

Geographic Distribution of *Neurospora* Spore Killer Strains and Strains Resistant to Killing

Barbara C. Turner

Department of Biological Sciences, Stanford University, Stanford, California 94305-5020

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Turner, B. C. 2001. Geographic distribution of *Neurospora* spore killer strains and strains resistant to killing. *Fungal Genetics and Biology* 32, 93–104. Spore killer strains, found in *Neurospora*, provided the first recognized example of meiotic drive in fungi. In the present study, natural populations throughout the world were examined for the presence of killer strains and strains that are resistant to killing. In *N. intermedia*, *Sk-2* and *Sk-3* are present but are rare. Killer strains were found at only five sites, in Borneo, Java, and Papua New Guinea. Nonkiller strains that are resistant to killing by *Sk-2* or *Sk-3* are frequent in that part of the world where the killer strains are present, but resistant stains were not found in regions where killers are absent. In *N. sitophila*, *Sk-1* killer strains are common in nature, but only 1 of 392 nonkiller strains was resistant. In *N. crassa*, no killer strain was found among >500, but widely scattered *Sk-2*-resistant strains were present, suggesting the past or present existence of killers. © 2001 Academic Press

Index Descriptors: meiotic drive; Spore killer; *Neurospora*; fungal populations; geographic distribution.

Meiotic drive elements are genes or gene complexes that result in transmission-ratio distortion. Meiotic products carrying them survive preferentially because presence of the element results in the death of all or some of the meiotic products that lack it (see Lyttle, 1991, for review). Spore killer strains, described by Turner and Perkins (1979), provided the first example of meiotic drive in the fungal kingdom. One killer element was found in cultures of *Neurospora sitophila* from Africa and another was de-

tected in a laboratory strain of *Neurospora crassa*, into which it had been introgressed from a wild *Neurospora intermedia* strain from Borneo. Extensive information on the genetics, chromosomal basis, and cellular expression of Spore killers was obtained in studies by Raju (1979), Campbell and Turner (1987), Turner *et al.* (1987), and Turner and Perkins (1991). Additional Spore killer strains were discovered among isolates obtained from natural populations (see Perkins *et al.*, 1976; Perkins and Turner, 1988; Turner *et al.*, 2001; for comprehensive reviews of the collection program, which was initiated in 1968).

The element responsible for killing in *N. sitophila* was named Spore killer-1 (*Sk-1*). Although Spore killer strains were common in *N. sitophila*, additional killers were found only in *N. intermedia*. These were of two distinct types, designated Spore killer-2 (*Sk-2*) and Spore killer-3 (*Sk-3*). For mapping and other genetic studies, *Sk-2* and *Sk-3* were introgressed from *N. intermedia* into the genetically better known *N. crassa* (Turner and Perkins, 1979). Killing (and self-resistance) in Spore killer-2 and Spore killer-3 segregated as a Mendelian factor in linkage group III.

Although *Sk-2* and *Sk-3* map in the same chromosomal region, the two differ in specificity of killing when crossed to strains from nature (Turner, 1977), and the two are mutually destructive. About 99.9% of ascospores abort in *Sk-2* × *Sk-3* crosses, and most of the rare survivors are aneuploid products of crossing-over (Turner *et al.*, 1988).

Killing is observed only in crosses that are heterozygous for a Spore killer element. In crosses homozygous for the same killer factor, e.g., *Sk-2* × *Sk-2*, each ascus contains eight black viable ascospores, just as in crosses not involving a Spore killer. In crosses between killer and sensitive, each ascus contains four ascospores that are normal size

(usually black and viable) and four that are small, transparent, and inviable. The viable spores are all killers. This situation is easily distinguished from other causes of ascospore abortion (Raju, 1994).

Nonkiller strains that were resistant to killing were discovered in *N. intermedia*, and resistance was shown to be due to genes that were linked to each other and to the killer elements. A *N. crassa* strain resistant to killing by *Sk-2*, but not itself a killer, carried a single gene for resistance, called *r(Sk-2)*, in the left arm of linkage group III (Turner and Perkins, 1979; Campbell and Turner, 1987). No strain resistant to *Sk-3* was found in *N. crassa*, but a gene, *r(Sk-3)*, that confers resistance to *Sk-3*, was introgressed from *N. intermedia* into *N. crassa*. It maps at approximately the same place as *r(Sk-2)* in crosses with the same markers, and there were no recombinants among 300 progeny from *r(Sk-2) × r(Sk-3)* (Campbell and Turner, 1987, and previously unpublished data). Additional resistance genes were discovered when a *N. crassa* strain from Penang, Malaysia was shown to be resistant to killing by *Sk-2* even though it had a sensitive allele at the *r(Sk-2)* locus. Resistance in this strain is conferred by resistance alleles at two linked loci that are located on the side of the linkage group III centromere opposite to that of *r(Sk-2)* and *r(Sk-3)*. One of the genes, *pr(Sk-2)*, confers a low level of resistance to killing in the absence of the second gene, *mod(pr)*, which has no known phenotype except to provide virtually complete resistance to killing when it is combined with *pr(Sk-2)* (B. C. Turner, unpublished).

At the time the first Spore killer was discovered in *Neurospora*, meiotic drive had been known already for many years in other organisms and had been studied most extensively in *Drosophila* and in the mouse. Much has now been learned about the molecular and chromosomal basis, cellular mode of action, and population-evolutionary aspects of the drive systems in those organisms (see Lyttle, 1991; Temin *et al.*, 1991; Ganetzky, 2000; Silver, 1993, 1996; Silver and Remis, 1987; Schimenti, 2000). The *Neurospora* Spore killer system provided an opportunity for studying meiotic drive in a very different organism. Such features as haploidy made *Neurospora* highly favorable for both genetic and molecular analyses of the elements responsible for drive. Presence or absence of a Spore killer element can be scored simply by visually examining asci from a cross to a known tester without it being necessary to isolate and test the progeny. As our worldwide collection of *Neurospora* isolates became available (Turner *et al.*, 2001), information could be obtained on the incidence and geographical distribution of strains that killed and

strains that were insensitive to killing. Questions such as whether killer strains have displaced strains sensitive to killing in one geographic area but not in another could be addressed.

In *Drosophila* and in the mouse, the drive elements are associated with a long chromosome segment that contains inversions. Crossing-over is therefore suppressed in the inverted regions, in heterozygous crosses. In these organisms, the segment is a complex containing not only the elements primarily responsible for meiotic drive but also various factors that modify their effectiveness.

The situation is similar in *Neurospora*. *Sk-2* and *Sk-3* are associated with a 30-map-unit segment of linkage group III within which crossing-over is suppressed in heterozygous crosses and within which are located various genes that confer resistance to killing. Crossing-over in this region is not blocked when nonkiller strains of *N. intermedia* are crossed to nonkiller strains of *N. crassa*. Absence of crossing-over when *Sk-2* or *Sk-3* is heterozygous in *N. crassa* is, therefore, not due to a species difference in chromosome structure. Recombination involving only resistant strains is essentially the same as recombination involving only sensitive strains. Crossing-over is not suppressed in crosses of resistant strains to standard normal-sequence laboratory stocks, which are sensitive to killing. Crossing-over is suppressed, however, in crosses between resistant strains and killer strains.

The objective of the present study was to throw light on the biological significance of meiotic drive in *Neurospora* by determination of the geographical distribution of strains that are Spore killers, strains that are sensitive to killing, and resistant nonkiller strains that are insensitive to killing. The strains used were obtained during the global study of *Neurospora* populations described by Turner *et al.* (2001).

MATERIALS AND METHODS

The following conventions are used: Genotypes, gene loci, and gene complexes (haplotypes) are symbolized using italics, e.g., *Sk-1*, *Sk-2*, *r(Sk-1)*, *r(Sk-2)*. A killer allele is symbolized without a superscript. For example, *Sk-2* is the genetic element (allele) responsible for the killer phenotype of Spore killer-2 strains. Sensitivity to killing is implied in the absence of a symbol. (In some contexts, killer and sensitive alleles may be designated explicitly, using superscripts, as *Sk-2^K* and *Sk-2^S*.) Strains and phenotypes are designated using nonitalicized words, e.g.,

TABLE 1
Strains Used for Genetic Analysis

Species	Type	Stock Nos. ^a and mating type
I. Spore killer and Spore killer-sensitive standard testers		
<i>N. sitophila</i>	Sensitive to <i>Sk-1</i>	5940A, 5941a
	<i>Sk-1</i>	2216A, 2217a
<i>N. intermedia</i>	Sensitive to <i>Sk-2</i> and <i>Sk-3</i>	3416A, 3417a
	<i>Sk-2</i> (Brunei allele)	3192A, 3675a
	<i>Sk-3</i>	3193A, 3194a
<i>N. crassa</i>	Sensitive to <i>Sk-2</i> and <i>Sk-3</i>	4317A, 4347a
	<i>Sk-2</i> (Brunei allele)	6648A, 6647a
	<i>Sk-3</i>	7076A, 7077a
II. Strains resistant to killing		
<i>N. intermedia</i>	<i>r(Sk-2)</i> (Indonesia)	P146a
	<i>r(Sk-2)</i> (P146 allele)	T468-3A
	<i>r(Sk-2)</i> <i>r(Sk-3)</i> (Australia)	P96a
	<i>r(Sk-2)</i> (Papua New Guinea)	P43A
	<i>r(Sk-2)</i> <i>r(Sk-3)</i> (Australia)	P130A
	<i>r(Sk-3)</i> (Papua New Guinea)	P34A
<i>N. crassa</i>	<i>r(Sk-2)</i> (U.S.A.)	2222A
	<i>r(Sk-2)</i> <i>acr-2</i>	3291A
	<i>r(Sk-2)</i> (Malaya)	P2604a
	<i>r(Sk-2)</i> (Brazil)	P3396a
	<i>r(Sk-2)</i> (Haiti)	P3453a
	<i>r(Sk-2)</i> (India)	P1110a
	<i>r(Sk-2)</i> (Ivory Coast)	P3591a

^a Numbers not prefaced by a letter are FGSC Stock Nos. Numbers prefaced with P or T identify strains in the Perkins collection that are not listed in the FGSC Stock List.

Spore killer, Spore killer-2, *Sk-2* resistant. The insensitive phenotype, called "resistant to killing by *Sk-n*," is usually shortened to "*Sk-n* resistant." Similarly, "sensitive to killing by *Sk-n*" is shortened to "*Sk-n* sensitive." Tester strains that contain a Spore killer element are called "killer testers;" those that are sensitive to killing are called "sensitive testers." The standard species tester stocks for *N. intermedia* and *N. crassa* are sensitive. Only for *N. sitophila*, for which killer strains are relatively abundant in nature, have species testers of both types, killer and sensitive, been deposited in the Fungal Genetics Stock Center as standard species testers.

Media and routine procedures were essentially as described by Davis and de Serres (1970). Tester strains are listed in Table 1. *N. intermedia* and *N. crassa* strains from nature were first screened by crossing to the standard species testers, which are sensitive to both *Sk-2* and *Sk-3*. They were then screened for resistance to killing by crossing them to killer testers carrying *Sk-2* or *Sk-3*. When

these killer testers are crossed to strains that are sensitive to the killer, each ascus contains four black viable ascospores (carrying the Spore killer allele) and four undersize, transparent, dead ascospores. When the killer testers are crossed to strains that are resistant to killing, many or all asci have eight viable black ascospores, of which four produce progeny with the killer phenotype and four produce progeny with the resistant phenotype. The strains being tested were normally used as fertilizing parents.

In the early years of the study, hundreds of crosses were scored by the opening of perithecia and the observation of intact asci under a dissecting microscope. Later, resistance was scored reliably and less laboriously by the examination of asci shot to the sides of the cross-tubes as groups of eight ascospores and the determination of whether each group consisted of eight black ascospores or four black and four aborted ascospores. Before fertilization, we cleaned the insides of the tubes with long sterile swabs to remove conidia and to make it easier to see the ascospores. Eventually, it was found that conidiation is greatly reduced by use of strips of filter paper as the carbon source. Cross-tubes were examined each day, starting 8 days after fertilization, to avoid the confusion of piled-up shot ascospores. Perithecia were removed from the tubes and dissected when there was difficulty in identifying discrete asci among the shot spores, when a cross had few shot spores, or when spore abortion from other causes was excessive.

Difficulties in scoring resistance occurred with individual strains from all species and from all regions, but the majority of problems occurred with *N. crassa*. Because no Spore killer strains have been found in *N. crassa*, killer testers were created by introgression of the Spore killer elements from *N. intermedia*. Fertile hybrid progeny are readily produced between *N. crassa* and *N. intermedia* in the laboratory, allowing genes and chromosomes to be transferred from one species to the other. Spore killer factors were introgressed into *N. crassa* (Turner and Perkins, 1979) by crossing *Sk-2* and *Sk-3* from *N. intermedia* to various *N. crassa* strains, all of United States origin. After a few backcross generations, numerous 4:4 asci (with varying numbers of large, pigmented ascospores) were seen both in the backcrosses and in the crosses to the standard *N. crassa* reference strains, indicating that the standard laboratory strains and the backcross parents were sensitive to both *Sk-2* and *Sk-3*. Spore killer strains obtained after at least 10 recurrent backcrosses into *N. crassa* were selected as killer testers (see Table 1). Mating types were alternated during the backcrosses to insure that the *N. intermedia* mating type chromosome was replaced by that of *N. crassa*. When the *Sk-2* (or *Sk-3*) testers of

opposite mating type were intercrossed, >90% of the ascospores were black and viable. Crosses of these killers to the standard sensitive laboratory strains of *N. crassa* produced normal numbers of asci, and ascospore development was normal for the four surviving ascospores in each ascus. However, when wild-collected *N. crassa* strains were crossed to the testers to screen for resistance to killing, many that had produced 60 to 90% viable ascospores with the standard sensitive testers were poorly fertile with the killer testers. Many of the full-size ascospores were only partially pigmented and many of the test crosses had large numbers of aborted asci. Consequently, scoring resistance by the presence or absence of asci with eight unaborted ascospores was often based on a small number of mature asci.

Some field-collected strains gave abundant black ascospores with the standard sensitive testers and few with the standard killer testers. This was not unexpected. The sensitive testers carry the *fl* mutation, which ordinarily increases fertility, but the *fl* mutation could not be included in the killer testers because it interacts with the Spore killers to produce high levels of ascus abortion. Several commonly used laboratory wild-type strains and field-collected strains from the United States were tried as recurring backcross parents. After several generations, inviable large ascospores continued to appear in sibling crosses and in crosses to sensitive testers. When several other field-collected *N. crassa* strains were substituted as introgression parents in later generations, the problem disappeared. Thus, a variety of parents was introduced into the pedigree. A useful alternate set of *Sk-2* killer testers was obtained by intercrossing the U.S.-background *Sk-2* killer testers to *N. crassa* strains from India (see Table 1). These derived India-background testers usually produced many scorable asci when crossed with strains from India, Thailand, and the Malay Peninsula.

Resistant strains of *N. crassa* may be slightly under- or overrepresented in the Results. Among crosses with mostly light-colored inviable ascospores, those that had asci with four large and four small ascospores were clearly sensitive and were scored. Those crosses that did not have 4:4 asci may have carried the allele for resistance, but in the absence of viable ascospores, they were not scored. This would result in underrepresentation. Conversely, the proportion of strains identified as resistant might be too high. In crosses to *Sk-2*, some *r(Sk-2)* strains tend to produce ascospores more readily than do *Sk-2*-sensitive strains from the same site, so there may have been a disproportionate number of sensitive strains among those that gave especially poor results.

When *N. sitophila* strains from culture collections or from nature are used as female parents, they are generally sterile or produce mostly defective ascospores. Fertile *N. sitophila* species-testers had already been bred and selected as standards before the existence of *Sk-1* was recognized (Perkins *et al.*, 1976). These standard reference testers were later found to be *Sk-1* killers. To complement the killer testers, we then developed fertile *Sk-1*-sensitive testers that were excellent for screening *N. sitophila* from all regions (Table 1). The first cross of newly acquired *N. sitophila* strains was sometimes to a *Sk-1* killer tester and sometimes to a *Sk-1* sensitive tester. If the resulting asci were 4:4, the phenotype of the new strain was clearly the opposite of that of the tester. If the asci were 8:0, the new strain was crossed to a tester of the other type. Except for one resistant strain, this second cross always resulted in 4:4 asci. The shot ascospores were almost always clearly either >95% black or about 50% black. Nevertheless, scoring was sometimes verified by opening perithecia.

RESULTS

Distribution of Spore killer strains. Most of the available wild-collected cultures of *N. sitophila*, *N. intermedia*, and *N. crassa* have been tested to determine whether they carry a Spore killer factor. In *N. sitophila*, *Sk-1* is common and is widely distributed among 566 isolates (Fig. 1, Table 2). In *N. intermedia*, *Sk-2* was found in only 4 cultures among ~3000 tested, 2 from Borneo and 1 each from Java and Papua New Guinea. *Sk-3* was found only once, at the same site as that with *Sk-2* in Papua New Guinea (Turner *et al.*, 1987; Perkins and Turner, 1988) (Fig. 2). No killers of either type were found among hundreds of *N. intermedia* isolates when sites in Papua New Guinea were revisited 15 years later. Disappearance of the killers is perhaps not surprising. Because selection for killers is "soft," the frequency of a newly arisen or newly introduced killer element is expected to remain very low for a long time, even if compensation occurs for the 50% of ascospores that are killed. New killers are perhaps more likely to be lost than to become established.

A second *Sk-2* strain from Borneo was obtained many years after the first, following an intensive search that was prompted by the earlier finding. In *N. crassa*, not a single Spore killer strain has been found among 523 wild-collected cultures.

***Sk-1* shows regional distribution in *N. sitophila*.** Spore killer strains are certainly not distributed randomly

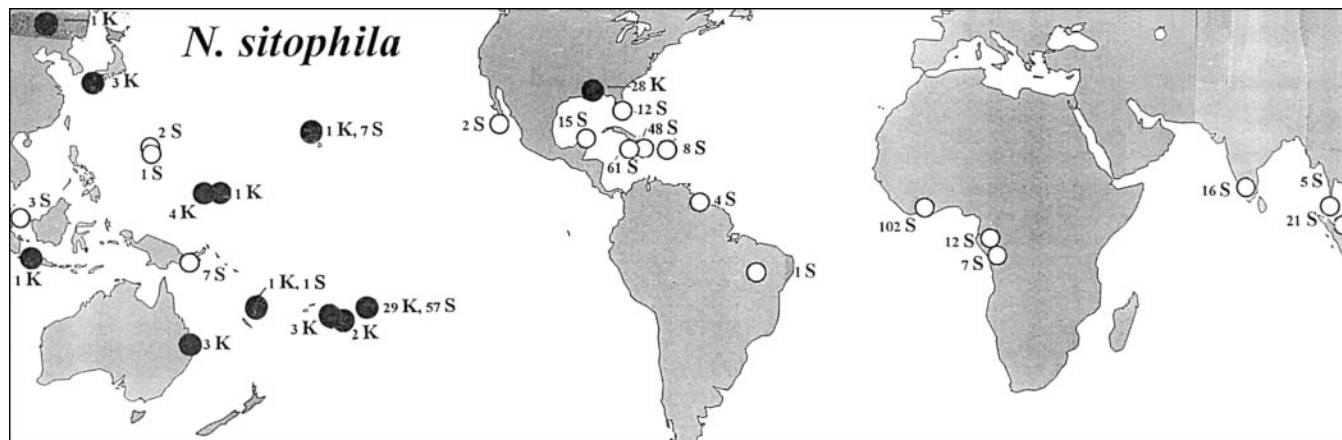


FIG. 1. Distribution of *N. sitophila* *Sk-1* killer strains (K) and strains sensitive to killing (S). Open circles identify locations within which samples consisted solely of sensitive isolates. A single nonkiller strain, from Gabon, was found to be resistant to killing by *Sk-1*. The *Sk-1* drive element was first detected in crosses between sensitive *N. sitophila* strains from Nigeria and killer strains from Virginia (Turner and Perkins, 1979).

throughout the world. A distinct geographic pattern is seen for those strains that were found growing out of doors on burned substrate (Fig. 1, Table 2). With one exception (Louisiana), *Sk-1* was not found in the Americas, Africa, India, or Southeast Asia. We screened all available *N. sitophila* strains for resistance to *Sk-1* and found only one resistant strain, from Gabon.

The data are insufficient to determine whether *Sk-1* has become fixed in any region. The only area with a large group consisting only of killers was in Louisiana, with 28 *Sk-1* strains, of which 20 were from one site. All *N. sitophila* strains from the nearest sites, in Florida, were sensitive.

N. sitophila is found growing indoors on food or food waste much more frequently than are other *Neurospora* species. Ten strains in the Perkins collection and 3 historic *Neurospora* strains from other culture collections came from such a sheltered environment in Australia and in areas of North America and Europe where winters are cold and where *Neurospora* had never been found on burned vegetation. All 13 of these strains are *N. sitophila*, and all but 1 carry *Sk-1*. Strains collected within buildings are not included in Table 2. The distribution and life style of these strains under conditions of climate control, rich medium, and lack of competition may not be comparable to that of the strains collected from a more challenging environment. Furthermore, their close proximity to human activity, including imported foods and fibers, raises the question of whether the breeding population from which they arose was local or whether the strain entered the site, or even the country, as a contaminant.

Five additional strains came from unburned substrates outdoors. These all carry *Sk-1*. Colonies growing on burned or scorched vegetation have been hypothesized to arise from ascospores germinated as a result of the fire and to represent a local breeding population (Perkins *et al.*, 1976). Whether these *N. sitophila* samples from unburned substrates are of local origin is more speculative, but the five isolates do not contradict the overall pattern of *Sk-1* distribution.

Resistance to Spore killer. Spore killer-2 and Spore killer-3 killer strains have not been found in *N. crassa* and are too rare to provide significant information about population variability in the *N. intermedia* populations sampled, but resistance to killing has provided phenotypes that vary within and between populations.

N. intermedia and *N. crassa* cultures from natural populations were crossed to *Sk-2* and *Sk-3* killer testers of the same species. In these test crosses, sensitive strains produced asci containing no more than four full-size ascospores (all killers, like the tester parent) and at least four aborted ascospores. (In a few of the test crosses in which killing was observed, up to 1% of the asci had five or more black ascospores. No attempt was made in the screening process to differentiate between such strains and the sensitive strains that produced 100% 4:4 asci.)

Some of the tested wild-collected strains of *N. intermedia* and *N. crassa* are resistant to *Sk-2*. Fewer strains of *N. intermedia* and no strains of *N. crassa* are resistant to *Sk-3*. When a fully resistant strain is crossed to a killer tester, about half of the progeny are killers and half are resistant, with few exceptions. This is as expected if resistance is

TABLE 2

N. sitophila Collected from Burned Vegetation, by Region or Country and Spore Killer-1 Phenotype

Region or country	Number of isolates	
	Sensitive	Killers
I. Regions where no killer isolates were found		
Florida (U.S.A.)	12	0
Mexico ^a	17	0
Grand Cayman, B.W.I.	61	0
Haiti	48	0
Puerto Rico	8	0
Guyana	4	0
Brazil	1	0
Ivory Coast	102	0
Gabon ^b	12	0
Congo	7	0
India	16	0
Thailand	5	0
Malaysia	21	0
Singapore	3	0
Rota	2	0
Guam	1	0
Papua New Guinea	7	0
II. Regions with both killer and sensitive isolates		
Hawaii	7	1
Vanuatu	1	1
Tahiti-Moorea	57	29
III. Regions with <i>Sk-1</i> isolates only		
China	0	1
Japan	0	3
Indonesia	0	1
Truk	0	4
Ponape	0	1
Australia ^c	0	3
Tonga	0	3
Rarotonga	0	2
Louisiana, U.S.A.	0	28

Note. See Fig. 1.

^a After this study was concluded, two strains obtained from Tucumanduba, northern Brazil did not show killing in crosses to the *Sk-1* killer testers (Ann Fairfield, personal communication). It is not known whether these strains were from burned substrate.

^b A nonkiller strain that was resistant to *Sk-1* was found in Gabon.

^c Two samples came from pollen baskets of bees harvesting *Neurospora* from burned substrate. A third sample came from lumber that had been steamed.

linked to the killer complex. *N. intermedia* strains from most regions are considered fully resistant if they give about the same results when they are test crossed to the appropriate killer tester as when they are crossed to a sensitive tester. Aborted asci may be present, but virtually every ascus that matures has eight full-size ascospores.

Some strains are only partially resistant. Each partially resistant strain has a consistent ratio of 8:0 vs 4:4 asci when crossed to Spore killer testers. We set an arbitrary requirement of at least 25% 8:0 asci for designating a strain as resistant. In practice, partially resistant strains almost always produce at least 50% 8:0 asci, and most of them produce more than 90% 8:0 asci. Some of the partially resistant strains were noted for the sake of possible future genetic studies, but scoring was usually recorded simply as *sensitive* or *resistant*, with partially resistant strains being classed as resistant.

Resistance to *Sk-2* and *Sk-3* in *N. intermedia* shows a distinctive pattern of geographic distribution. In some areas, one-third to over one-half of *N. intermedia*

TABLE 3

Sensitivity and Resistance to Killing by *Sk-2* and *Sk-3* in *N. intermedia*

Region or country	Number of isolates			
	Sensitive to <i>Sk-2</i>	Resistant to <i>Sk-2</i>	Sensitive to <i>Sk-3</i>	Resistant to <i>Sk-3</i>
I. Regions with resistance to both Spore killers ^a				
Thailand	5	6	7	8
Malaya	35	28	51	11
Singapore	1	7	5	3
Taiwan	15	4	19	1
Philippines	8	1	8	1
Borneo	19	19	6	10
Indonesia	63	21	70	14
Papua New Guinea	27	37	60	19
Australia	37	42	49	26
Ponape	24	11	27	8
Tahiti, Moorea	8	3	5	7
II. Regions with resistance to <i>Sk-2</i> but not to <i>Sk-3</i>				
India	32	28	61	0
China	4	2	6	0
Japan	5	2	7	0
III. Regions without resistance to either Spore killer				
U.S.A. (southeastern)	153	0	153	0
Haiti	20	0	20	0
Puerto Rico	132	0	134	0
Guyana	8	0	2	0
Brazil (São Paulo)	64	0	64	0
Congo	29	0	29	0
Ivory Coast	39	0	21	0

Note. Numbers refer to strains tested successfully. Totals may therefore not be the same for *Sk-2* as for *Sk-3*.

^a Thirty-two of 52 strains from Rota and Guam were resistant to *Sk-2*. These were not tested for resistance to *Sk-3*. They would be expected to fall in Group I.

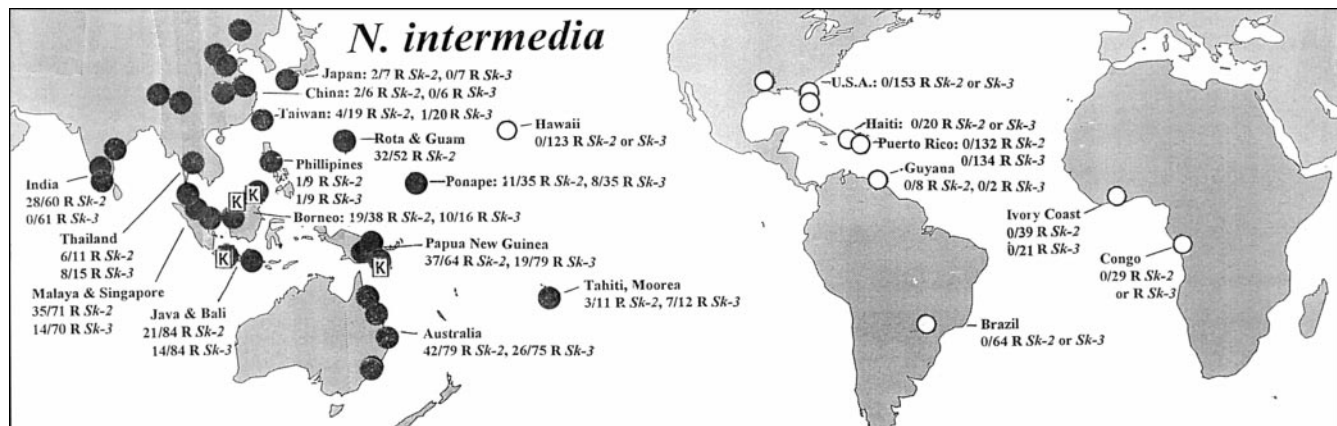


FIG. 2. Distribution of resistance to killing by *Sk-2* and by *Sk-3* in *N. intermedia*. Open circles mark locations from which no resistant isolates were obtained. *Sk-2* killer strains have been found only at the four sites marked "K." *Sk-3* killer strains were found only once, at the same site as that with *Sk-2* in Papua New Guinea. *Sk-2*-resistant strains from Rota and Guam included some that were not tested for *Sk-3* resistance.

strains are resistant to *Sk-2* (Table 3, Fig. 2). *r(Sk-2)* strains have been found in an area that can be thought of as radiating from the points where *Sk-2* was found. Resistant strains were found at collection sites ranging from Japan in the north to Australia in the south, and from India to the west, through the western Pacific (Rota, Guam, Ponape) and across the south Pacific to Tahiti. Resistant strains were generally scattered across many of the sites in areas where they occurred and not concentrated at any particular location.

The geographic range of *r(Sk-3)* is enclosed within that of *r(Sk-2)* (Fig. 2). The only significant difference between the two distributions is in India, where *r(Sk-2)* is abundant but no *r(Sk-3)* was found. Japanese and Chinese populations may also differ with respect to *r(Sk-2)* vs *r(Sk-3)*, but sample sizes are small. *r(Sk-2)* is as frequent in India as it is in the areas where *r(Sk-3)* is found.

Four types of non-killer strains were found in *N. intermedia*: doubly sensitive, *r(Sk-2)* only, *r(Sk-3)* only, and doubly resistant *r(Sk-2) r(Sk-3)*. The doubly resistant strains were found wherever *r(Sk-2)* and *r(Sk-3)* occur in the same population. All four types were often found at the same site or at neighboring sites. In fact, there is not one broad area where *r(Sk-3)* was found but doubly resistant strains were not, nor is there an area where doubly resistant strains were found but *r(Sk-3)* alone was not.

Resistance in *N. crassa*. Resistance to killing by *Sk-2* occurs as a minority phenotype throughout the range of *N. crassa* even though *Sk-2* has not been found in that species (Table 4). No strains resistant to *Sk-3* were found among the 85 strains crossed to *Sk-3* testers. The known range of

N. crassa extends from Yucatan, Mexico and the southeastern United States eastward through the Caribbean, northern South America, Africa, India, and Thailand, to Penang, Malaysia (see Fig. 3 of Turner *et al.*, 2001). No *N. crassa* samples were found among the cultures collected from the Malay Peninsula or the Pacific area, and except

TABLE 4
Resistance to Killing by *Sk-2* and *Sk-3* in *N. crassa*

Region or country	Number of isolates			
	Sensitive to <i>Sk-2</i>	Resistant to <i>Sk-2</i>	Sensitive to <i>Sk-3</i>	Resistant to <i>Sk-3</i>
U.S.A. (southeastern)	102	5	60	0
Mexico (Yucatan)	14	1	11	0
Haiti	12	3	15	0
Puerto Rico	11	2	11	0
Venezuela	5	0	4	0
Guyana	2	0	2	0
French Guiana	1	0	0	0
Brazil (northern)	12	1	8	0
Ivory Coast	9	12	23	0
Gabon	1	0	1	0
Congo	5	0	6	0
Pakistan	9	0	4	0
India	70	8	73	0
Bangladesh	1	0	1	0
Thailand	5	3	4	0
Malaya (Penang Island only)	35	4	42	0

Note. Numbers refer to strains tested successfully. Totals may therefore not be the same for *Sk-2* as for *Sk-3*.

for equatorial Africa, *N. crassa* has not been found in the Southern Hemisphere.

Distribution of *Sk-2* resistance in *N. crassa* is different from that in *N. intermedia* in two ways. First, *r(Sk-2)* strains were found in every region where *N. crassa* is found (Table 4), and second, the incidence is usually much lower in *N. crassa* than in *N. intermedia*. Only for the small group of populations from west Africa (Ivory Coast, Gabon, Congo) was the proportion of *r(Sk-2)* strains in *N. crassa* (44%) similar to that in *N. intermedia* (43% overall, for the areas in which *Sk-2* resistance occurs). Even where just a few samples were collected from a limited area, resistance was found in every country or area with 10 or more *N. crassa* samples and in some with fewer than 10. Not all available *N. crassa* strains were tested for resistance. The primary question addressed for each geographic area was whether *r(Sk-2)* and *r(Sk-3)* are present. Only for areas with the greatest number of strains available was an attempt made to gather sufficient data to estimate the proportion of strains with *r(Sk-2)*.

The widespread incidence of *r(Sk-2)* may mean that the *Sk-2* killer now exists or once existed in *N. crassa*. In fact, *Sk-2* may be present in *N. crassa* and its incidence may be just as great as that in *N. intermedia*. About 3000 *N. intermedia* strains were screened, compared to only 467 for *N. crassa*. Obviously, we did not obtain and test enough samples to be sure that *Sk-2* does not exist in *N. crassa* at the present time.

Breakdown of fertility as protection against invasion by a Spore killer. Complete breakdown of fertility in crosses with invading Spore killer strains would be an effective way to prevent a killer element that was introduced by mutation or immigration from becoming established in a population. In the laboratory, the first indication that a wild-collected strain possesses such a fertility barrier would be that the strain was fertile with the standard species-tester but that it made virtually no viable ascospores when crossed with the standard killer tester. Strains and local populations fitting this description may have been observed in *N. intermedia*.

Typically, fertility is similar when a wild strain is crossed to a sensitive tester and to a killer tester. However, some *N. intermedia* strains that are reasonably fertile with the sensitive testers are atypical in giving very infertile crosses with the killer testers. In fact, crosses to the killer produce so few asci that it is not even possible to score for sensitivity versus resistance. Although the atypical strains produce more than 50% viable ascospores with the sensitive *N. intermedia* species-testers, most asci abort and $\ll 1\%$ of the shot ascospores are viable in crosses of the same wild

strains with the killer testers. Some of the atypical strains are individual isolates from scattered sites, but all the *N. intermedia* strains from two areas, in New Zealand and in Yucatan, Mexico, consistently produce few if any progeny with the killer testers. Attempts to develop killer testers compatible with the New Zealand and Yucatan strains were unsuccessful. After two generations of introgression of *Sk-2* into strains from these two areas, fertility was not improved. For example, when five of the wild-collected New Zealand strains were crossed pairwise to 10 killer progeny of the second backcross generation, viable ascospores were as scarce as in crosses of the atypical strains to the standard killer testers that were used at the beginning. These two populations thus appear to possess a general sterility phenotype that would prevent invasion by killer elements.

Strains that behave in this way have not been found in *N. crassa*. Although some *N. crassa* strains from nature were poorly fertile in crosses to the standard killer testers, the same strains were much more fertile when crossed to other testers that provided a more compatible background into which the Spore killer factors had been introgressed. The original poor fertility cannot therefore be attributed to interaction between the wild *N. crassa* strains and the Spore killer elements per se.

DISCUSSION

This is the first extensive geographic survey bearing on meiotic drive in populations of a haploid organism. Information was obtained on the incidence and distribution of Spore killer strains and strains resistant to killing in heterothallic species of *Neurospora*, providing data from wild populations to complement what had previously been learned in the laboratory about the genetic basis and mode of action of the Spore killer drive elements.

Evolutionary implications of the data. Species of *Neurospora* were found to differ markedly from one another in their geographic distribution (Perkins *et al.*, 1976; Perkins and Turner, 1988; Turner *et al.*, 2001). This was not a foregone conclusion. At the time Spore killers and genes conferring resistance to killing were discovered, the existence of distinct, genetically different populations within the individual *Neurospora* species had not been demonstrated. The possibilities existed that species had been homogenized by natural or human-mediated dispersal and that separate populations would not be found to differ in their gene frequencies. However, when Spieth

(1975) examined allozyme patterns for *N. intermedia* cultures from two widely separated regions, he found distinct differences between the regions. Our results agree with his in showing that geographically separated populations of *N. intermedia* are genetically different. It is therefore possible to look at the distributions of the Spore killer strains and strains differing in sensitivity to killing and to ask what inferences can be drawn about the population dynamics of meiotic drive in *Neurospora*. Does the presence of resistance signal the previous existence of Spore killers in the population? Can a Spore killer enter and take over a population?

Differences among species and among Spore killers.

Differences between *N. crassa* and *N. intermedia* in the geographic distribution of the various types support the hypothesis that these differences result from isolation between populations. *Neurospora* species differ from one another in the relative frequencies of killer strains and resistant strains. In *N. sitophila*, killers comprise a large minority of the strains (Table 2) but only one resistant strain was found. The reverse is true in *N. intermedia* and *N. crassa*, in which many resistant strains were found but killer strains were rare (~0.1%) or absent.

In contrast to the situation in *Neurospora*, Kathariou and Spieth (1982) found a high frequency of Spore killers among 225 strains of *Fusarium moniliforme*, mostly from Italy and California. Of these strains, 81% were killers, only 15% were sensitive, and the rest were of a "mixed" type—neither killer nor sensitive and differing in their characteristics from the resistant strains of *Neurospora*. The only other study of drive elements in nature is by van der Gaag *et al.* (2000), who found that 23% of strains from a Netherlands population of the pseudohomothallic ascomycete *Podospora anserina* carried a Spore killer element in heterokaryotic condition. The dynamics of meiotic drive in a pseudohomothallic fungus is expected to differ from that in a heterothallic species, reflecting the survival of sensitive nuclei when they are sheltered in the same heterokaryotic ascospore with a killer nucleus (Raju and Perkins, 1991).

Sk-2 and *Sk-3* are tightly linked in the same long segment of linkage group III. It is therefore reasonable to suppose that they are related in their evolutionary history. *Sk-2* was collected five times, whereas *Sk-3* was found only once, and the *r(Sk-2)* gene is much more widely distributed than *r(Sk-3)*. These observations suggest that *Sk-2* is more common than *Sk-3*.

The question arises whether *r(Sk-2)* and *r(Sk-3)*, which differ in specificity of action, are at separate loci or whether they represent a single resistance locus with mul-

iple alleles—(1) the standard sensitive allele, (2) *r(Sk-2)*, (3) *r(Sk-3)*, and (4) an allele conferring resistance to both *Sk-2* and *Sk-3*. We think that *r(Sk-2)* and *r(Sk-3)* probably represent two loci with some crossing-over between them. Strains showing resistance only to *Sk-3* and strains resistant to both *Sk-2* and *Sk-3* are found consistently in the same areas. Strains resistant only to *Sk-2* are also present in these same areas and in a much wider area.

The frequencies of killing and of resistance are clearly different for *Sk-1* in *N. sitophila* than for *Sk-2* and *Sk-3* in *N. intermedia*. *Sk-1* killer strains are abundant and widely distributed, whereas only a single *Sk-1*-resistant strain has ever been found. The question might be asked whether *Sk-1* differs from the killers in other species in its underlying molecular and cellular basis, resembling *Sk-2* and *Sk-3* only in having a similar end effect. Alternatively, the differences might be due to species-specific differences in ecological preferences or in the importance of sexual reproduction, or they might simply be due to random events in evolutionary history. Segregation distortion can result from any of a variety of developmental alterations that have in common only that they result in a deficit of one class of ordinarily viable meiotic products when a drive element is heterozygous. Meiotic drive has arisen independently in widely differing organisms and is not expected to be functionally identical in all of them. For example, both Segregation distorter in *Drosophila* (Temin *et al.*, 1991) and the *t* haplotype in *Mus* (Silver and Remis, 1987) affect only male meiotic products, but cytological and molecular analyses reveal profound differences in the mechanisms by which this is brought about in the two organisms (Lyttle, 1991; Ganetzky, 2000; Schimenti, 2000). Successful production of ascospores in *Neurospora* requires many developmental steps culminating in a changeover to the state of dormancy. It is reasonable to suppose that meiotic drive could have arisen independently in different *Neurospora* lineages as a result of changes in genes that differ in their primary mode of action, affecting different aspects of development. Thus, one Spore killer may interact with a particular resistance mutation while another does not, and one Spore killer may be detrimental in the vegetative phase while another is not.

Other explanations should be considered. The distribution patterns in *N. sitophila* populations may reflect differences at the level of the organism or differences in ecology rather than differences in the underlying molecular or cellular mechanisms. For example, the sexual cycle may not be as important for survival in *N. sitophila* as in other species, or species-specific substrate and climate

preferences may affect the expression of *Sk-1*. Yet another alternative is that species differences in the proportion of killer, sensitive, and resistant strains may reflect chance historical or evolutionary events rather than species-specific differences in the Spore killer elements or their mode of action. For example, killer elements or genes conferring resistance may be of more recent origin in one species than in another.

Differences in geographic distribution. In *N. intermedia*, *r(Sk-2)* strains are abundant in one part of the range and missing in the rest. Since the part of the world where *r(Sk-2)* is found centers on the places where *Sk-2* was collected, it is tempting to think that the resistance genes owe their existence primarily to their function of conferring resistance.

r(Sk-3) is closely linked to *r(Sk-2)* in *N. intermedia*. After introgression, the same *r(Sk-3)* allele from *N. intermedia* is also closely linked to the *r(Sk-2)* allele found in *N. crassa* (0/300 recombination). *Sk-2*-resistant strains occur in both species in a region of overlap that includes India, Malaysia, and Thailand. In addition to determining the diversity of resistance alleles within populations, DNA sequencing may eventually reveal whether resistance alleles have spread from one species to the other.

In most areas, *N. sitophila* is far less abundant than *N. intermedia* or *N. crassa*, and there is no way to enrich for it as populations are being sampled. We have maintained skepticism about the apparent distribution patterns that emerge from the very small samples of *N. sitophila*.

Relationship of resistance genes to meiotic drive genes. Genetic and molecular information is needed not only to compare resistance genes among themselves and Spore killer complexes among themselves, but also to compare killer strains with resistant strains. We would like to know whether the same gene or genes that confer resistance on insensitive ("neutral") non-killer strains are also responsible for conferring self-resistance on the killer strains, which experience no killing in homozygous crosses.

We have suggested that the distribution of resistance genes may provide clues to the prior distribution of distorter genes. However, preadaptation is a possibility. Genes that happen to confer resistance might already be present in a population in the absence of any killer, and their effect on distorter gene expression might be secondary to some other function. Hiraizumi *et al.* (1960) posed this question for the *SD* system in *Drosophila*, and ambitious programs were undertaken to seek an answer (see, for example, Hiraizumi and Thomas, 1984). Although meiotic drive is suppressed both by linked genes integral to

the system and by unlinked genes, data regarding the natural occurrence of these suppressors are inconclusive with respect to their function and distribution independent of the *Sd* drive element.

Rick (1971), discussing the gamete-eliminator system in the tomato (*Lycopersicon esculentum*) and its wild relative *L. pimpinellifolium*, suggested that resistance genes might be a necessary precondition for the spread of a distorter from one subpopulation to another or for the establishment of a newly arisen distorter. In *Neurospora*, crosses between subpopulations that differ in the meiotic drive elements that they carry would, like tomato plants heterozygous for distorter genes, produce fewer progeny. If they differed for two linked Spore killers such as *Sk-2* and *Sk-3*, they would produce virtually no progeny. However, some strains from a population that was free of Spore killers but polymorphic for appropriate resistance genes would be able to cross productively with a foreign Spore killer strain, allowing invasion of the killer element, which would be expressed only in later generations, in recombinant progeny that were not resistant.

Polymorphism for resistance might be thought of as essential to establishment of a two-element drive system, wherein there is interaction between a distorter gene on the killer chromosome and a closely linked target locus on the sensitive homologous chromosome. If a new Spore killer element were introduced into a sensitive chromosome by mutation or invasion, that chromosome would quickly be eliminated. For the new drive element to survive for long and to function as a Spore killer, the target locus either must be capable of conferring resistance or must be coupled to a gene that can do so.

Other loci that may interact with meiotic drive. Hurst and Pomiankowski (1991) presented the case for expecting populations to evolve generalized "antidrive" mechanisms rather than relying simply on suppressors of specific meiotic drive haplotypes. Such a mechanism might prevent the establishment of new drive elements by conferring sterility on crosses between an invading element and a strain possessing the antidrive mechanism. We have described behavior of some *N. intermedia* strains from New Zealand and Mexico that might be explained in this way. Our attempt to introduce a Spore killer factor into the strains from those two collections was essentially a laboratory simulation of the invasion of a killer strain into the populations from which the wild strains were sampled. Overall, only a few dozen progeny were obtained, even though many different crosses were made, with conditions for crossing probably being better than those in nature. If the New Zealand and the Mexico strains had been fertile

with *Sk-2*, whether sensitive or resistant, these crosses would have produced millions of Spore killer progeny. If the laboratory results are indicative of what happens in nature, a Spore killer gene would exist in these areas only as long as vegetative propagules of the original strain survived (and vegetative propagules are less resistant to environmental stress than ascospores). In contrast with the mere decrease of a Spore killer element effected by the presence of genes conferring resistance to killing, an infertility mechanism would make resistance unnecessary because it prevented the initial invasion of the killer element.

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