SPORE KILLER POLYMORPHISM IN *FUSARIUM MONILIFORME*

SOPHIA KATHARIOU[1] AND PHILIP T. SPIETH[2]

Department of Genetics, University of California, Berkeley, California 94720

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ABSTRACT

A Spore killer trait, which exhibits genetic and cytological properties analogous to those previously found in Neurospora, exists in natural populations of the fungal plant pathogen *Fusarium moniliforme*. The genogeography of the polymorphism in *F. moniliforme* differs from the situation in *Neurospora intermedia*. It is more akin to the situation in *N. sitophila*, although more extreme with respect to the prevalence of killer alleles: more than 80% of tested isolates of *F. moniliforme* carry the killer allele. Nevertheless, sensitive alleles are widely distributed and have been found in California, Italy, Greece and Central America.

AN anomalous class of genetic variants has been found in a wide variety of organisms. The common characteristic of these variants is their ability, when in heterozygous condition, to be preferentially included among functional products of meiosis. Notable examples include Segregation Distorter in Drosophila, Pollen-killer in wheat, and Gamete-eliminator in tomato [for review see ZIMMERING, SANDLER and NICOLETTI (1970)]. Among the fungi, examples of this class, designated "Spore killer", have been documented by TURNER and PERKINS (1979) in natural populations of *Neurospora sitophila* and *Neurospora intermedia*; PADIEU and BERNET (1967) found a similar case in *Podospora anserina*.

Spore killer in Neurospora is governed by a nuclear gene (or, perhaps, a tightly linked gene complex) and is distinct from "killer" phenomena that are caused by cytoplasmic agents in Saccharomyces and Ustilago. Mature asci from a cross between a Neurospora strain carrying the killer allele (Sk^K) and one with the sensitive allele (Sk^S) typically contain only four viable ascospores, each of which is Sk^K; by contrast, crosses between two Sk^K strains or between two Sk^S strains typically produce asci with eight viable ascospores. Cytological observations of RAJU (1979) indicate that meiosis occurs normally in a cross of $Sk^K \times Sk^S$, but the four Sk^S spores degenerate during the eight spore stage when spore walls are formed around the ascospores.

We report here the widespread occurrence of a Neurospora type of Spore killer phenomenon among natural populations of *Fusarium moniliforme* (Sheld.) emend. Snyd. & Hans, which is an ascomycetous plant pathogen with a worldwide distribution. All isolates discussed here belong to Mating Group A as defined by HSIEH, SMITH and SNYDER (1977). Mating Group A is cosmopolitan, occurring on maize and sorghum around the world. Isolates of Mating Group A are also found on sugarcane, rye, cotton, asparagus, and occasionally on rice.

[1] Current address: Department of Microbiology, Cornell University, Ithaca, New York 14850.
[2] To whom reprint requests should be addressed.

MATERIALS AND METHODS

Our data on Spore killer were obtained during a broad survey that looked for relationships between electrophoretic variation and crossing compatibility with tester strains of mating group A. The data are based on 225 isolates of *F. moniliforme* that were individually collected from natural populations in southern Europe and North and Central America. The number of fields and the host crops are indicated in Table 1. The southern Italian isolates were obtained from fields located along an 80-km coastal stretch on the Gulf of Taranto. Northern Italian sites were located 10–40 km S.E. of Milan. California collections were made in the vicinities of Stockton and Berkeley.

Crosses were made on V-8 juice agar which consisted of 20% (v/v) V-8 juice, 2% (w/v) agar and 0.2% (w/v) $CaCO_3$. Protoperithecial (female) parents were grown on plates of V-8 agar for 10 days before being fertilized with spore suspensions of the male parent. Optimum results were obtained with incubation at 21–25°C and 12 hr light and darkness per day. Mature perithecia were obtained from compatible crosses in 2–3 weeks after fertilization.

Achieving sexual reproduction between two strains of *F. moniliforme* is subject to the requirements that (1) both strains belong to the same "mating group", (2) the strains be of opposite mating type, and (3) at least one of the strains be competent to act as a female (protoperithecial) parent and the other as a male parent [see HSIEH, SMITH and SNYDER (1977) for background information, especially with respect to identification of mating groups]. Our Spore killer data are restricted to isolates that, by virtue of crossing with known testers, can be assigned to mating group A. Mating type in *F. moniliforme* behaves in a fashion typical of heterothallic ascomycetes such as *Neurospora crassa* and is governed by what appears to be single locus with two alleles, + and −.

Isolates of *F. moniliforme* show great variability in sexual competency in laboratory crosses. Many isolates fail to cross when used as female parents. The observable result of such incompetency is failure to produce any signs of a sexual reaction and perithecial development and is distinct from problems that lead to abnormal perithecial, ascus, or spore development. We do not know whether the problem of sexual incompetency affects natural populations or whether it is merely an artifact of the laboratory environment.

The practical consequence of variability for sexual competency is that surveys of crossing compatabilities within natural populations must be conducted using tester strains that have been identified and (carefully) maintained in the laboratory for their reliability as crossers. The primary testers used for our survey are F80(+), Sk^K, isolated from sorghum in the San Joaquin delta of California, and F237(−), Sk^S, isolated from maize near Visalia, California. Both of these strains, which were obtained before the collections listed in Table 1, are notable for their reliability in laboratory crosses. Unpublished evidence from electrophoretic studies suggests that almost any isolate of Mating Group A can serve as the conidial (or male) parent with the one of these two strains that is of opposite mating type. Other tester strains that were used, generally to double check strains crossed with F237, are F10(−), Sk^K, an early tester with poor reliability as a female parent, and F223(−), Sk^K. Both are from maize fields near Visalia, California.

No reliable tester with Sk^S and the + mating type was found, although one could presumably be produced by sufficient backcrossing of an Sk^S(+) isolate with F237. Assignment of these testers to Mating Group A and designation of their mating types derive from crosses among the tester strains and a cross between F237(−) with strain M-4(+) of HSIEH, SMITH and SNYDER (1977). Systematic analysis of the results of crossing Sk^K and Sk^S with themselves and with each other is based primarily upon crosses involving the tester strains and F1127(+), Sk^S, which was isolated from maize in Greece and which functions only as a male parent in laboratory crosses.

RESULTS

Natural isolates of *F. moniliforme* are typically Sk^K. The sensitive allele, Sk^S, was first noticed in the tester strain F237. Sk^K and Sk^S alleles behave genetically as described for Neurospora. That is, $Sk^S \times Sk^K$ crosses in either direction give 95% asci with four viable Sk^K spores and four inviable Sk^S spores, whereas homozygous crosses with Sk^K result in over 95% of the asci having eight viable ascospores. Preliminary cytological observations by N. B. RAJU (personal communication) indicate that meiosis and the postmeiotic mitosis are normal regardless of the cross, again analogous to Neurospora.

The phenotypes of 225 wild strains with respect to mating-type and *Sk*, as deduced from crosses to F237 (Sk^S,−) and F80 (Sk^K,+), are given in Table 1. Since each of these strains gave perithecia with only one of the two testers, assignment of mating type is unambiguous. Strains that gave ≥95% eight-spored asci with F80 and strains that gave ≥95% four-spored asci with F237 are classified as Sk^K; strains that gave ≥95% four-spored asci with F80 are classified as Sk^S as are those that gave eight-spored asci with F237.

TABLE 1

Occurrence of spore killer alleles among F. moniliforme *isolates of mating group A*

Location: field and host	Sk^S		Sk^{Mx}		Sk^K	
	(+)	(−)	(+)	(−)	(+)	(−)
Southern Italy[a]	(4)	(10)	(2)	(1)	(19)	(53)
1. Maize	0	0	0	0	1	9
2. Rice	0	4	0	0	1	3
3. Maize	1	2	0	0	5	15
4. Maize	0	1	0	0	0	0
5. Maize	3	3	2	1	7	17
6. Maize	0	0	0	0	5	9
Northern Italy	(3)	(10)	(0)	(0)	(26)	(63)
1. Maize	0	3	0	0	3	8
2. Maize	1	0	0	0	2	4
3. Asparagus	0	0	0	0	0	6
4. Sorghum	0	1	0	0	0	0
5. Maize	0	2	0	0	8	16
6. Rice	0	3	0	0	5	3
7. Maize	0	0	0	0	3	7
8. Rice	0	0	0	0	2	12
9. Maize	2	1	0	0	3	7
Greece						
1. Maize	1	2	0	4	1	1
California[b]	(0)	(2)	(0)	(0)	(5)	(7)
1. Maize	0	2	0	0	1	2
2. Maize	0	0	0	0	0	3
3. Maize	0	0	0	0	1	1
4. Maize	0	0	0	0	3	1
Central America	(0)	(1)	(0)	(0)	(4)	(6)
Costa Rica						
1. Maize	0	0	0	0	0	1
2. Maize	0	0	0	0	0	1
Guatamala						
1. Maize	0	0	0	0	2	2
2. Maize	0	1	0	0	2	2
Total numbers	8	25	2	5	55	130
Percent	4%	11%	1%	3%	24%	57%

[a] Regional totals are given in parentheses.

[b] California fields 1-3 are from the San Joaquin Delta; field 4 from Berkeley.

Homozygous crosses between two Sk^S isolates predominantly produce asci with eight viable spores; however, the results are not always as clear-cut as with the other crosses. Table 2 shows the results of test crosses with the eight $Sk^S(+)$ isolates listed in Table 1. Four give clean results when crossed with F237, but the other four produce a substantial number of asci with only four spores when crossed with F237. However, in all four cases where $Sk^S(+)$ isolates have been crossed with Sk^K strains, the Sk^S show the typical response for sensitive strains.

F1051 is exceptional. In crosses with F10 (Sk^K), F1051 is seen to be sensitive. In crosses between F1051 and the sensitive tester F237, the proportion of asci with eight spores differs greatly for reciprocal crosses. This is the only known case in which reciprocal crosses give different results; however, because few natural isolates perform as female parents in the laboratory, comparisons of reciprocal crosses were possible in only a few cases.

Seven isolates from two fields—one in southern Italy and one in Greece—are classified as intermediate forms designated Sk^{Mx}. The crossing results of these isolates are given in Table 3. The five mating type (−) isolates, when crossed with the killer tester F80, showed partial resistance to the killer allele. The percentage of asci with eight spores in those crosses varied from 39% to 76%. Assignment of the two mating type (+) isolates—F743 and F744—to the category Sk^{Mx} is somewhat arbitrary because it is based solely upon crosses with the sensitive tester F237, and the results for these two isolates differ only in degree from that of, say, F753 in Table 2. Beyond taking note of its occurrences within the survey, Sk^{Mx} has not been investigated further.

Table 1 summarizes data on the frequencies of Spore killer alleles within our

TABLE 2

Tests of $Sk^S(+)$ *isolates*

Isolate	Location	Tester	Asci produced		
			8-Spores	4-Spores	% 8-spores
F1127	Greece	F237 (Sk^S)	650	17	97
		F223 (Sk^K)	18	440	4
F874	N. Italy	F237 (Sk^S)	480	19	96
		F223 (Sk^K)	7	250	3
F1110	N. Italy	F237 (Sk^S)	470	26	95
F791	S. Italy	F237 (Sk^S)	415	22	95
F741	S. Italy	F237 (Sk^S)	563	68	89
F753	S. Italy	F237 (Sk^S)	60	21	74
F712	S. Italy	F237 (Sk^S)	294	112	72
		F10 (Sk^K)	0	70	0
F1051	N. Italy	F237 (Sk^S)—female	231	536	30
		F237 (Sk^S)—male	380	93	80
		F10 (Sk^K)—male	2	122	2

TABLE 3

Tests of Sk^{Mx} *isolates*

Isolate	Location	Tester	Asci produced		
			8-Spores	4-Spores	% 8-spores
F743(+)	S. Italy	F237 (Sk^S)	359	339	51
F744(+)	S. Italy	F237 (Sk^S)	184	416	31
F747(−)	S. Italy	F80 (Sk^K)	352	114	76
F1124(−)	Greece	F80 (Sk^K)	446	708	39
F1128(−)	Greece	F80 (Sk^K)	255	277	48
F1129(−)	Greece	F80 (Sk^K)	193	198	49
F1131(−)	Greece	F80 (Sk^K)	127	81	61

collection of 225 group A isolates of *F. moniliforme*. The Sk^K allele is clearly the most abundant. Over 80% of our isolates exhibit Sk^K. No pattern in the geographical distribution is apparent other than a slightly higher frequency of Sk^S in Europe than in America. The isolates from the single maize field in Greece and from field 5 in Southern Italy show high frequencies of Sk^S and Sk^{Mx} that may be the result of clonal propagation within the particular fields. The overall frequencies of Spore killer alleles, especially Sk^{Mx}, in Table 1, therefore, may be somewhat exaggerated by these two fields. Nevertheless, the Sk^S allele is widely distributed and is found in geographically distinct populations.

DISCUSSION

The results just described were obtained during a broad survey of genic variation and mating structure in *F. moniliforme*. The primary observation to be noted here is the existence of a Spore killer polymorphism within natural populations of *F. moniliforme* that parallels the polymorphisms previously found in Neurospora. TURNER and PERKINS (1979) give a detailed analysis of the Spore killer phenomenon in Neurospora with a comparison of Spore killer to similar phenomena in higher organisms. Our information on *F. moniliforme*, although not as extensive as the information on Neurospora, is consistent with the conjecture that the Spore killer in Fusarium has genetical and cytological properties identical to those found in Neurospora.

In *Neurospora intermedia*, TURNER and PERKINS (1979) found resistant, nonkiller strains that produce asci with eight spores whether crossed with killer strains or with sensitive ones. We have no evidence of such fully resistant strains in *F. moniliforme*; however, since the 130 Sk^K(−) strains listed in Table 1 were crossed only with F80 (with which they produced asci with eight spores), we cannot exclude the possibility that some of them might be resistant, nonkiller strains. On the other hand, in the four crosses in which Sk^S(+) isolates (identified by producing asci with eight spores in crosses with F237 (Sk^S)) were crossed with F223 (Sk^K), the isolates proved to be strictly sensitive and not resistant, nonkillers (Table 2). The five anomalous Sk^{Mx}(−) isolates in Table 3 are the only examples we have found of any kind of partial resistance and sensitivity.

The Fusarium polymorphism differs from the Neurospora situation with respect to the frequency of the Sk^K allele and its geographical distribution.

Table 1 demonstrates the common occurrence of both Sk^K and Sk^S alleles among widespread populations of *F. moniliforme*. The predominance of Sk^K contrasts with the situation found by TURNER and PERKINS (1979) in Neurospora. Among *N. sitophila* isolates, Sk^K and Sk^S alleles occur with approximately equal frequencies. In *N. intermedia*, Sk^K alleles are rare, although there are two different Sk^K phenotypes, genetically distinguishable from each other and from the one in *N. sitophila*. In *N. intermedia* the situation is further complicated by the widespread occurrence of resistant, nonkiller strains in those geographical areas where the killer strains are found. Within our collection of *F. moniliforme* there is no evidence of fully resistant types. The rare Sk^{Mx} phenotype is the only indication of any sort of resistance to the killer trait in *F. moniliforme*.

These differences in genogeography for *F. moniliforme*, *N. sitophila*, and *N. intermedia* may reflect differences in as yet undetected selective disadvantages associated with the various Sk^K alleles that check their intrinsic spread within their respective gene pools. Alternatively, we may be witnessing in Fusarium and Neurospora three different intermediate stages in a transient process governed by the origin, spread and fixation of Sk^K alleles. In either event, we have within two relatively unrelated groups of ascomycetous fungi strikingly similar instances of a curious genetic polymorphism.

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