and it is therefore of great interest to obtain information on the extent of meiotic drive in natural populations. This is not easy to study because in animals and plants a driving genetic element requires a specific phenotype to be observable. For this reason it is understandable that an appreciable number of known cases of meiotic drive involve genes affecting the sex ratio. However, fungi in which the haploid nuclei resulting from meiosis are linearly arranged within an ascus provide unique opportunities to analyze abnormal segregation, for precisely the same reason that they have played such a big role in the classical experiments by Lindegren and others on fundamental aspects of linkage, meiotic recombination, and gene conversion (see Whitehouse 1973; Perkins 1992). Any meiotic drive system in such fungi—provided the elimination of the nuclei containing the nondriving allele occurs in an early stage after the completion of meiosis, as it does in all known meiotic drive systems—will be observed in a cross between a driving and a sensitive strain as *spore killing*: the degeneration and early abortion of half the ascospores in a certain proportion of the asci. This is not the only distinguishing feature of drive systems in fungi. The ascospores are the products of meiosis as well as the progeny. Thus distortion in fungi also affects the number of offspring produced and reduces the fecundity, which has important consequences for the population genetics of meiotic drive in fungi (Nauta and Hoekstra 1993).

The earliest analysis of two segregation distorters in fungi, then called ascospore abortion factors, is by Padieu and Bernet (1967) in the ascomycete Podospora. Turner and Perkins (1979, 1991) identified such abortion factors in Neurospora as Spore killers. Other fungi in which distorters have been found are

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Gibberella fujikuroi (= *Fusarium moniliforme*) and *Cochliobolus heterostrophus* (see Raju 1994, 1996 for a review). However, the best-studied example of meiotic drive in ascomycetes is Spore killer (*Sk*) in Neurospora. Haploid Spore killer strains of Neurospora were originally identified because asci always contained four viable black and four small inviable unpigmented spores in crosses with standard wild-type strains. All the viable spores carry the Sk^K allele. In crosses homozygous for a killer allele ($Sk^K \times Sk^K$) each ascus contains eight viable black ascospores, as in normal sensitive crosses ($Sk^S \times Sk^S$), indicating that killing occurs only in crosses heterozygous for the killing factor (Turner and Perkins 1979, 1991).

Several Spore killer types have been characterized in Neurospora: *Sk-1*^{K} from *Neurospora sitophila* and a *Sk-2*^{K} and *Sk-3*^{K} from *N. intermedia*. Only *Sk-1*^{K} occurs widespread in nature (Turner and Perkins 1979). Both *Sk-2*^{K} and *Sk-3*^{K} were introgressed into the genetically better-characterized *N. crassa* and both mapped to a region of 30 map units across the centromere of linkage group III. This region, ~3% of the total genomic map, was found to contain a recombination block (Campbell and Turner 1987). No evidence was found for large inversions or chromosome rearrangements, though small inversions might exist between markers (Bojko 1988; Turner and Perkins 1991). The killer complex must therefore be considered as a haplotype. Whether *Sk-1*^{K} is associated with a recombination block is unknown (Turner and Perkins 1979, 1991).

Meiosis is normal in crosses between Spore killers and sensitives. Both nuclear types coexist within the same ascus cytoplasm and ascus development is typical until after postmeiotic mitosis when the nuclei are enclosed by ascospore walls. Both nuclear types can coexist as well in vegetative heterokaryons, as is apparent from rare occasions when they are included together in the same ascospore (Raju 1979; Raju and Perkins 1991; Turner and Perkins 1991).

Sk-2 and *Sk-3* have been introgressed into the secondarily homothallic *N. tetrasperma*, which normally makes asci with four large spores that are heterokaryotic for mating type and any other centromere-linked markers that are heterozygous in the cross. Crosses of *N. tetrasperma* heterozygous for the centromere-linked killers *Sk-2* and *Sk-3* all produced four-spored asci as predicted from the behavior of these killers in the eight-spored species. The sensitive nuclei were protected in heterokaryotic $Sk^K + Sk^S$ ascospores, but killing occurred in this species when exceptional small homokaryotic ascospores were formed (Raju and Perkins 1991).

Podospora anserina grows on dung of herbivores and is also a secondarily homothallic ascomycete. It also produces four binucleate spores per ascus (Figure 1). For the behavior of Spore killers in *P. anserina* the following aspects of ascospore formation are relevant. (1) Programming of nuclear positioning in the Podospora ascus is such that following meiosis and postmeiotic mitosis, the two ascospores in one-half of the ascus each receive two nonsister nuclei descending from the two meiotic products from the same half tetrad. The other two ascospores each contain two nonsister nuclei from the other half tetrad. As a result, ascospores are homokaryotic for all markers showing first divison segregation (FDS) and heterokaryotic for those markers that show second division segregation (SDS; see Esser 1974; Raju and Perkins 1994). Due to an obligate single crossover between the centromere and the mating-type locus, (nearly) all spores are heterokaryotic for mating type (*mat*+ and *mat*−). (2) In crosses heterozygous for a Spore killer element, ascospores that receive only sensitive nuclei abort, whereas ascospores that are homo- or heterokaryotic for a killer nucleus survive. Therefore, the frequency of asci showing two viable and two inviable ascospores (two-spored asci) reflects the frequency of FDS for the Spore killer element (Figure 1). This fraction of two-spored asci is hereafter referred to as the spore-killing percentage. This study describes the results of our search for meiotic drive elements in a natural *P. anserina* population. We have identified and characterized seven different types of Spore killers, indicated by the abortion of half of the ascus progeny, among 99 recently isolated Dutch strains of *P. anserina* and 3 older isolates originating from France. Six of these Spore killer types can be attributed with certainty to meiotic drive elements. We have also analyzed the interactions

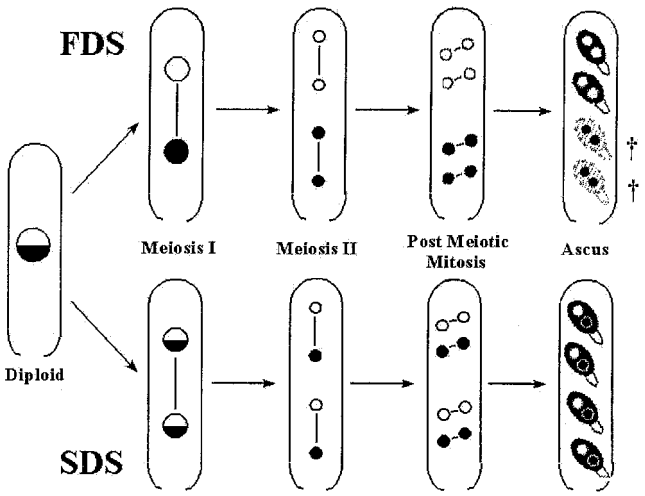


Figure 1.—Model to explain spore killing in *P. anserina* as the segregation of a meiotic drive element. The figure shows a cross between two strains carrying a Spore killer (○) and a sensitive (●) allele. Two nonsister nuclei descending from a half tetrad are enclosed in each ascospore. FDS for the Spore killer element results in an ascus with two surviving spores, each homokaryotic for the killer element, and two aborted spores, each homokaryotic for the sensitive alleles. SDS results in a four-spored ascus, in which each ascospore survives because it carries both a nucleus with the killer allele and one with the sensitive allele.

between the different killer types. Finally, mapping data of the Spore killer elements are presented.

MATERIALS AND METHODS

Strains and culture methods: *P. anserina* strain S, isolated in Normandy, France, in 1937 was used as a standard tester strain. Spore killer strains Y and Z originated from Picardy, France, in 1937 (Belcour *et al.* 1997). *P. comata* Spore killer strain T (Picardy, France, 1937) was previously described as *P. anserina* but was renamed later on the basis of morphology and mitochondrial type. It is interfertile with *P. anserina* (Padieu and Bernet 1967; Belcour *et al.* 1997). All other *P. anserina* Spore killer strains (Wa numbers) were collected in Wageningen, The Netherlands, during 1991–1997 from dung (van der Gaag *et al.* 1998). Recombinants of the Spore killer strains (XS numbers) with the genetic background of sensitive strain S were obtained by five recurrent backcrosses.

Marker strains used in this study were *Cs3* (16% SDS, LG I, cold sensitive), *136* (0.5% SDS, LG II, green spores), *Cs2* (3% SDS, LG III, cold sensitive), *187* (76% SDS, LG III, green spores), *rd1* (84% SDS, LG III, round spores), *Lys2* (0% SDS, LG IV, lysine requiring), *As7* (0% SDS, LG V, paromomycin resistant), *Cs18* (0–7% SDS, LG VI, cold sensitive), *Cs12* (0–3% SDS, LG VII, cold sensitive, paromomycin hypersensitive; Picard 1971; Marcou *et al.* 1990). All marker strains were derived from wild-type strain S.

Culture conditions and media have been described by Esser (1974). Cornmeal agar was used as a standard growth medium with 100 mg/liter lysine added for the *Lys2* marker. Tests for the *Lys2* marker were performed on minimal medium 2 (MM2) without lysine. Paromomycin hypersensitivity and resistance were tested on MM2 supplemented with 270 μg/ml paromomycin (Sigma, St. Louis). All cultures were grown at 27°. Cold sensitivity was tested at 11° (Picard-Bennoun and Le Coze 1980).

Crosses were performed on moistened copromes (horse dung tablets that were sterilized by γ-irradiation; Wood and Cooke 1984) on a sterile filter paper on top of the agar in a plate to improve crossing ability. Crossing occurred either by spermatization of monokaryotic strains with microconidia or by confrontation of mycelia of opposing mating type. Backcrosses of progeny to the parental strains were performed using the spermatization technique.

Genetic mapping: Methods of genetic analysis have been described by Esser and Kuenen (1967). In short, Spore killer strains were crossed with centromere-linked marker strains to identify the linkage group. Two-spored asci resulting from FDS for the Spore killer element (see Figure 1) were tested for the occurrence of the marker. Such markers show FDS, and thus the two surviving ascospores will be homokaryotic either for the marker or the wild-type allele. Nonparental ditypes (NPD, *i.e.*, the two surviving spores show the marker) and parental ditypes (PD, *i.e.*, the two surviving spores are wild type) will be equally frequent in the progeny when killer and marker are unlinked. When linked, NPDs appear very rarely, because they require a four-chromatid double crossover between the killer locus and the marker. When using a centromere-linked tester strain, SDS for the marker is rare and can be neglected.

Markers more distal from the centromere were used for the establishment of linkage on chromosomal arms. Four-spored asci, resulting from SDS for the Spore killer element, were tested for the absence or occurrence of the marker. A low percentage of SDS for only the Spore killer element compared to SDS for both Spore killer and marker indicates linkage to that chromosomal arm (depending on the distance of the markers).

RESULTS

Spore killing in Podospora reflects meiotic drive: To assess the extent of meiotic drive in a natural population we sampled the local *P. anserina* population of Wageningen, The Netherlands. During the period 1991–1997 we obtained a total of 99 new isolates of *P. anserina*. Species determination was based on morphological criteria, as well as on fertility with the standard strain S, or with wild-type strains sexually compatible with S (Mirza and Cain 1969; van der Gaag *et al.* 1998). We observed that 23 isolates produced up to 95% two-spored asci in these crosses, instead of the expected four-spored asci (Table 1, Figure 2). In addition to the new wild-type strains, we analyzed three French Podospora strains, T, Y, and Z, isolated in 1937 and showing similar spore abortion in specific crosses (Belcour *et al.* 1997). Strain T was previously characterized as a Spore killer strain (Padieu and Bernet 1967; Turner and Perkins 1991) and was recently renamed *P. comata* on the basis of morphological and molecular data (Belcour *et al.* 1997). Progeny grown from two-spored and four-spored asci were backcrossed to both mating types of the parental strains to confirm that the observed spore killing is caused by meiotic drive. The results from such an analysis of strain Wa58 (*Psk-7*) are shown in Table 2 and may be summarized as follows:

1. Selfing of the progeny from two-spored ascus progeny always yielded normal four-spored asci (no abortion).
2. Backcrossing of the progeny of the two-spored asci to the S^+ and S^- strains showed spore killing, whereas backcrossing to the $Wa58^+$ and $Wa58^-$ strains gave normal four-spored asci.
3. Selfing of the four-spored progeny showed ascospore abortion.
4. Backcrosses of the four-spored progeny produced spore killing in the backcross to S^- and $Wa58^+$ or to S^+ and $Wa58^-$, but not to both.
5. In addition to these observations, it must be added that there is no effect of the mating type or of the sexual role (maternal or paternal) of the strains involved.

These data support the meiotic drive model of spore killing as presented for Neurospora (Turner and Perkins 1979) and applied to the genetic system of *P. anserina* (Figure 1). Spores from a cross between a strain carrying a Spore killer element and a sensitive strain would be either homokaryotic (*i.e.*, show FDS for the Spore killer element, reflecting no crossover in the centromere proximal region) or heterokaryotic for the Spore killer element (SDS, reflecting a crossover). Only spores carrying a Spore killer nucleus survive; thus FDS

TABLE 1

Spore killer types found in isolates of Podospora

Spore killer type	*P. anserina* strain	Year of isolation	% two-spored asci (first cross)	% two-spored asci (fifth backcross)
Psk-1	Wa1	1991	95.7 (1480 asci)	90.9 (956 asci)
	Wa2	1991	94.6 (1460 asci)	ND[a]
	Wa6	1992	96.3 (1112 asci)	93.6 (109 asci)
	Wa12	1993	94.3 (457 asci)	ND
	Wa52	1994	91.3 (1412 asci)	89.0 (845 asci)
	Wa53	1994	92.5 (681 asci)	91.6 (490 asci)
	Wa86	1997		
	Wa87	1997		
	Wa98	1997		
Psk-2	Wa28	1993	78.5 (960 asci)	73.0 (333 asci)
	Wa38	1993	77.2 (228 asci)	77.7 (323 asci)
	Wa49	1994	72.4 (1204 asci)	82.5 (388 asci)
	Wa85	1997		
	Wa97	1997		
Psk-3	Wa20	1993	Variable	ND
	Wa21	1993	Variable	ND
	Wa25	1993	Variable	ND
	Wa27	1993	Variable	ND
Psk-4	Wa46	1994	45.1 (2671 asci)	ND
Psk-5	Y	1937	94.4 (1239 asci)	96.0 (250 asci)
Psk-6	Wa47	1994	45.7 (1835 asci)	ND
	Wa89	1997		
	Wa90	1997		
Psk-7	Wa58	1994	53.6 (2007 asci)	53.8 (143 asci)
	Z	1937	48.9 (6340 asci)	51.3 (903 asci)
a_2 *or (Sk-1)*	T[b]	1937	70.8 (861 asci)	ND

Isolates are classified in different killer types based on spore killing frequency (FDS) and killing interaction among Spore killer isolates (Table 5). Percentage of spore killing (FDS) is based on crosses to sensitive strain S (number of asci shown in parentheses). Backcrossed strains were obtained through five recurrent backcrosses with sensitive strain S. All Wa strains were isolated around Wageningen, The Netherlands, during 1991–1997. Strains T, Y, and Z were isolated in Picardy, France, during 1937. The 1997 Wa strains were classified by interaction with other Spore killers.

[a] No backcrosses were made for these strains nor could be obtained due to infertility.

[b] Strain T was previously identified as *P. anserina* by Padieu and Bernet (1967), but reclassified *P. comata* on the basis of morphological and molecular data (Belcour *et al.* 1997). Killer-type classification of strain T was done by Turner and Perkins (1991).

for the Spore killer results in two-spored asci, the aborted spores carrying only sensitive alleles. SDS would result in four-spored asci. The sensitive nuclei in the heterokaryotic four-spored asci are viable as can be seen in the selfings and backcrosses. Results similar to those described in Table 2 were found for all other Spore killer isolates that were tested. In all cases, standard strain S behaved as the sensitive isolate.

There are at least six different Spore killer types in the Wageningen population of *P. anserina*: The Spore killer strains were initially classified on the basis of (1) spore killing frequency in a cross to a standard sensitive strain (FDS percentage) and (2) the interaction between the Spore killers (Tables 1 and 3). In this way at least six types of Spore killers could be identified among the 99 natural isolates. An additional seventh type was discovered in the French *P. anserina* strain Y. All Spore killer strains of the same type showed a constant and repeatable spore-killing frequency when crossed to strain S and absence of spore killing when intercrossed. When intercrossed (Table 3), however, Spore killer strains of different types show killing, similar to the behavior of $Sk\text{-}2^K \times Sk\text{-}3^K$ in Neurospora.

Figure 2 shows some rosettes of a normal cross and different spore-killing reactions. The killer types *Psk-1* and *Psk-5* show the highest frequency of two-spored asci, >90% (Figure 2B). *Psk-4*, *Psk-6*, and *Psk-7* produce the lowest killing percentage; only half of the asci contain two spores; the remaining asci carry four spores as is the normal condition (Figure 2D). The *Psk-2* strain and *P. comata* strain T show intermediate levels of spore killing; ~75% of two-spored asci are found (Figure 2C). Spores homokaryotic for the sensitive allele can be observed only for a short time in these killer crosses. They completely degrade at the start of spore wall formation.

The group of *Psk-3* strains is different from the others

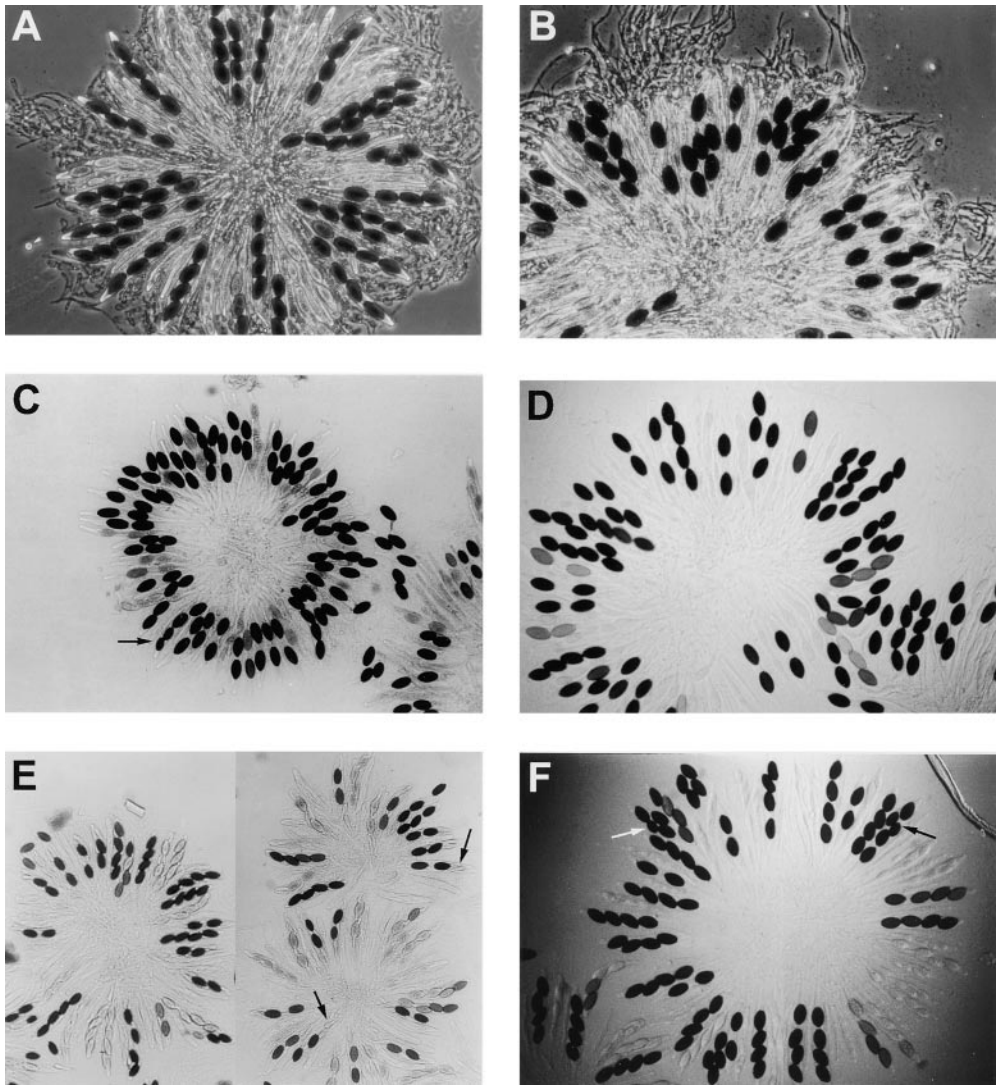


FIGURE 2.—Rosettes of asci from crosses between *P. anserina* strains. Asci with darker ascospores are more mature than those with lighter spores. (A) A rosette from a normal cross showing only four-spored asci. (B) A cross of Wa6 *(Psk-1)* × S showing a high percentage of two-spored asci. (C) A cross of Wa28 *(Psk-2)* × S showing ~70% two-spored asci. An ascus containing two dikaryotic and two small monokaryotic killer spores can be seen (black arrow). Both monokaryotic sensitive spores have been aborted. (D) A cross of Wa58 *(Psk-7)* × S showing 50% two-spored asci. (E) A cross of Wa20 *(Psk-3)* × Wa16 showing rosettes with different killing percentages. Aborted spores are also visible within the asci (black arrows). (F) A cross between Wa52 *(Psk-1)* × Wa58 *(Psk-7)* showing ~30% two-spored asci. The five-spored ascus (black arrow) indicates that both smaller mononucleate spores contain a killer locus (as expected for a parental ditype). A four-spored ascus (white arrow) containing a mononucleate spore indicates SDS for one of the killer loci, resulting in the segregation of an (aborted) sensitive nucleus.

TABLE 2

Progeny tests of cultures from two- and four-spored asci of the cross Wa58 (*Psk-7*^{K}) × S (*Psk-7*^{S})

Ascospore no.	Occurrence of two-spored asci[a] in backcrosses to				Selfing	Inferred genotype of ascospores
	Wa58^{+}[b]	Wa58^{-}	S^{+}	S^{-}		
	Two-spored asci (six complete ascus progeny tested)					
1	No	No	Yes	Yes	No	*Psk-7*$^{K+}$/*Psk-7*$^{K-}$
2	No	No	Yes	Yes	No	*Psk-7*$^{K+}$/*Psk-7*$^{K-}$
	Four-spored asci (five complete ascus progeny tested)					
1	No	Yes	Yes	No	Yes	*Psk-7*$^{S+}$/*Psk-7*$^{K-}$
2	No	Yes	Yes	No	Yes	*Psk-7*$^{S+}$/*Psk-7*$^{K-}$
3	Yes	No	No	Yes	Yes	*Psk-7*$^{K+}$/*Psk-7*$^{S-}$
4	Yes	No	No	Yes	Yes	*Psk-7*$^{K+}$/*Psk-7*$^{S-}$

[a] With respect to the killing percentage of the killer strain, if killing occurs, approximately half of the asci show two viable and two aborted ascospores, and the other asci have four viable ascospores. The percentage of two-spored asci is like that found in the parental cross.

[b] + and − refer to mating type of the nuclei in the ascospores or parental strains.

TABLE 3

Interactions between different Spore killer types

Strains	*Psk-1* (Wa6)	*Psk-2* (Wa28)	*Psk-4* (Wa46)	*Psk-5* (Y)	*Psk-6* (Wa47)	*Psk-7* (Z)
Psk-1 (Wa6)	0					
Psk-2 (Wa28)	94.3 (1594 asci)	0				
Psk-4 (Wa46)	90.1 (243 asci)	62.5 (557 asci)	0			
Psk-5 (Y)	25.5 (825 asci)	92.4 (607 asci)	89.9 (715 asci)	0		
Psk-6 (Wa 47)	92.2 (141 asci)	72.0 (1009 asci)	26.5 (895 asci)	93.8 (727 asci)	0	
Psk-7 (Z)	23.2 (992 asci)	53.4 (1301 asci)	61.8 (845 asci)	44.1 (860 asci)	67.9 (505 asci)	0
Sensitive (S)	96.3 (1112 asci)	78.5 (960 asci)	45.1 (2671 asci)	94.4 (1239 asci)	45.7 (1835 asci)	48.9 (6340 asci)

The percentage of two-spored asci is shown for crosses between one member of each Spore killer type. Spore killer strains of any one type have similar spore-killing percentages when crossed to a standard sensitive strain and do not show killing when intercrossed (Table 1). For comparison, the percentage killing in a cross with strain S is also given. The number of asci analyzed is shown in parentheses.

in that the frequency of spore killing is highly variable among perithecia of the same cross (Figure 2E). Fruiting bodies with any combination of two- and four-spored asci can be observed. Furthermore, ascospore abortion is found only in crosses between specific strains and even between some *Psk-3* strains (Table 4). Another distinguishing feature of the *Psk-3* group is that the aborted spores do not disintegrate as in the other killer types, but remain in the asci as tiny, shriveled spores. Because of the erratic expression of spore abortion, further genetic analysis of these *Psk-3* isolates has not been performed. It is therefore not certain that this group contains true meiotic drive elements.

Spore killers are stable upon recurrent backcrossing: Several strains belonging to different Spore killer types were backcrossed five times with the sensitive strain S to assess the stability of the Spore killers and, at the same time, to obtain a more identical genetic background and to increase fertility for further analysis. The fraction of two-spored asci of the fifth recurrent backcross did not differ from the percentage found in the first cross for *Psk-1*, *Psk-2*, *Psk-5*, and *Psk-7* (Table 1). These Spore killer types all show a stable percentage of two-spored asci. We were not able to proceed in backcrossing *Psk-4* and *Psk-6* killers, owing to fertility problems.

TABLE 4

Spore killing found in crosses with *Psk-3* isolates

Psk-3 strains	Spore killing with sensitive strains	Spore killing with *Psk-3* members
Wa20	Wa14, Wa15, Wa16, Wa33	Wa25
Wa21	Wa15, Wa17, Wa18, Wa23, Wa26, Wa30, Wa41, Wa51, Wa57, Wa63	Wa25, Wa27
Wa25	Wa14, Wa17, Wa21, Wa26	Wa20, Wa27
Wa27	S, Wa3, Wa4, Wa14, Wa15, Wa16, Wa26, Wa50, Wa57, Wa63	Wa21, Wa25

All *Psk-3* strains show variable killing percentages within a cross.

High frequency and diversity of Spore killers in a natural population of Podospora: The incidence of Spore killer strains in the *P. anserina* population of Wageningen appears remarkably high. Of the 99 Wa strains isolated between 1991 and 1997, 23 contain a driving element. Spore killers were found during all years of isolation, except for 1995. In 1996 no strains were isolated. *Psk-1*, *Psk-2*, and *Psk-6* strains were found over several years in the population; *Psk-4* and *Psk-7* were isolated only in 1994 (Table 5).

Among six strains isolated in 1937 in Picardy, France, two contained a meiotic drive element. Also the *P. comata* Spore killer strain T was isolated on that occasion (Belcour *et al.* 1997). No Spore killer strains were reported among isolates from other French regions (L. Belcour, personal communication). Both the Dutch Spore killer strain Wa58, isolated in 1994, and the French strain Z, originating in 1937, belong to the same killer type *Psk-7* (Table 1).

In contrast to Neurospora, no neutral strains, *i.e.*, strains that are not killed but do not themselves kill, were found. However, only a selection of the Wageningen

TABLE 5

The occurrence of Spore killer strains among natural isolates from the *P. anserina* population of Wageningen, The Netherlands

Year	No. of isolates	No. of Spore killers	Spore killer type					
			Psk-1	*Psk-2*	*Psk-3*	*Psk-4*	*Psk-6*	*Psk-7*
1991	5	2	2	—	—	—	—	—
1992	1	1	1	—	—	—	—	—
1993	32	7	1	2	4	—	—	—
1994	31	6	2	1	—	1	1	1
1995	14	0	—	—	—	—	—	—
1997	16	7	3	2	—	—	2	—
Total	99	23	9	5	4	1	3	1

isolates before 1994 was tested against every new Spore killer isolate. Exceptions are the strains from the *Psk-3* group that show killing behavior only in specific crosses (Table 4). Other nonkiller strains are sexually incompatible, or produce four-spored asci in crosses with *Psk-3* members, and seem to act like neutral strains. Among the different Spore killer types some killer types are resistant to killing by other killer types. This is discussed below in more detail.

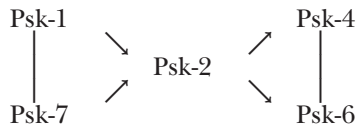
Spore killer types show dominant epistatic or mutual resistant interactions: We have crossed the Spore killer strains to each other and measured the fraction of spore killing. The results of the initial crosses are shown in Table 3 for one representative of each killer type. Results for other strains were similar. No empty perithecia or completely aborted progeny were found in any of these crosses. Two types of interaction between killer elements were observed. (1) Most commonly, the fraction of two-spored asci was similar to that found for one of the parents when crossed to a sensitive tester strain. We refer to this type of interaction as dominant epistasis. For example, the cross between *Psk-1* and *Psk-2* shows the killing percentage characteristic of *Psk-1* (Table 3). Backcrosses of two-spored progeny from these interactions produced results similar to those for backcrosses of two-spored progeny from *Psk-1* and a sensitive tester. (2) In some interactions between killers, a much lower percentage of two-spored asci was observed than was found for any of the parents when crossed to the standard testers. This type of interaction can be described as mutual resistance of the unlinked killer elements (for explanation see below). For example, in the cross *Psk-1* × *Psk-7* a relatively low percentage of two-spored asci was observed (Figure 2F and Table 3). We have analyzed the two-spored progeny by backcrosses to the parental Spore killer strains, by selfing, and by crosses with a sensitive strain. The results of the analysis of the *Psk-1* × *Psk-7* progeny are shown in Table 6 as an example of mutually resistant Spore killers. These results can be summarized as follows:

1. Backcrosses with both parental strains did not show any killing. Every nucleus in the two-spored progeny therefore contains both killers.
2. Crosses with the sensitive strain produced a similar killing percentage as the original cross between *Psk-1* and *Psk-7* did. This verifies that the two surviving ascospores contain both killers.
3. Selfing of the progeny from two-spored asci yielded only four-spored asci. This is also consistent with the surviving ascospores being homokaryotic for both killer elements.

Spore killing between mutually resistant Spore killers is reminiscent of the interaction of unlinked duplicate genes. Ascospores survive when at least one killer element is present, and spore killing is limited to the NPD class of asci with FDS for both killer elements. In these asci, the surviving two spores carry both *Psk-1* and *Psk-7*. The two aborted spores carry neither. The low percentage (23%) of two-spored asci is consistent with the two killer loci being unlinked. Tightly linked Spore killers would have given a high percentage of two-spored asci. *Psk-4* and *Psk-6* were also found to be mutually resistant.

Not every backcross with each wild-type strain was fertile, but usually enough information could be extracted when backcrosses of other strains of the same killer type were taken into consideration. This was, however, not the case with analysis of the four-spored asci, although the incomplete results obtained in these backcrosses did not contradict the analysis of the two-spored asci.

The relation of the Spore killer types found in Wageningen to each other is summarized in the scheme below:



The straight line represents a mutually resistant interac-

TABLE 6

Progeny tests of cultures from two-spored asci of crosses between *Psk-1* and *Psk-7*

Ascospore no.	Occurrence of two-spored asci in backcrosses						Selfing	Inferred genotype of ascospores
	Psk-1^{+a}	*Psk-1*$^{-}$	*Psk-7*$^{+}$	*Psk-7*$^{-}$	S^{+}	S^{-}		
Asci from Wa6 *(Psk-1)* × Wa58 *(Psk-7)* (progeny from two complete asci tested)								
1	No	No	ND	No	ND	ND	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$
2	No	No	ND	No	ND	ND	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$
Asci from Wa1 *(Psk-1)* × Z *(Psk-7)* (six complete ascus progeny tested)								
1	ND	No	No	No	Yes	Yes	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$
2	ND	No	No	No	Yes	ND	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$
Asci from Wa6 *(Psk-1)* × Z *(Psk-7)* (three complete ascus progeny tested)								
1	ND	No	ND	No	ND	Yes	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$
2	ND	No	ND	No	ND	Yes	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$
Asci from Wa52 *(Psk-1)* × Z *(Psk-7)* (five complete ascus progeny tested)								
1	No	No	No	ND	Yes	Yes	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$
2	No	No	No	ND	Yes	Yes	No	*Psk-1*^{K}/*Psk-7*$^{K-}$ *Psk-1*^{K}/*Psk-7*$^{K+}$

Crosses were performed and analyzed as in Table 2. Initial killing percentages were 30.5% (Wa6 × Wa58; 525 asci), 15.5% (Wa1 × Z; 774 asci), 23.2% (Wa6 × Z; 992 asci), and 24.3% (Wa52 × Z; 1671 asci). Crosses to sensitive strains produced the original percentage of asci that show killing. Not every test produced perithecia in all backcrosses with the parents (ND, no data due to infertility of the cross), but usually enough information could be extracted from the other test crosses. Results of crosses between other killer types are summarized in the text.

[a] + and − refer to mating type of the nuclei in the ascospores and strains used.

tion, whereas the arrow indicates a dominant epistatic interaction. All Spore killer types to the right of the arrowhead are sensitive to killing by the killer types on the left. Thus *Psk-7* and *Psk-1* are the most effective killer types in the Wageningen population. They are mutually resistant but kill *Psk-2*, *Psk-4*, and *Psk-6*. *Psk-4* and *Psk-6* are killed by all the other killer types but are mutually resistant. The French *Psk-5* Spore killer type has a more complex interaction to the other killer types. *Psk-5* is sensitive to *Psk-7*, mutually resistant to *Psk-1*, and kills all other killer types.

Most Spore killers are assigned to LG III, but not tightly linked to the centromere: All Spore killer strains were crossed to centromere-linked marker strains to identify the linkage group of the killer element. The linkage analysis for one representative per Spore killer type is presented in Table 7. Other strains of the same killer type showed similar results. All *Psk-1*, *Psk-2*, *Psk-5*, and *Psk-7* killer strains showed linkage to the linkage group III centromere marker *Cs2*, as indicated by the low percentage of NPD asci. Linkage could not be established with certainty for *Psk-6*, but LG III (10.8% NPD) seems more likely than LG IV (18% NPD). Spore killer type *Psk-4* seems to be located on linkage group IV, although a possible location on LG V cannot be excluded. Not all unlinked markers, however, show an equal 50% segregation pattern (Table 7). This can be due to negative fitness aspects, which lead to an underrepresentation of the marker involved. Furthermore, interference as often observed in *P. anserina* may hamper linkage group assignment for certain genes (M. Picard, personal communication).

Psk-1 and *Psk-5* were crossed to a strain with two other markers on both sides of the LG III centromere to determine their location on the chromosome arm. A green spore marker, 187, is located on the left arm of the chromosome, whereas *rd1*, a round spore marker, is situated on the right arm. Results of these crosses indicated a strong interference (Table 8). The SDS percentage for *rd1* was reduced from >80% to ~25% when a crossover for *Psk* occurred. This reduction was found in all combinations of killer and markers (data not shown). A control cross of *rd1* with *187* did not show this interference. Whether the interference is due to, or merely detected by, the presence of the Spore killer cannot be concluded from these data. These results can be explained by strong chromosomal interference across the centromere or by positive chromatid interference, if a crossover for *rd1* is followed by a specific second crossover involving the same chromatids. In the first case, the killer must be present on the left arm, whereas in the second case the killer is present on the right arm of the linkage group. Alternatively, centromere misdivision or occurrence of spindle overlap at the second division could simulate double crossovers across the centromere with positive interference (D. D. Perkins, personal communication).

Also, attempts were made to further localize *Psk-7* in crosses with LG III markers *rd1* and *187* (Table 8). Chromosomal arm linkage would be most strongly indi-

TABLE 7

Linkage group analysis of *P. anserina* Spore killer types in crosses with centromere-linked marker strains

Linkage group	Centromere marker	*Psk-1* (Wa6)	*Psk-2* (Wa38)	*Psk-4* (Wa46)	*Psk-5* (Y)	*Psk-6* (Wa47)	*Psk-7* (Z)
I	*Cs3*	19/56	24/45	13/61	50/113	31/140	89/218
II	*136*	500/892[a]	424/719[a]	116/224	87/172	255/418	172/341
III	*Cs2*	0/97[b]	5/74[b]	15/79	0/228[b]	11/101[b]	12/122[b]
IV	*Lys2*	13/97	31/72	1/74[b]	101/305	9/50	39/113
V	*As7*	11/57	18/40	ND[c]	11/30	9/12	28/141
VI	*Cs18*	23/57	30/69	25/81	19/45	35/94	43/193
VII	*Cs12*	16/53	44/74	12/25	21/48	14/40	100/232

Only data of one strain per Spore killer type are shown. Other strains of the same Spore killer type show identical results. Spore killers were crossed to marker strains carrying a centromere-linked marker. The fraction of NPD as the number of two-spored asci showing the centromere marker/total number of two-spored asci analyzed is shown.

[a] Random spores are analyzed.

[b] Linkage indicated by a low fraction of nonparental ditype asci.

[c] ND, no data available due to infertility of the cross.

cated by a low tetratype (T) fraction of two-spored asci (FDS for *Psk-7*) for that marker. The T class for *187* is combined with the PD class, since neither can be distinguished phenotypically. If the PDs comprise a considerable part of the combined classes, linkage to the left arm is possible. The large T class for the *rd1* marker also indicates linkage to the left chromosomal arm.

DISCUSSION

Spore killer types in Podospora: We have found segregation distorters showing meiotic drive in natural isolates of the secondarily homothallic ascomycete *P. anserina*. These meiotic drive elements cause the abortion of two of the four spores in the ascus. The two surviving ascospores contain the distorter, whereas the aborted spores contain alleles sensitive to it. Other causes of ascospore abortion, such as translocations (Perkins 1974; Perkins and Barry 1977; Bronson 1988), lethal mutations (Delange 1981), or spore color mutants (Marcou *et al.* 1990; Raju 1994), can be excluded. Reciprocal translocations are not expected to produce two-spored asci in Podospora, and nonreciprocal translocations could produce at most 50% of such asci. Lethal genotypes and spore color mutants can be excluded because both killer and sensitive strains behave normally during selfing and in homokaryotic condition. Our data on backcrosses, killing percentages, and localization of the killer complex demonstrate that the observed spore killing in Podospora is caused by meiotic drive elements.

Rescue of sensitive alleles was first shown in crosses of Neurospora *Sk-2* and *Sk-3* with the mutant *Banana*, which is sensitive to both killers. These crosses produce giant ascospores containing four killer and four sensitive nuclei of both types (Raju 1979). Also, when *Sk-2* and *Sk-3* were introgressed into pseudohomothallic *N. tetrasperma*, no killing was found in heterokaryotic ascospores. This fungus produces four binucleate spores, but unlike *P. anserina*, the spores are heterokaryotic for centromere-linked markers. The sensitive nuclei are saved here because tight linkage of the killer genes to the centromere ensures that every ascospore receives a killer nucleus (Raju and Perkins 1991). As in Neurospora, sensitive alleles can be rescued in Podospora by inclusion in an ascospore with a nucleus containing the killer allele. This is the case in four-spored asci. The sensitive nucleus proved fully functional in backcrosses of the four-spored progeny with the sensitive and killer parents and shows that no irreversible damage occurs to the nucleus during or after meiosis.

Segregation distortion in Podospora differs in an important common aspect from other meiotic drive systems found in nature (Lyttle 1991). Absence of tight linkage to the centromere was observed, which is reflected in the lower fraction of asci in which ascospores are killed in four of the seven Spore killer types. Also the failure to detect resistant or suppressor alleles differs from the other drive systems. The Podospora ascospore abortion factors resemble in their behavior the Spore killer complexes found in the sibling species *P. comata* (Padieu and Bernet 1967) and the heterothallic ascomycetes *C. heterostrophus* (Bronson *et al.* 1990), *G. fujikuroi* (= *F. moniliforme*; Kathariou and Spieth 1982), *N. intermedia*, and *N. sitophila* (Turner and Perkins 1979). However, a significant difference in the reproductive system exists between the secondarily homothallic Podospora and the other ascomycetes, which are heterothallic. This sexual strategy has consequences for the presence of Spore killers. As in *N. tetrasperma*, each ascospore of Podospora contains two nuclei and is heterokaryotic for mating type. SDS for the Spore killer locus results in shielding of the sensitive nucleus within

TABLE 8

Linkage group analysis of *P. anserina* Spore killer types in crosses with noncentromere-linked marker strains

		SDS gene 1		FDS gene 1					
		SDS gene 2[a]	FDS gene 2	SDS gene 2	FDS gene 2		SDS %		
Parent 1	Parent 2	PD/NPD/T	T	T	PD	NPD	Gene 1	Gene 2	Total no. asci
Psk-1 crosses									
XS-Wa6-*rd1*	*187*	156	65	1537[b]		391	10.3	70.6	2139
XS-Wa6-*rd1*	*187*	52	169	1741	126	71	10.3	83.8	2139
XS-Wa6-*rd1*	*187*	40	20	116	42	3	27.1	70.6	221[c]
XS-Wa6-*187*	*rd1*	224	90	2258	615	—[b]	9.9	71.3	3187
XS-Wa6-*187*	*rd1*	96	218	2509	218	116	9.9	81.7	3187
XS-Wa6-*187*	*rd1*	61	163	35	48	8	71.3	30.6	314[c]
Psk-5 crosses									
XS-*Y*-*rd1*	*187*	37	14	552[b]		121	7.1	72.5	723
XS-*Y*-*rd1*	*187*	7	44	605	62	6	7.1	84.6	723
XS-Y-*rd1*	*187*	5	2	32	10	2	13.7	72.5	51[c]
Psk-7 crosses									
Wa58	*rd1*-*187*	55	17	97[b]		15	39.1	76.4	184
Wa58	*rd1*-187	56	16	90	10	12	39.1	79.3	184
Wa58	*rd1*-*187*	44	12	11	4	1	77.8	76.4	72[c]

The *Psk-1*, *Psk-5*, and *Psk-7* Spore killer strains were used in combinations with LG III markers *187* (76% SDS) and *rd1* (84% SDS) to determine the location on the chromosome arm. Only data of one cross reciprocal for the markers are shown for *Psk-1*. Results were similar in all crosses with other possible combinations of used markers. Control crosses of markers with sensitive strains all showed normal segregation patterns. The ascus-type distribution is given for the underlined markers of each cross.

[a] The PD, NPD, and T classes cannot be distinguished from each other in dikaryotic spores.

[b] No distinction can be made in two-spored asci for spores homokaryotic and heterokaryotic for the wild type, and the tetratype T is combined with either PD or NPD. The SDS percentage of the marker is therefore based on the four-spored asci.

[c] Percentages based on the four-spored asci (SDS for *Psk*).

the ascospores and the formation of a normal four-spored ascus whose spores are heterokaryotic for the Spore killer. Killing frequencies in this secondarily homothallic fungus can range from 0% (complete SDS) to 100% (complete FDS), as exemplified by this study. Thus, the percentage of SDS for the Spore killer locus influences the percentage of two-spored asci found. In contrast, the ascospores of heterothallic ascomycetes are homokaryotic and a crossover only leads to a shift in the linear order of the nuclei in the ascus. All four sensitive nuclei are still killed, and the four spores containing the killer nuclei remain. Thus the percentage of SDS does not have any influence on the killing percentage in heterothallic fungi.

An SDS percentage of nearly 100% in Podospora, as found for the mating-type locus, would automatically lead to a nonkilling phenotype. This led Perkins and co-workers to propose that secondary homothallism (in *N. tetrasperma*) evolved as a mechanism to escape Spore killer elements in the heterothallic precursor species (Turner and Perkins 1991; Raju and Perkins 1994). However, this proposition only holds for driving elements in *N. tetrasperma* that are closely linked to the centromere. A Spore killer located more distantly from the centromere would still give a spore-killing phenotype in *N. tetrasperma*, though such high frequencies as found in *P. anserina* would not be expected. No Spore killer elements have been identified in *N. tetrasperma*. However, ascospore abortion is high in outcrosses between wild-collected *N. tetrasperma* strains (Jacobson 1995), and the basis of the ascospore death remains undetermined. Drive elements are not excluded.

We have identified seven different Spore killer types, six of which occurred in a sample of 99 wild-collected strains from Wageningen, The Netherlands. *Psk-2* shows a percentage of killing comparable to the percentage found in *P. comata Sk-1* (or -*a2*; Padieu and Bernet 1967; Turner and Perkins 1991). It is probable that Spore killer complexes found in *P. anserina* and *P. comata* are related, since the two species are relatively interfertile.

One set of Spore killers, the *Psk-3* group, possesses some unique properties different from other killer types. First, the two aborted spores remain visible within the ascus as small unpigmented spores together with the two normal-sized black ascospores. Second, the percentage of killing varies between fruiting bodies within the same cross. Last, *Psk-3* killers show the spore-killing

phenotype only in crosses with specific strains. Most other strains are apparently resistant to *Psk-3* killing. This variable killing percentage superficially resembles the ascospore abortion found in Podospora by Bernet (1965) in crosses between strains S and s. Ascospore abortion occurred in the s perithecia at the restrictive temperature of 18°. The amount of killing found varied over time; perithecia that were initiated later had a decreased amount of two-spored asci. No killing was found in the S perithecia, nor at normal growth temperatures. All the surviving spores in the two-spored asci belonged to the s genotype. The maternal effect is associated with the s prion, which is also involved in the heterokaryon incompatibility reaction in this fungus (Coustou *et al.* 1997). However, the *Psk-3* killing occurs at normal growth temperatures (27°) and does not have a maternal effect.

In the heterothallic *G. fujikuroi*, a mixed Spore killer type, Sk^{mx}, was also found. This killer type causes the abortion of half of the spores in 23–70% of the asci. The remaining asci are normally eight-spored (Kathariou and Spieth 1982; Sidhu 1984). Crosses between Sk^{mx} strains result in a variety of asci containing two, four, six, or eight viable spores (Sidhu 1984, 1988). Sk^{mx} strains are also partially resistant to normal *Sk* strains. A variable killing percentage occurs also in *N. intermedia* with certain partially sensitive or resistant strains. Strains are called resistant in Neurospora when at least 25% of the asci contain eight spores, but the partially resistant strains found in nature produced at least 50% eight-spored asci (Turner 1977 and personal communication). It is possible that the killing reaction of the Podospora *Psk-3* group is caused by a few remaining partially resistant strains, whereas the other strains are fully resistant. The killing of the *Psk-3* group with other specific strains, but not the variability of killing, can also be explained by synthetic lethals (Thompson 1986), *i.e.*, epistatic genes that affect viability only in specific combinations.

Interaction between Spore killer types: The Spore killer types found in *P. anserina* show either dominant epistasis or mutual resistance. In a dominant epistatic interaction one killer strain behaves like a killer and the other like a normal sensitive strain. In the Wageningen population, *Psk-1* and *Psk-7* show dominant epistasis to all other killer types, whereas *Psk-4* and *Psk-6* are sensitive to killing by all the other Spore killers. The dominant epistatic interaction resembles the interaction between Sk^{mx} and *Sk* in *G. fujikuroi*, where Sk^{mx} appears to be dominant epistatic to *Sk*, even though Sk^{mx} kills less efficiently than *Sk* (Kathariou and Spieth 1982).

Interactions between mutually resistant Spore killers exhibit a much lower killing percentage than that observed in either parent when crossed to a normal sensitive. Ascospores from two-spored asci from these crosses are recombinant (NPD) types that now possess both killer elements. These recombinant double killers are less efficient distorters, since sensitive alleles can be rescued by each single killer type. We did not find double killer strains in our sample, but the strain studied by Padieu and Bernet (1967) contained two unlinked killer elements. We have localized six of the Spore killer types by crossing killer strains with sensitive centromere-linked marker strains. Remarkably, almost all Spore killer types are found in linkage group III. Also the *het*-s locus involved in the above-mentioned spore killing between strains s and S is located in linkage group III. The only exception, *Psk-4*, is probably in LG IV.

Recombination can easily occur between unlinked or distally linked killer types, as found for the interactions *Psk-4* × *Psk-6* and *Psk-1* × *Psk-7*. However, the observed recombination percentage of some interactions, *e.g.*, *Psk-1* × *Psk-5*, is hard to understand. Both killer types show a high percentage of FDS and are possibly located on the same arm of LG III. Normal recombination cannot produce such high recombination values for tightly linked markers. A very specific interference type has to be assumed, or perhaps some other factor interferes with the spore killing pattern. In *C. heterostrophus*, the analysis of a Spore killer was complicated by the presence of a translocation (Taga *et al.* 1984; Bronson 1988; Bronson *et al.* 1990).

No mutually sensitive killer strains were found, in contrast to *N. intermedia*, in which Spore killer *Sk-2* and *Sk-3* kill each other when crossed (Turner and Perkins 1979, 1991). However, Turner and Perkins (1991) in their analysis of the data from Padieu and Bernet (1967) with the *P. comata* killer strains show that the results are consistent with mutual killing of the *a* and *b* genotypes. Apparently a mutually sensitive reaction may exist in Podospora, although such Spore killer types have not been encountered in our *P. anserina* sample.

Natural populations: Of the 99 newly isolated *P. anserina* strains from Wageningen, 23% contain a meiotic drive element. As argued in the Introduction, fungi with ordered tetrads linearly arranged in asci provide a unique possibility to observe the genome-wide extent of meiotic drive because any meiotic drive element present in a cross heterozygous for the driving allele will cause spore killing. Viewed in this way, meiotic drive can be concluded to be common in this population. On the other hand, assuming that the number of coding genes per genome is in the order of 10^4, the probability per locus of a segregation-distorting allele is in the order of 10^{-5}, implying that non-Mendelian segregation at nuclear loci is rare indeed. Data on spore killing in other fungal populations show roughly a similar picture. In *N. sitophila*, the overall incidence of *Sk-1* is 19%, but geographic regions exist where sensitive or killer strains were not obtained. The frequencies of *Sk-2* and *Sk-3* in *N. intermedia* in nature are extremely low and killer strains are restricted to the southeast Asian archipelago. The highest number of Spore killers was found in worldwide isolates of *G. fujikuroi* var. *moniliforme*. Here, a total

frequency of 88% *Sk* and *Sk*^{mx} killers was observed (Kathariou and Spieth 1982). However, a later study by Sidhu (1988) of midwestern United States isolates showed a reduced frequency of <50% *Sk* and *Sk*^{mx}. For *G. fujikuroi* var. *subglutinans* Sidhu (1984) found results comparable to those of Kathariou and Spieth (1982). The worldwide incidence of Spore killers in *C. heterostrophus* is ~50% in Race O field isolates, but no killers have been found in the Race T isolates. Spore killers in Race O were restricted to the United States mainland and could be subdivided into regions that were polymorphic or consisted only of killer isolates (Bronson *et al.* 1990).

Most of the Spore killer types originating from Wageningen could be recovered over several years, indicating a relative stability of the killer genes within the natural population. The finding of the *Psk-7* killer type in the recently isolated Dutch population and the French strains isolated almost 60 years earlier also supports the idea of a stable population of killers and sensitives. A prerequisite for the maintenance of a stable polymorphism of driving and sensitive alleles at a distorter locus in fungi is the existence of neutral or resistant strains as predicted by the model of Nauta and Hoekstra (1993). No such neutral strains have been found for *P. anserina* yet, though several killer types are resistant to other killer types. The same situation exists in *G. fujikuroi* where *Sk*^{mx} is partially resistant against *Sk* (Kathariou and Spieth 1982). No resistant types were observed in *C. heterostrophus* (Bronson *et al.* 1990) and strains resistant for *Sk-1*^{K} are rare in *N. sitophila* (Turner and Perkins 1991; B. C. Turner, personal communication). In *N. intermedia*, fully and partially resistant strains to *Sk-2*^{K} and *Sk-3*^{K} or both were found in nature. Genes conferring resistance to killing have been mapped closely linked to the killer complex. *r(Sk-2)-1* is at the left end of the recombination block, while two interacting resistance genes were mapped at loci flanking the right end of the recombination block. Widespread resistance for *Sk-2*^{K} has been found in *N. crassa* and in *N. intermedia* even though the *Sk-2* killer haplotype has not been found in *N. crassa* (B. C. Turner, personal communication).

P. anserina and *N. tetrasperma* are able to reproduce by selfing and do not depend on outcrossing. Selfing protects the offspring from being harmed by Spore killers both because it avoids the introduction of killer elements from other strains and because the program of ascus development results in ascospores that are heterokaryotic for genes far from the centromere (*P. anserina*) or near the centromere (*N. tetrasperma*). Chances for meiotic drive by spore killing to occur depend on the occasional outcross of a sensitive strain with a Spore killer. This aspect of the reproductive system will affect the population genetics of meiotic drive and has not been taken into account in the model of spore killing analyzed by Nauta and Hoekstra (1993). Also the chromosomal location of the distorter locus relative to the centromere was not considered in this population genetics model. One would expect that any linked suppressor of recombination between a distorter and the centromere would be selected, since centromere-linked distorters are maximally effective. Remarkably, we have found some distorters with appreciable amounts of SDS. It is of interest to study further the population genetics of meiotic drive in *P. anserina*, not only taking the reproductive system and the location into account, but also the implications of interactions between abundant Spore killer types for retention of sensitive alleles. Segregation distorters, once established in a population, may probably linger on for a longer time than in heterothallic species.

To understand the evolutionary consequences of spore killing, it is important to know more about the ecology of spore killing. In this study we detected Spore killers in roughly one-quarter of the natural isolates. However, all crosses were done under standardized laboratory conditions at a constant temperature of 27°, while, *e.g.*, the *het*-s locus of *P. anserina* only shows meiotic drive when strain s is used as maternal parent in a cross to S at low temperature (18°). It is important to analyze the effect of fluctuations in environmental conditions such as temperature on spore killing. Also fitness consequences of Spore killers should be studied; *e.g.*, are there differences in size or number of spores from perithecia of crosses homozygous or heterozygous for Spore killers as compared to sensitive strains?

Finally, a mechanistic understanding of spore killing awaits molecular characterization of the genes and gene products involved. At the same time such a molecular approach may yield insight into the evolutionary origins of meiotic drive in fungi.

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