SPORE KILLER, A CHROMOSOMAL FACTOR IN NEUROSPORA THAT KILLS MEIOTIC PRODUCTS NOT CONTAINING IT[1]

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ABSTRACT

Three chromosomal factors called Spore killer (*Sk*) have been found in wild populations of *Neurospora sitophila* and *N. intermedia*. *Sk* resembles other examples of meiotic drive such as Segregation Distorter in Drosophila, Pollen killer in wheat, and Gamete eliminator in tomato. In crosses heterozygous for *Sk*, each ascus contains four viable black ascospores and four inviable, undersize, clear ascospores, with second-division segregations infrequent. The survivors contain the killer allele Sk^K, while unlinked markers segregate normally. Reciprocal crosses are identical. When crosses are homozygous for an allele of *Sk*, all eight ascospores are viable and black in most asci. (Many homozygous crosses have a background level of randomly occurring inviable spores; however, the pattern of 4 viable : 4 small clear ascospores is not found in any of the asci of *Sk*-homozygous crosses.)——Killer ($Sk\text{-}1^K$) and sensitive ($Sk\text{-}1^S$) alleles occur in about equal numbers among a worldwide sample of *N. sitophila* strains, following no geographic pattern. No killer allele has been found in *N. crassa*. $Sk\text{-}2^K$ and $Sk\text{-}3^K$, found in *N. intermedia*, are rare. Most *N. intermedia* strains are $Sk\text{-}2^S$ and $Sk\text{-}3^S$, but some are wholly or partially resistant to one or both of the killer alleles, while not themselves acting as killers. $Sk\text{-}2^K$ and $Sk\text{-}2^R$ are both specific in conferring resistance to $Sk\text{-}2^K$, but not to $Sk\text{-}3^K$. Likewise $Sk\text{-}3^K$ and $Sk\text{-}3^R$ are resistant specifically to $Sk\text{-}3^K$, but not to $Sk\text{-}2^K$. Resistance segregates as an allele of Sk^K.——*Sk-2* and *Sk-3* have been mapped near the centromere of linkage group III after introgression into *N. crassa*, where crossing over is normally 11% between the proximal III markers *acr-2* and *leu-1*. But crossing over is absent in this region when either of the killer alleles is heterozygous ($Sk\text{-}2^K \times Sk\text{-}2^S$, $Sk\text{-}3^K \times Sk\text{-}3^S$ and $Sk\text{-}2^K \times Sk\text{-}2^R$ have been examined).

STUDIES of natural populations of Neurospora, employing wild-collected isolates from different localities and regions, were initiated in 1968 (PERKINS, TURNER and BARRY 1976). Crosses within the same species are usually fertile, with abundant black ascospores. However, especially with crosses involving widely separated populations, perithecia with defective ascospores are sometimes produced. In the extreme case, the developmental process is aborted even before ascospores are delimited. More frequently, viable black ascospores are mixed randomly in the asci with various proportions of brown (sometimes viable), yellow, white, and clear shrunken ascospores. A few strains are found to make regu-

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lar patterns of black *vs.* white ascospores that can be shown to result from heterozygosity for chromosome rearrangements (see PERKINS 1974); in this case ascospore inviability reflects the production of deficiencies by meiotic recombination. When crossed to standard tester strains or any other strains with standard chromosome sequence, each of these rearrangement strains produces its own consistent pattern of black *vs.* white ascospores, but always with more than one type of ascus (see DISCUSSION). Now a different kind of regular pattern, in which four of the eight ascospores in every ascus are very shrunken and transparent (Figure 1), has been found. This behavior is the result of a new type of chromosomal gene (or gene complex), Spore killer (*Sk*). Heterozygosity in meiosis for the killer (Sk^K) and sensitive (Sk^S) alleles results in death of the four ascospores that receive the wild-type allele, Sk^S. This is an example in fungi of meiotic drive (ZIMMERING, SANDLER and NICOLETTI 1970).

The Spore killer factors in Neurospora resemble in many respects the extensively studied Segregation Distorter (*SD*) factors in Drosophila (see HARTL and

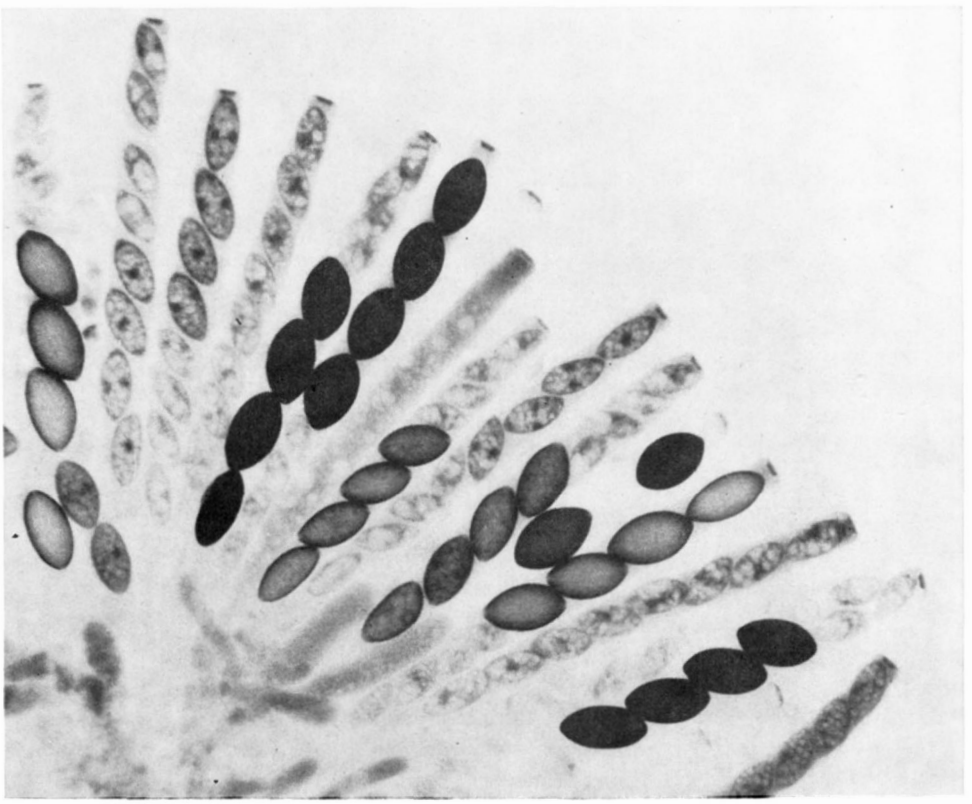

FIGURE 1.—Asci from *Sk-2*^{K} × *Sk-2*^{S} in *Neurospora crassa*, showing progressive degeneration of Sk^S ascospores and progressive enlargement and darkening of Sk^K ascospores as the ascus matures. Asci originate asynchronously in Neurospora. The asci shown differ in age and stage of development. The mature black ascospores measure about 29 × 15 μm. Photograph by N. B. RAJU.

HIRAIZUMI 1976; CROW 1979). Other similar systems are "sex ratio" in *D. peudoobscura* (see POLICANSKY and ELLISON 1970), the Pollen-killer gene (*Ki*) of wheat (LOEGERING and SEARS 1963), the Gamete-eliminator gene (*Ge*) in tomato (RICK 1966, 1970), and two genes that result in death of ascospores in the fungus, Podospora (PADIEU and BERNET 1967). There may be similarity also to killing of homologs by specific alien chromosomes, which has been described in Nicotiana (CAMERON and MOAV 1957) and in wheat (MAAN 1975, 1976; ENDO and TSUNEWAKI 1975).

The term "killer" has been used for quite different phenomena in *Saccharomyces cerevisiae* (HERRING and BEVAN 1974), *Paramecium aurelia* (SONNEBORN 1959) and *Ustilago maydis* (KOLTIN and DAY 1976), where it has been applied to phenotypes involving cytoplasmic agents that actively kill the sensitive strain. No such agents have been found in the Spore killer strains of Neurospora.

The present paper describes preliminary genetic results with three different Spore killer genes, all of which were found in nature. (The individual factors will be referred to as genes, although a tightly linked complex is not ruled out.) A companion paper (RAJU 1979) provides cytological descriptions of the three Spore killers and reports cytogenetic experiments bearing on the mode of action of one of them. These results have been partially reported in abstract form (TURNER and PERKINS 1976; RAJU 1976; TURNER 1977).

MATERIALS AND METHODS

Three related species of Neurospora were used: *N. crassa*, *N. intermedia*, and *N. sitophila*. Their relationship to each other and criteria for distinguishing them are discussed in PERKINS, TURNER and BARRY (1976). *N. crassa* and *N. intermedia* are closely related. Asci are formed in experimental crosses between the two, and ascospores are produced; most of these are inviable, but usually a small percent are black and viable and, from these, fertile hybrid cultures can be obtained. Crosses of *N. sitophila* with either *N. crassa* or *N. intermedia* are highly sterile; only rarely is a combination found that produces any viable ascospores. Standard wild-type reference strains of the three species are described by PERKINS, TURNER and BARRY (1976).

In the present study, the standard *N. sitophila* strains P8085 *A*, P8086 *a*, or acon *A*, acon *a* were used as *Sk-1*^{K} (killer) references. (These are derived from Arlington (Virginia) stocks.) *N. sitophila* strains FGSC 1134 *A* and FGSC 3191 *a* were used as *Sk-1*^{S} (sensitive) testers. (FGSC signifies Fungal Genetics Stock Center. FGSC 1134 *A* was collected in Panama and FGSC 3191 *a* was derived from a cross of FGSC 1134 *A* × FGSC 2493 *a* from Malaya. *N. sitophila* stocks FGSC 2009 *A* and 2010 *a* originated from Naukka, East Nigeria, and were obtained from D. P. MAHONEY.)

The standard *N. intermedia* reference strains, FGSC 1766 and 1767, were used as *Sk-2*^{S} and *Sk-3*^{S} testers. The *Sk-2*^{K} strains used in most of this work were derived from a strain of *N. intermedia* collected from forest soil in Brunei, Borneo, by J. G. WARCUP. The original stock (now FGSC 3257) was obtained from A. M. SRB; *Sk-2*^{K} was discovered in *nit-4* derivatives of it (BLAKELEY and SRB 1962), which came from FGSC. Origins and derivations will be given in the text for other *N. intermedia* stocks, including *Sk-3*^{K}.

In *N. crassa*, the standard strains OR23-1VA, ORSa, or *fl A*, *fl a* were used as *Sk-2*^{S} and *Sk-3*^{S} testers. FGSC 2222 is a *Sk-2*^{R} *Sk-3*^{S} *N. crassa* strain collected in Louisiana. *Sk-2*^{K} and *Sk-3*^{K} were introduced into *N. crassa* from *N. intermedia* by recurrent backcrosses to the sensitive standards. Tester stocks with *Sk2*^{K} and *Sk-3*^{K} in *N. crassa* background were then selected. These *N. crassa Sk-2*^{K} and *Sk-3*^{K} stocks are the only strains used in this study that have mixed

ancestry involving a second species. All sensitive strains and all resistant strains carry *Sk* alleles that originated in the same species.

In all three species a number of different testers were used as work progressed. Those found to be most useful have been deposited in FGSC (see BARRATT and OGATA 1978 and subsequent stock lists).

The mutants of *N. crassa* and *N. sitophila* used in this study are documented by BARRATT and OGATA (1978). *N. intermedia* mutants requiring pyridoxine (*pdx*) and asparagine (*asn*) were obtained from SHEW (1977, 1978). Origins of most wild-collected strains are given by PERKINS, TURNER and BARRY (1976). The "multicent" linkage tester (FGSC 2015) used for mapping *Sk-2* in *N.* crassa contains markers near the centromeres of all seven linkage groups (PERKINS 1972).

Individual isolates were scored for *Sk* by crossing to tester strains of Sk^K and Sk^S. The test crosses were scored for the presence of asci with four of the ascospores aborted (clear and small). These test crosses were usually carried out on slants of synthetic cross medium (WESTERGAARD and MITCHELL 1947) in 13 × 100 mm tubes; the tester strain was used as protoperithecial parent and was fertilized four to seven days after inoculation (25° throughout). Approximately ten days later, both viable and defective ascospores had been shot to the walls of the tubes. In $Sk^K \times Sk^S$ crosses, about half of the ascospores are unpigmented and distinctly smaller than their normal siblings. Because they are much less conspicuous, they may appear to the eye to be less numerous; however, it is often possible to discern discrete asci. These appear as four black ascospores with an associated small clump of clear ascospores. Shot ascospores are often completely adequate for scoring crosses between laboratory strains for which *Sk* heterozygosity is known to be the only plausible reason for the production of defective ascospores. When one of the parents is an isolate from nature or is known to produce defective ascospores in crosses homozygous for *Sk*, it is usually necessary to open perithecia in a drop of water or 15% sucrose solution and examine intact asci.

RESULTS

Three different Spore killer genes have been discovered. *Sk-1* was found in *N. sitophila* from numerous sources. In *N. intermedia*, *Sk-3* was found in a wild strain from only one locality, while *Sk-2* was found in three localities. *Sk-2* and *Sk-3* map in the same region of Linkage Group III, but they differ in several respects and may be at separate loci. *Sk-1* resembles the other two in showing little second-division segregation, but it has not been assigned to a linkage group.

RAJU (1979) has shown that early development is normal in asci of $Sk^K \times Sk^S$ crosses, and abnormalities appear only after walls have been formed around the eight ascospores. Four of the ascospores then cease development and begin to degenerate. Each mature ascus characteristically contains four viable black ascospores, which are Sk^K, and four inviable white ascospores, which are Sk^S. For photographs of asci from $Sk^K \times Sk^S$, see Figure 1 of the present paper and Figures 1 and 2 of RAJU (1979). In mature asci, the killed ascospores are generally smaller and less conspicuous with *Sk-3* than with *Sk-1* or *Sk-2*.

Spore-killer-1 in Neurospora sitophila

Sk-1 was first detected in crosses between two *N. sitophila* strains from Nigeria (FGSC 2009 and 2010) and two from North America (Arlington, Virginia, FGSC 2019 and 2020). Nigeria *A* × Nigeria *a* and Arlington *A* × Arlington *a* made mostly normal asci with all of the ascospores black. In contrast, both intercrosses between the Arlington and Nigeria strains made mostly abnormal asci

that contained four opaque black ascospores and four small, clear inviable ascospores. Other *N. sitophila* strains were examined, and all resembled either the Arlington or the Nigeria strains.

In all intercrosses between Arlington-type strains and Nigeria-type strains, mature asci contained 4 black: 4 clear aborted spores; at least 95% were arranged linearly in typical first-division segregation patterns, with four like-spores grouped together at each end of the ascus. This result was identical in crosses reciprocal with respect to mating type and did not depend on which parent was used as female (protoperithecial) parent and which as fertilizing parent. When the viable (black) ascospores were germinated and tested, almost all f_1's behaved in crosses like the Arlingtons, and not like the Nigerias. On this basis, the factor carried by the Arlington-type strains was named "Spore killer" (Sk^K), and the Nigeria-type strains were called "sensitive" (Sk^S). With the discovery of different Spore killer factors in other strains, the factor in *N. sitophila* was designated *Sk-1*.

When 71 *N. sitophila* strains from nature were tested by crossing to $Sk\text{-}1^K$ and $Sk\text{-}1^S$ standard testers, Spore-killer and sensitive types were both frequent. No intermediate or alternative types were found. There is no apparent relation between the *Sk-1* genotype and geographical origin, although the collection is worldwide. In some areas both $Sk\text{-}1^S$ *and* $Sk\text{-}1^K$ were found (numbers are total *N. sitophila* strains collected in that area): Kyushu, Japan (3), Kauai, Hawaii (4), Panama (3), Palo Alto, California (3). In some other areas only sensitive strains were found: Karnataka, India (15), Malaya (2), Florida (1), Brazil (1). Yet other areas yielded only killer strains: Australia (2), Java (1), Nigeria (2), France (1), England (4), Pennsylvania (1), Virginia (2), Louisiana (26).

$Sk\text{-}1^S$ is highly susceptible to killing by $Sk\text{-}1^K$. In six $Sk\text{-}1^K \times Sk\text{-}1^S$ crosses, only eight $Sk\text{-}1^S$ progeny were recovered among 337 germinants from randomly collected ascospores. Apparently these were chance survivors rather than genetic recombinants with a more resistant phenotype, for they produced the typical 4:4 pattern when testcrossed to $Sk\text{-}1^K$. To further examine this question, shot asci were tabulated from a cross between standard strains $Sk\text{-}1^K$ (FGSC 2116) × $Sk\text{-}1^S$ (FGSC 3191) (for method, see PERKINS 1974). The percentage of asci with five or more black ascospores varies from cross to cross; for these parents it was about 5%. It is assumed that such asci contain four $Sk\text{-}1^K$ ascospores and one or more $Sk\text{-}1^S$. Results consistent with this expectation were obtained when ascospores from these exceptional asci were germinated and tested.

In most $Sk\text{-}1^K \times Sk\text{-}1^K$ crosses virtually all of the ascospores are black and viable. (It may be worth noting that the species standard strains were selected for exactly this characteristic.) In $Sk\text{-}1^S \times Sk\text{-}1^S$ crosses, there are often some brown and tan ascospores, but virtually all of the ascospores are of normal size and have some amount of pigmentation, unlike the aborted ascospores of $Sk\text{-}1^K \times Sk\text{-}1^S$ crosses.

Sk-1 is not linked to mating type, to the *N. sitophila* markers *pdx* (FGSC 348), *thi* (FGSC 347), and *acon* (FGSC (2664), or to several morphological markers that originated in wild-collected strains. All these markers segregate 1:1 in the surviving progeny. The very low fertility of interspecific crosses with *N. crassa*

creates serious difficulties in assigning *Sk-1* to a linkage group of *N. crassa*. Furthermore, it appears that the *N. crassa* strains used so far are not sensitive to *Sk-1*^{K}.

Spore killer-2 in Neurospora crassa *genetic background*

Sk-2 was discovered in crosses with *N. crassa* stocks into which a *nit-4* mutant had been introgressed from *N. intermedia*, and *Sk-2* was then traced back to the single *N. intermedia* ancestor from which the marker had originated, a strain collected in Borneo.

Defective ascospores were noticed in routine crosses of nitrate nonutilizing strains *nit-4 A* (FGSC 1170) and *a* (FGSC 1171) with standard *N. crassa* wild types. All asci were found to contain 4 black : 4 clear aborted ascospores, and virtually all asci showed the arrangements expected from first-division segregation. All F_1 strains from the viable black ascospores produced the same kind of asci when crossed to various strains of *N. crassa*. The responsible factor, called *Sk-2*^{K}, segregated independently of *nit-4* and of mating type. Maturing asci of *Sk-2*^{K} × *Sk-2*^{S} are shown in Figure 1.

Both large pigmented and small clear ascospores are shot from the perithecia of crosses heterozygous for *Sk-2*^{K}, in approximately equal numbers. The clear ascospores are usually distinctly smaller than their normal siblings, although considerable size variability is found. When ejected ascospores from *Sk-2*-heterozygous crosses are collected as groups of eight, representing unordered tetrads, almost all groups are seen to consist of 4 black and 4 small clear ascospores (Figure 2A).

Crosses homozygous for *Sk-2*^{K} (Figure 2E, F) are distinctly different from all heterozygous crosses. All the ascospores in *Sk-2*^{K} × *Sk-2*^{K} crosses are of normal size or nearly so, and a majority of the asci contain eight viable ascospores. However, the viability of the ascospores depends on the genetic background. Most *Sk-2*^{K} × *Sk-2*^{K} crosses in standard *N. crassa* (Oak Ridge) background are not completely normal, even after ten generations of backcrossing (Figure 2F). A significant fraction of ascospores, although normal in size, are only partially pigmented (pigmentation is closely correlated with viability). Nevertheless, these homozygous crosses are still in marked contrast to heterozygous crosses (Figure 2A), where each ascus typically has four aborted ascospores much smaller than normal.

We have now developed *Sk-2*^{K} strains that give very few defective ascospores when crossed to each other or to their progeny (Figure 2E). This was accomplished by outcrossing *Sk-2*^{K} to a series of *N. crassa* strains from nature, each time selecting the progeny that gave the most normal crosses to each other and to their *Sk-2*^{K} parent. Considering the excellent results shown in Figure 2E, it is evident that the defective ascospores produced in *Sk-2*^{K} × *Sk-2*^{K} crosses with Oak Ridge background are not due to the *Sk-2* locus alone. However, we have reported the data in Figure 2F because many of our marked stocks have Oak Ridge background, and therefore we are obliged to work with such crosses.

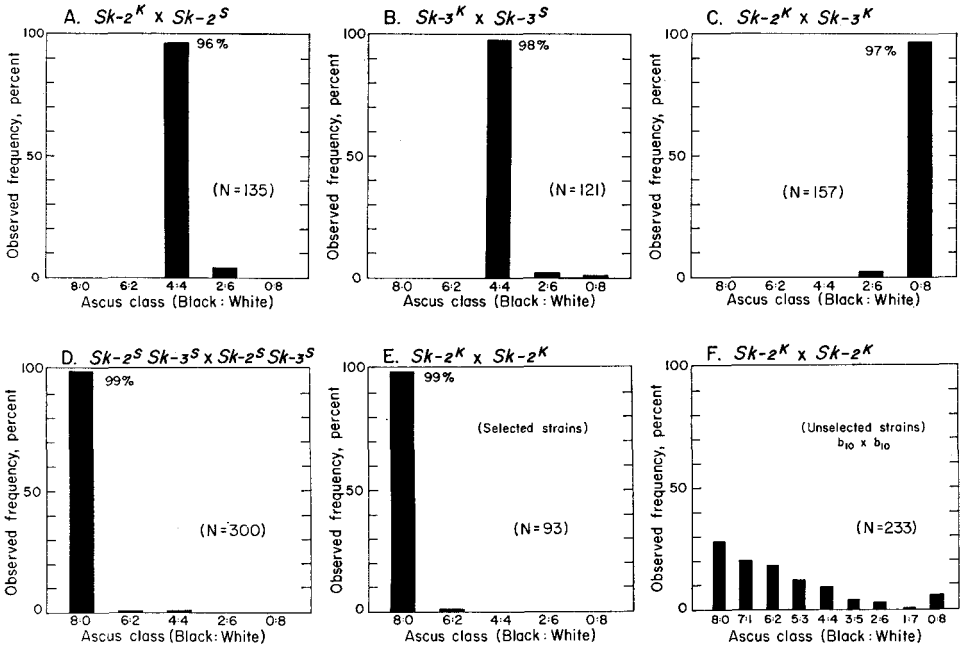

FIGURE 2.—Frequencies of unordered ascus types in crosses that involved various *Sk-2* and *Sk-3* genotypes in *Neurospora crassa* genetic background. Asci were collected as groups of eight ascospores shot together from the perithecia, as described by PERKINS (1974). Histograms show the frequencies of asci containing the indicated numbers of ascospores of the two classes, normal (large, usually viable, black) and defective (inviable, usually clear, small). The five major classes range from eight ascospores normal (8:0) to eight ascospores defective (0:8). *N* is the observed number of asci. Except for cross F the odd classes 7:1, 5:3, 3:5, and 1:7 are very infrequent and have been ignored. The defective ascospores in F are mostly full size, and many are partially pigmented; they are attributed to some cause other than Spore killer. *Sk-2*^{K} had been backcrossed recurrently to standard inbred *N. crassa* laboratory strains to obtain the parents for F, whereas the parents for E were obtained selectively from outcrosses of *Sk-2*^{K} to wild-collected *N. crassa* strains.

Chromosomal location of Sk-2: When crossed with the seven-group linkage tester, multicent, *Sk-2* showed complete linkage to the linkage-group III centromere marker, *acr-2*, and segregated independently of markers in the other six groups. Attempts have been made to obtain recombinants between *Sk-2*^{K} and *acr-2*, as well as other centromere-linked genes in III. These attempts have been completely unsuccessful for the markers *acr-2*, *sc*, *spg*, *thi-4*, *un-6* and *leu-1*, occupying a proximal region within which 11% crossing over is normally expected to occur.

In these crosses, all proximal markers necessarily entered the cross in coupling with *Sk-2*^{S}. In this situation, noncrossover progeny carrying the markers will usually be killed, but any recombinants where a marker has crossed over into the *Sk-2*^{K} chromosome should survive. Our experience with crosses between *Sk-2*^{K} and marked sensitive strains has been that the proximal markers were usually not recovered at all. Occasionally some viable progeny with a proximal marker were

obtained as rare exceptions (1% or less), but these invariably proved to be *Sk-2*^{S} rather than *Sk-2*^{K}—genotypic *Sk*-sensitives that had somehow escaped being killed. For example, in crosses of *Sk-2*^{K} by *Sk-2*^{S} strains carrying the III-centromere marker, scumbo, one progeny among 238 was *sc*. This exceptional *sc* progeny was not a recombinant, however, but rather an *Sk*-sensitive parental type. With the acriflavin-resistant marker, *acr-2*, a selective procedure made it possible to obtain larger numbers of exceptional acriflavin-resistant F_1's. Ascospores from *Sk-2*^{K} *acr-2*^{S} × *Sk-2*^{S} *acr-2*^{R} were germinated on sorbose medium containing acriflavin. A total of 143 *acr-2*^{R} progeny were obtained from an estimated 70,000 germinated ascospores. All of these were *Sk-2*^{S}, like the *acr-2*^{R} parent. The closely linked morphological marker *spg* (sponge) was present in coupling with *acr-2* in the crosses that produced 63 of the *acr*^{R} progeny, and *spg*, like *acr-2*, failed to recombine.

Possible association of Sk *with a chromosome rearrangement*: Crossing over in *Sk-2*^{K} × *Sk-2*^{S} is completely suppressed between *acr-2* and *leu-1*, a region that is normally about 11 units long (Table 1a, b). In contrast, crossing over is not eliminated in the more distal regions of linkage group III, to the right of *leu-1*. *Sk-2*^{K} has not been separated from the crossover suppression during at least ten recurrent backcrosses to *N. crassa* reference strains. A less inbred strain of *Sk-3*^{K} shows the same effect (Table 1c). These observations suggest that the Spore killer chromosomes may differ structurally from standard sequence in a region adjoining or including *Sk*^{K}. (Table 1d and e will be discussed later under "Genetic analysis of resistance.")

There is no genetic or cytological evidence of chromosomal interchange involving the Spore killer chromosome. Markers in all other linkage groups of *N. crassa* segregate independently of *Sk-2*. There are also no patterns of aborted ascospores of the types that would be expected if a translocation were present in addition to the *Sk* factor.

The possibility of intrachromosomal rearrangement is more difficult to evaluate because inversions, especially short ones, escape detection both genetically and cytologically. (No example of a simple short inversion is, in fact, known in Neurospora, although other rearrangement types are well represented—see PERKINS and BARRY 1977.) A brief cytological examination of meiotic chromosome pairing and disjunction has been made by E. G. BARRY (personal communication), using *N. crassa Sk-2*^{S} × *Sk-2*^{K} crosses. The preparations were not satisfactory for detecting structural rearrangements by pachytene analysis, but frequencies were determined of dicentric bridges and acentric fragments in telophase nuclei. An elevated frequency of these would provide *prima facie* evidence of a paracentric inversion. Bridge and fragment production in the *Sk-2* heterozygotes did not exceed the background level for normal-sequence homozygotes. Therefore, it is not likely that the *Sk-2*^{K} chromosome contains a large paracentric inversion. A short inversion is not excluded. Alternatively, the local suppression of crossing over could be due to the presence in *Sk*^{K} of an altered control element that blocks recombination (CATCHESIDE 1974).

TABLE 1

Effect of Sk-2^K *and* Sk-3^K *on meiotic crossing over in linkage group III in* N. crassa

Zygote genotype and recombination %	Parentals	Single crossovers *Sk-acr*	Single crossovers *acr-leu*	Single crossovers *leu-his*	Double crossovers
a. No Sk^K present:					
Sk-2^S + + *his-7*	28	..	3	5	0
Sk-2^S *acr-2* *leu-1* +	18	..	4	8	0
11 20					
b. *Sk-2^K* × *Sk-2^S*:					
Sk-2^K + + *his-7*	60	0*	0	20	0
Sk-2^S *acr-2* *leu-1* +	0	0	0	0	0
0 0 25					
c. *Sk-3^K* × *Sk-3^S*:					
Sk-3^K + + *his-7*	62	0*	0	8	0
Sk-3^S *acr-2* *leu-1* +	2*†	0	0	0	0
0 0 11					
d. *Sk-2^R* × *Sk-2^K*:					
Sk-2^R *acr-2* *leu-1* *his-7*	15	0	0	2	0
Sk-2^K + + +	46	0	0	17	0
0 0 24					
e. *Sk-2^R* × *Sk-2^S*:					
Sk-2^R + + +	28	5	2	2	0
Sk-2^S *acr-2* *leu-1* *his-7*	28	8	4	1	2‡
19 10 4					

The *Sk-2^K* and *Sk-3^K* alleles were introgressed from *N. intermedia*.

The order *acr-2 leu-1 his-7* is based on normal crossing over as shown in the first cross. The centromere is located near *acr-2*. *Sk^K* has arbitrarily been shown at a locus left of *acr-2*. Crossing over has not been studied in crosses homozygous for *Sk^K* because *Sk^K* stocks are not available that contain markers closer than *his-7*. The same *acr-2 leu-1* parent was used in crosses a, b, and c. Like all *N. crassa* laboratory stocks tested, it was found to be *Sk-2^S Sk-3^S*. The *Sk-2^R* allele in crosses d and e was found in a wild-collected *N. crassa* strain from Louisiana (FGSC 2222).

The top number of each pair of complementary classes represents progeny of the genotype that contains the top allele of the leftmost marker. Isolation number of markers: *acr-2* KH5, *leu-1* 33757, *his-7* Y152M31. Germination was 80 to 82% in all crosses, among the black ascospores that were isolated.

* *acr-2^S* progeny were not scored for *Sk*, but were assumed to be *Sk^K* on the basis of many thousand isolates screened from similar *Sk^K* × *Sk^S* crosses.

† These two *Sk*-3-sensitive progeny illustrate the occasional survival of sensitive progeny from *Sk^S* × *Sk^K* crosses.

‡ Double crossovers in regions 1 and 2.

There is also the question of whether the suppression is due to a difference between *Sk^K* and *Sk^S* or to a difference between *N. crassa* and all *N. intermedia*, regardless of *Sk* genotype. It appears to be the former, for crossing over at near-normal levels has been observed between *acr-2*, *leu-1*, and *his-7* in several crosses of *N. crassa* × *N. intermedia* in the absence of *Sk^K*.

The killing behavior of *Sk-2^K* remains unchanged in structurally heterozygous crosses with a translocation strain that involves the same region. *T(II;III) T54M140b* has one of its interchange points located between *acr-2* and *sc* in the

centromere region of III where *Sk-2* is located. When *Sk-2^S T sc un-6* was crossed × *Sk-2^K Normal Sequence* + +, the surviving progeny were all *sc*$^+$ *un*$^+$.

Spore killer-2 and Spore killer-3 in Neurospora intermedia

The original *Sk-2^K* strain from Borneo was crossed to other *N. intermedia* strains to identify them as *Sk-2^K* or *Sk-2^S* and to derive testers that would be more morphologically normal and fertile than the original (which cannot grow on standard medium because it carries *nit-4*, which prevents utilization of nitrate).

Crosses involving *Sk-2^K* are similar in *N. intermedia* and *N. crassa*. Homozygous *Sk-2^K* × *Sk-2^K* crosses produce asci with eight normal-sized ascospores. In some of these crosses there are pale or light brown ascospores scattered among the black ones. This is quite common for *N. intermedia* crosses, and defective ascospores of this type are easily distinguished from the smaller, clear ascospores produced because of Spore killer in heterozygous *Sk-2^K* × *Sk-2^S* crosses. The *N. intermedia Sk-2^K* testers used were derived from the original Borneo strain by three generations of crosses to the standard *Sk-2^S a* tester, and no serious attempt has been made to obtain stocks that would not have the background of large defective ascospores.

Sk-3^K was discovered in a population from New Guinea, as described below.

The Rouna, New Guinea, collection of N. intermedia: After the discovery of *Sk-1* and *Sk-2*, our records of test crosses of wild-collected Neurospora were screened for evidence of other Spore killer strains. The most intriguing results involved a very unusual group of cultures of *N. intermedia* collected near Rouna Falls, Papua (New Guinea). (These are listed below in Table 3.) Many different kinds of spore patterns were found in crosses among Rouna strains and between the Rounas and other strains of *N. intermedia*. Some crosses gave varying proportions of nonviable spores, sometimes all full sized and sometimes including small clear spores such as those produced by *Sk^K* × *Sk^S*. A group of crosses gave 4:4 asci just like those produced by crosses heterozygous for *Sk-1^K* or *Sk-2^K*.

Analysis of the Rouna group was exceedingly laborious. Two of the strains proved to contain chromosome rearrangements, and some of the problems related to faulty ascospore genesis have not yet been resolved. At the time analysis was undertaken, *Sk-2* was known only in *N. crassa*, and for both *Sk-2* and *Sk-1* only the killer and sensitive phenotypes were known. That is, for *Sk-2* in *N. crassa* and *Sk-1* in *N. sitophila*, all strains gave asci with eight normal-sized spores with the *Sk^S* tester and 4:4 asci with the *Sk^K* tester, or *vice versa*, and no other pattern of behavior had been observed.

Two of the major questions originally presented by the Rouna collection were: "Is the *Sk* in Rouna the same as *Sk-2* discovered in *N. crassa*?" And "Why do some crosses of *Sk^K A* × *Sk^K a* from Rouna make over 99% aborted ascospores?" (P757 × P758 did so, for example.)

It turned out that the answer to the first question was not simple, but once found it led directly to answering the second. The Rouna collection proved to contain not only *Sk-2^K* but also a second, linked Spore killer factor with different properties—*Sk-3^K*. (Strain P757 proved to be *Sk-2^K Sk-3^S*, while P758 was *Sk-2^S*

Sk-3^K.)*Sk-3* resembles *Sk-2* in behavior and chromosomal location, but the two differ in specificity of killing and resistance, each being vulnerable to killing by the other.

Diagnosis was based on the following sequence of observations: When *Sk-2*^{K} from Borneo became available in *N. intermedia*, it was crossed to "the *Sk* from Rouna" (P758), and vast numbers of ascospores were produced, almost all of them small and clear, with a scattering of black, viable ascospores. This was exactly the pattern produced by the mysterious intra-Rouna crosses. *Sk-2*^{K} testers were then crossed to strain P757 and the other Rouna cultures that were known to make 99% aborted ascospores with P758, and these crosses produced asci with eight normal-sized ascospores, many of which were viable. Thus, strain P757 fit the criteria for *Sk-2*^{K}, and the Spore-killer allele carried by strain P758 was designated *Sk-3*^{K}.

In addition to being mutually sensitive, *Sk-2*^{K} and *Sk-3*^{K} were found to differ in their interactions with a large number of wild-type strains. Some strains are resistant (*Sk*^{R}); that is, they are not *Sk*^{K} (because when crossed to *Sk*^{S}, they produce asci with eight black viable ascospores), but neither are they *Sk*^{S} (because when crossed to *Sk*^{K}, they produce asci with eight black viable ascospores). Some strains are *Sk-2*^{R} (resistant to *Sk-2*^{K}); some strains are *Sk-3*^{R} (resistant to *Sk-3*^{K}); and some are *Sk-2*^{R} *Sk-3*^{R} (resistant to both). This phenomenon was first discov-

TABLE 2

Types of asci produced in crosses with Sk *tester strains*

Constitution of tested strain		Constitution of tester parent		
Sk-2	*Sk-3*	*Sk-2*^{K} *Sk-3*^{S}	*Sk-2*^{S} *Sk-3*^{K}	*Sk-2*^{S} *Sk-3*^{S}
K	*S*	8:0	0:8	4:4
S	*K*	0:8	8:0	4:4
S	*S*	4:4	4:4	8:0
R	*S*	8:0	4:4	8:0
S	*R*	4:4	8:0	8:0
R	*R*	8:0	8:0	8:0

S: Sensitive to Killer. *K*: Killer; *R*: Resistant.

The predominant ascus type is listed for each cross. Many isolates from nature are poorly fertile (in general or with specific testers), but most can be diagnosed even when random ascospore abortion is superimposed on the *Sk* effects. "Bubble" asci (eight shrunken, hyaline ascospores which usually are not shot) are very common in Neurospora and are usually ignored in studies related to ascospore genesis. They are ignored here when accompanied by large numbers of asci that contain viable ascospores. Some tested strains give a preponderance of aborted ascospores in all crosses with available testers and therefore cannot be scored for *Sk*; such strains have a variety of problems unrelated to *Sk* and are not covered by this table.

Explanation of entries:

0:8 In *Sk-2*^{K} × *Sk-3*^{K} crosses, masses of undersize, clear ascospores are shot. A very small proportion of asci have one pair of viable ascospores.

4:4 In *Sk-2*^{K} × *Sk-2*^{S} or *Sk-3*^{K} × *Sk-3*^{S} crosses most asci have four undersize, clear ascospores and four that are full-size and pigmented. In some crosses a few asci with five or six viable ascospores are found.

8:0 Most asci have eight full-size ascospores, not necessarily all dark. (In some *Sk-2*^{K} × *Sk-2*^{R} or *Sk-3*^{K} × *Sk-3*^{R} crosses there are in addition up to 50% partially aborted mainly 4:4 asci, with the percent consistent for each specific *Sk*^{R} strain. Such asci are not seen in 8:0 crosses other than *Sk*^{K} × *Sk*^{R}.)

ered during the analysis of the intercrosses of Rouna strains. Table 2 shows the ascospore patterns of crosses among the six known *Sk* genotypes of *N. intermedia*. As noted, resistance is sometimes only partial. Preliminary information on the genetic behavior of $Sk\text{-}2^R$ and $Sk\text{-}3^R$ will be presented in a later section.

When the original Borneo $Sk\text{-}2^K$ allele and the Rouna $Sk\text{-}2^K$ allele were crossed to a number of strains from nature, results were parallel. All strains were either sensitive to both or resistant to both. (The Rouna $Sk\text{-}2^K$ strains have not been tested further, and statements about $Sk\text{-}2^K$ in other sections of this paper refer only to the Borneo allele.) When $Sk\text{-}3^K$ was crossed to the same strains from nature, the strains that were found to be resistant to $Sk\text{-}3^K$ were randomly divided among those that were $Sk\text{-}2^S$ and those that were $Sk\text{-}2^R$. This is additional evidence that $Sk\text{-}2^K$ and $Sk\text{-}3^K$ are different from each other.

The designations eventually assigned to the 15 Rouna cultures are listed in Table 3. Cultures P32 and P35 were both mixtures at the time of collection, and ascospore progeny were obtained from both cultures. At least one component of each mixture was lost between collection and stock preservation; the starred phenotypes are those inferred from analysis of the progeny.

The standard $Sk\text{-}3^K$ testers were obtained by three backcrosses to the *N. intermedia* standards. They are very similar to the $Sk\text{-}2^K$ testers in producing some full-size pale spores in homozygous crosses. For either $Sk\text{-}2^K$ or $Sk\text{-}3^K$, progeny from both homozygous and heterozygous crosses are often found to be deficient in their ability to make normal fertile crosses with the Sk^K parent or with Sk^S testers. Fertility problems just like these have been found repeatedly in other *N. intermedia* studies unrelated to Spore killer, and it would probably be a mistake to consider them intrinsic to the essential nature of the *Sk* phenomenon,

TABLE 3

Sk *constitution of* N. intermedia *isolates collected in a series between Rouna Falls and Port Moresby, New Guinea*

Strain No.	Constitution *Sk-2*	*Sk-3*	Strain No.	Constitution *Sk-2*	*Sk-3*
P32–1*	*S*	*R*	P38A†	*R*	*S*
P32–2(P757a)	*K*	*S*	P39A	*R*	*S*
P32–3(P758A)	*S*	*K*	P40a	*R*	*S*
P33a	*R*	*R*	P41a	*R*	*R*
P34a	*S*	*R*	P42a	*R*	*R*
P35–1*	*K*	*S*	P43A	*R*	*S*
P35–2*	*R*	*R*	P44a	*R*	*S*
P35–3A	*R*	*S*	P45A	*R*	*S*
P36A	*S*	*R*	P46a	*R*	*S*
P37A†	*R*	*S*			

Diagnosis of the *Sk* constitution was based on behavior with tester strains, as in Table 2.

Strains are numbered in the order of collection, along the 35 km road from Rouna Falls Lookout to Port Moresby. P32 to P36 came from a single site at the lookout.

* P32 and P35 were mixed cultures. Starred strains were not carried into the preserved cultures and are inferred from progeny analysis.

† These strains contained a reciprocal translocation.

especially considering that some *Sk-2*^{K} × *Sk-2*^{K} and *Sk-3*^{K} × *Sk-3*^{K} crosses produce 95% black, viable ascospores. As noted previously, excellent crosses are readily obtained with *Sk-1*^{K} × *Sk-1*^{K}.

Spore killer-3 in Neurospora crassa *background*

After introgression into *N. crassa*, *Sk-3*^{K} resembled *Sk-2*^{K} in all essential respects except specificity of killing and resistance. Like *Sk-2*, *Sk-3* is linked to the centromere of III.

When *Sk-3*^{K} is heterozygous, asci that show ascospore arrangements characteristic of second-division segregation are more frequent than for *Sk-2*, but they are still rare (1 or 2% at most). Crossing over is suppressed between *acr-2* and *leu-1*, but not in distal IIIR regions, just as was true for *Sk-2* (Table 1c). The types and frequencies of unordered asci from *Sk-3*^{K} × *Sk-3*^{S} resemble those in crosses heterozygous for *Sk-2* (Figure 2.—Compare B with A). The killed ascospores are usually smaller with *Sk-3*^{K} than with *Sk-2*^{K}.

When *Sk-3*^{K} *is* homozygous, asci with five to eight black ascospores are seen, and the spectrum of ascus types is clearly distinct from that of heterozygous *Sk-3*^{K} and *Sk-3*^{S} crosses, and more nearly normal. In contrast to *Sk-3*^{K} × *Sk-3*^{K} in *N. intermedia*, no completely normal homozygous *Sk-3*^{K} × *Sk-3*^{K} crosses have been found in *N. crassa*. Instead, the asci resemble those graphed for *Sk-2* in Figure 2F. The abnormal ascospores are attributed to causes other than *Sk-3*^{K} itself, and an attempt will be made to select *Sk-3*^{K} strains free of the defect, as was done with *Sk-2* to obtain better results of the type shown in Figure 2E.

Crosses between Sk-2 *and* Sk-3

Sk-2^K and *Sk-3*^{K} strains have been intercrossed in various combinations, in both *N. intermedia* and *N. crassa*. In every cross, the majority of asci have contained only aborted ascospores, but in each perithecium several asci have been found that contain 2 large:6 aborted ascospores. (See, for example, Figure 2C.) In many crosses, such as those with standard *Sk*^{K} stocks as parents, the large ascospores are black and viable. Because the great majority of ascospores in these crosses look just like the aborted ascospores in *Sk*^{K} × *Sk*^{S} crosses, it is inferred that *Sk-2*^{K} and *Sk-3*^{K} are mutually sensitive. The characteristics of the survivors are now under study; they appear to be *Sk-2*^{K} *Sk-3*^{S}, but they have a colonial morphology, and preliminary genetic analysis suggests that in the *Sk* region they are not identical to the *Sk-2*^{K} parent.

Incidence of Spore killer genes in natural populations of N. intermedia

Over 500 cultures of *N. intermedia* from many geographical regions have been crossed to various tester strains of *Sk-2*^{K}, *Sk-3*^{K} and *Sk-2*^{S} *Sk-3*^{S}. In addition to the Rouna and Borneo strains described previously, only one strain from West Java was found to be a Spore killer; this appears to be *Sk-2*^{K}. A few other strains still under study are possible Spore killers. The rarity of Spore-killer strains in *N. intermedia* contrasts with their abundance in *N. sitophila*, where more than half of the available strains are Spore killers, all having the same specificity.

Another, and probably related, major difference between the species is the presence in *N. intermedia* of resistant or "neutral" strains. Resistance to *Sk-2*^{K} (*Sk-2*^{R}) appears to be distributed in populations independently of resistance to *Sk-3*^{K} (*Sk-3*^{R}). A site-by-site analysis is being made.

Resistant alleles are widespread in *N. intermedia* in much of the world. Of the cultures from the eastern hemisphere, 43% are *Sk-2*^{R} and 18% are *Sk-3*^{R}. However, neither *Sk-2*^{R} nor *Sk-3*^{R} has been found in collections from Hawaii (101 cultures) or the southern United States (27 cultures). The widespread occurrence of resistant alleles suggests that the Spore killer phenomenon may be more significant for *N. intermedia* than would be inferred from looking only at the low incidence of the killer alleles.

Genetic analysis of resistance to Sk-2^K *and* Sk-3^K

Resistant strains make 8 black:0 white asci and segregate 1:1 for resistance when crossed to *Sk*^{S}. When crossed with *Sk*^{K}, they produce from 0 to 50% 4 black:4 white asci (the remainder mostly have eight full size ascospores), and randomly collected progeny are 25% to 50% *Sk*^{R}. A few apparent *Sk*^{S} recombinants from *Sk*^{K} × *Sk*^{R} are under study.

A preliminary screening for nonallelic resistance loci in *N. intermedia* was made by crossing strains with *Sk-2*^{R} *Sk-3*^{R} and *Sk-2*^{R} *Sk-3*^{S} phenotype from widely separated locations to *Sk-2*^{K}, to *Sk-3*^{K} and to an arbitrarily selected *Sk-2*^{R} *Sk-3*^{R} tester. In general almost all progeny were of the two parental types only, but it appears that there are unlinked or loosely linked loci segregating in some crosses which modify the degree of resistance of *Sk*^{R} strains.

An *Sk-2*^{R} allele was found in a wild-collected *N. crassa* strain, and it was used to study the effect of *Sk-2*^{R} on crossing over. Table 1D and E show the results of two crosses heterozygous for *Sk-2*^{R} and for linkage group III markers. The *K/R* heterogyzote resembles *K/S* in showing no recombination of *Sk-2* with *acr-2* or *leu-1*. In contrast, the cross heterozygous for *Sk-2*^{R} and *Sk-2*^{S} shows crossing over not only of *acr-2* with *leu-1*, but also of *Sk-2*^{R} with both *acr-2* and *leu-1*. Thus, *Sk-2*^{R} does not itself suppress crossing over and does not alleviate the suppression of crossing over by *Sk-2*^{K}. The simplest hypothesis is that this *Sk-2*^{R} allele and *Sk-2*^{S} have the same chromosome sequence in the centromere region of linkage group III, while *Sk-2*^{K} has a different sequence. No other marker is known that maps left of *acr-2* in linkage group III, where *Sk-2*^{R} seems to be located.

Heterokaryons between Sk-2^K *and* Sk-2^S

The Spore killer genes resemble heterokaryon-incompatibility (*het*) genes in that killing is locus-specific and occurs only when nuclei of two different genotypes are present. *Sk* and *het* differ in that the *het* genes are not expressed in the ascus or during the sexual phase of the life cycle, but only vegetatively in heterokaryons or heterozygous partial diploids. Experiments were therefore carried out using [*Sk-2*^{K} + *Sk-2*^{S}] heterokaryons, to determine whether *Sk-2*^{K} has any effect on *Sk-2*^{S} nuclei during the vegetative phase. These tests employed

heterokaryon-compatible forced heterokaryons of *N. intermedia*. Inocula in the heterokaryon-compatibility tests were 3 mm squares cut from one-day cultures and placed together on restrictive media at 30°.

All *pdx*; *Sk-2*^{K} *a* progeny from a cross of *Sk-2*^{K} *A* × *pdx a* proved to be heterokaryon-compatible with *asn a*. Differences in *Sk-2* constitution did not affect the ability of *asn* and *pdx* strains to grow as a heterokaryon. Four different *Sk-2*^{K} *pdx a* progeny from the same cross were grown as heterokaryons with *Sk-2*^{S} *asn a*, and all grew as vigorously as the control with *Sk-2*^{S} *asn a* + *Sk-2*^{S} *pdx a*. Neither component of the heterokaryon grew when tested alone under the same conditions. Another control employed components of opposite mating type [*Sk-2*^{K} *pdx A* + *Sk-2*^{S} *asn a*]. This failed to grow on minimal medium, showing that complementation was due to heterokaryosis rather than cross feeding (and, incidentally, showing for the first time that the mating-type alleles of *N. intermedia* resemble those of *N. crassa* in showing vegetative incompatibility and acting as *het* genes during the vegetative phase of the life cycle).

The finding that there is no killing reaction when *Sk-2*^{K} and *Sk-2*^{S} nuclei are together in a heterokaryon is consistent with the observation of RAJU (1979) that *Sk-2*^{S} nuclei survive when they are included in the same ascospore with *Sk-2*^{K}.

DISCUSSION

Comparison of Sk *in Neurospora to similar systems in higher organisms*

Spore killer exhibits meiotic drive as broadly defined by ZIMMERING, SANDLER and NICOLETTI (1970).

Our results with the Spore-killer genes indicate a number of striking similarities with Segregation Distorter (*SD*) in Drosophila (reviewed by HARTL and HIRAIZUMI 1976), Pollen killer (*Ki*) in wheat (LOEGERING and SEARS 1963), and Gamete eliminator (*Ge*) in tomato (RICK 1966, 1970). In all cases the only manifestation of the killer allele is in the production of nonfunctional meiotic products from heterozygous meioses, and there is no other effect in the rest of the life cycle. In all three organisms, killer strains have been found recurrently in nature, as have resistant or neutral strains. For Pollen killer (*Ki*), 12 strains of *Triticum aestivum* were tested. All of these are highly domesticated and therefore not comparable to the studied populations of Neurospora and Drosophila. Nevertheless, the three types were found—killer, sensitive, and neutral—even in the small and selective sample. All three types were found also among both wild and cultivated tomatoes.

Close linkage to a centromere was found for *Sk*, *SD* and *Ge*, but not for *Ki*.

While it is tempting to think of *Sk* in terms of the *SD* system in Drosophila and to frame hypotheses in accordance with the abundance of data amassed about that system, it would be unwise to ignore differences that are known to exist. Outstanding among these are the observations on homozygous fertility and on the effect of heterozygous partial diploids in the meiotic products. In Neurospora, *Sk*^{K} × *Sk*^{K} crosses are fully or partially fertile (as are *Ki/Ki* plants in wheat), but for most of the *SD* alleles in Drosophila, homozygous males are virtually

sterile. When Sk^S and Sk^K are present in a common cytoplasm of multinucleate giant ascospores (RAJU 1979), the Sk^S nucleus survives, as if it were in some way protected by the presence of the Sk^K nucleus in the same spore. In contrast, in one study of Drosophila males heterozygous for a translocation and for *SD*, meiotic products containing both *SD* and SD^+ apparently did not survive (SANDLER and CARPENTER 1972).

Other differences between Neurospora *Sk* and Drosophila *SD* may be superficial, but are not necessarily so. For example, there is so far no cytological evidence that the *Sk*'s are associated with chromosome rearrangements, as are most occurrences of *SD*. However, crossing over is suppressed locally in crosses heterozygous for $Sk\text{-}2^K$ or $Sk\text{-}3^K$, where Spore killer has been introgressed from *N. intermedia* into *N. crassa*. This suggests structural heterozygosity in the vicinity of *Sk*. The suppression is not due to structural differences between the species, because crossing over occurs between *acr-2* and *leu-1* in interspecific crosses where Spore killer is not present. The well known *SD-72* chromosome carries a small pericentric inversion in the *SD* region (see HARTL and HIRAIZUMI 1976).

The *SD* system consists of two linked but separable elements, *Sd* (Distorter) and *Rsp* (Responder) (GANETSKY 1977; SANDLER and CARPENTER 1972). and a third, enhancer, component has been identified in the same region. It remains to be seen whether the Spore killer region of Neurospora can be similarly resolved into component units.

Ge in tomato eliminates male and female meiotic products with equal intensity, whereas *Ki* in wheat and *SD* in Drosophila act only in male gametogenesis. Since there is no sexual differentiation during ascospore genesis in Neurospora, sex limitation provides no basis for deciding whether *Sk* resembles one of these systems more than another. The mating-type alleles, on another chromosome, segregate independently of *Sk*.

POLICANSKY and ELLISON (1970) showed that the "sex ratio" system in *D. pseudoobscura* is functionally the same as *SD*. Studies of "sex ratio" in the various Drosophila species may be useful in suggesting lines of inquiry with regard to *Sk*; for example, VOELKER (1972) rediscovered and elucidated the action of *msr*, a condition in *D. affinis* that reverses the effect of sex ratio (*sr*) so that male rather than female progeny predominate.

The *t* alleles of mice have not been considered here, mainly because the characteristics of that system (*cf.*, BENNETT 1975) do not seem directly applicable to *Sk*. It may be noted, however, that *t* alleles that show meiotic drive also frequently suppress crossing over in their vicinity. BRADEN *et al.* (1974) have drawn up a detailed comparison for the segregation-distorting alleles in Drosophila and mouse. In time it will be useful to do this also for Neurospora and the other organisms discussed above.

Spore-killer genes in Podospora

PADIEU and BERNET (1967) have described experiments originating with two wild strains of Podospora that differ from one another at two loci responsible for ascospore abortion. Crosses homozygous at these loci are normal, but heterozygous

intercrosses show a complex pattern of ascospore abortion. *Podospora anserina* is a pseudohomothallic ascomycete that has asci with four large ascospores, each of which normally encloses two genetically unlike nuclei derived from different haploid products of meiosis by a precise sequence of cytological events (see ESSER 1974).

The two Podospora loci concerned with ascospore abortion segregate independently, showing first-division segregation frequencies of 80% and 45%, respectively. In an elegant analysis, PADIEU and BERNET (1967) showed that a binucleate ascospore aborts whenever its two nuclei are both a_1 or both b_2 or both a_1b_2, but that an ascospore does not abort if it contains at least one a_2 and one b_1. Exceptional small ascospores are sometimes formed that contain only one nucleus. Among these, the only viable survivors are of genotype a_2b_1.

Each of the Podospora loci thus resembles *Sk* in Neurospora. If one substitutes the symbols *Sk-a*^{K} for a_2, *Sk-a*^{S} for a_1, *Sk-b*^{K} for b_1, and *Sk-b*^{S} for b_2, and postulates that the mode of action of each of the Podospora genes is identical to that of the *Sk* genes of Neurospora, all the results observed in the remarkable study by PADIEU and BERNET (1967) can be predicted.

A comparison of Sk *with other conditions that result in defective ascospores in Neurospora*

Spore-killer genes were discovered because of their visible effects on ascospore maturation. Three other quite different genetic conditions are known which also produce defective ascospores: (1) mutant genes that autonomously affect ascospore maturation and pigmentation (*e.g.*, *asco*, tan spore, white spore. STADLER 1956; NAKAMURA 1966; PHILLIPS and SRB 1967); (2) chromosome aberrations whose meiotic products include deficiencies (see PERKINS 1974), and (3) meiotic mutants that produce aneuploid products as a result of nondisjunction (e.g., SMITH 1975). These are all distinct in their manifestations.

With Spore killer (in the absence of extraneous complications), all asci from heterozygous crosses contain 4 black, viable and 4 small, clear, inviable ascospores, and the survivors are killers. Crosses homozygous for either *Sk*^{K} or *Sk*^{S} are capable of producing asci in which all ascospores are black and viable. In contrast, with autonomous ascospore-color mutants the four survivors in asci from heterozygous crosses are the normal wild type, while homozygous mutant × mutant crosses result in asci where the ascospores are all defective. With most chromosome rearrangements, defective ascospores are produced only in structurally heterozygous crosses; such crosses produce not only asci with 4 black:4 white ascospores, but also asci with 8:0 and with either 6:2 or 0:8, and the surviving progeny include both parental genotypes. With meiotic mutants that result in frequent nondisjunction, many white deficiency ascospores are produced, but these do not fall into simple patterns in the asci or simulate the 4:4 asci from *Sk*.

Mechanism of Sk *action*

Our knowledge is not sufficient at present to warrant the elaboration of explicit

models. One general hypothesis is that Sk^K kills by altering Sk^S nuclei temporarily, rendering them unable to perform functions needed for survival during ascospore maturation. Not only must the Sk^K factor render Sk^S ascospores inviable, it must also confer on itself immunity to the lethal action. In ascospores containing Sk^K in addition to Sk^S, the active Sk^K nucleus would then be able to perform all essential functions and to rescue the spore.

Another general hypothesis is that the Sk^K allele mediates two effects, one before ascospore delimitation and one after. First, a relatively stable early product made under direction of Sk^K is distributed in the developing ascus so as to be included in all ascospores. Then, later, Sk^K interacts with the stable product so that any ascospores containing Sk^K survive and mature normally, whereas ascospores containing only Sk^S do not.

The mechanism of inactivation could well be regulatory. Many functions must be turned on or shut down at the stages critical to *Sk* expression, and ascospore survival depends upon the correct regulation of each function. When wild-collected Neurospora strains are intercrossed (PERKINS, TURNER and BARRY 1976), many pairs of strains show very high percentages of ascospore abortion, as might be expected from regulatory dysfunction or incompatibility.

The well known phenomenon of heterokaryon (vegetative) incompatibility has several similarities to Spore killer. Heterokaryon incompatibility is locus-specific and is expressed only when unlike alleles are present. Expression is also limited to a specific phase of the life cycle. At least ten heterokaryon incompatibility genes (*het* loci) have been identified in Neurospora and many more are inferred to exist (MYLYK 1975). Each of the three *het* loci tested produces a substance that is lethal when injected into strains carrying another allele of that locus, but each strain is immune to its own *het* substances (WILSON, GARNJOBST and TATUM 1961). Possibly Spore killer might be a *het* gene with abnormal regulation, so that unlike all other *het* genes it is turned on during the sexual phase, but does not kill during vegetative growth.

There are some difficulties with this hypothesis. Killing is undirectional for Spore killer, whereas vegetative killing by *het* genes in Neurospora is bidirectional (*het-c* is possibly a partial exception—WILSON, GARNJOBST and TATUM 1961). However, vegetative killing is sometimes expressed unilaterally in other organisms, *e.g.*, in myxomycetes (CLARK and COLLINS 1973; CARLILE 1976). A second difficulty is that the simplest incompatibility model does not predict that a sensitive nucleus can survive and be rescued when included in the same ascospore with Sk^K, as has been observed by RAJU (1979). This makes an incompatibility hypothesis less attractive, but does not necessarily eliminate it in modified form.

Some other considerations regarding the action of *Sk* are discussed by RAJU (1979).

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