How to distinguish a Spore killer from alternative causes of ascospore abortion.

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Background: The known Spore killer elements were discovered (and killer strains are scored) because when a cross is heterozygous for a killer, asci are produced asci in which four of the eight ascospores are inviable and fail to darken, and the four viable black survivors are all Spore killers (Figure 1). In homozygous killer × killer crosses, all eight ascospores are pigmented and viable (Turner and Perkins 1979).

But there are other ways of getting asci with four black and four nonblack ascospores (4B:4W). 4B:4W asci are produced when crosses are heterozygous for an autonomously expressed mutant allele of a gene that is required for ascospore pigmentation (e.g., *ws-1*, *per-1*, *cys-3*) (Figure 2). 4B:4W asci may also originate from crosses parented by a chromosome rearrangement that produces duplication/deficiency meiotic products that are ascospore-lethal (e.g., a translocation) (Figure 3).

4B:4W-producers of the different types are readily distinguished. An autonomously expressed mutant allele is recognized as such because the viable progeny are nonmutant and do not produce 4B:4W asci when they are crossed to a sensitive tester. A chromosome rearrangement is recognized as such because both parental types -- rearrangement and normal sequence --are recovered among the viable progeny of a heterozygous cross. Also, most heterozygous rearrangements produce asci of types other than 4B:4W. The diagnostic differences are diagrammed in Figure 4 of Raju (1994).

Presumptive evidence for a Spore killer is provided if all (or most) mature asci from a heterozygous cross are 4B:4W, with no 8B:0W, 6B:2W, or 0B:8W, and if nonkiller progeny are absent (or rare) when viable f_1 progeny are test-crossed. Critical proof is provided if homozygous killer × killer crosses produce 8B:0W asci, and if the progeny of these crosses produce 4B:4W asci when crossed by a sensitive wild type strain. To accomplish this critical test, killer strains of both mating types must be available.

Procedure: Cross a presumptive Spore killer strain by a sensitive tester and examine shot asci. Open perithecia to observe rosettes of linear asci. Obtain asci as shot groups of eight ascospores, isolate and tube black ascospores. Cross individual f_1 progeny by a sensitive tester. Recommended testers are listed below in Table 1, taken from Turner and Perkins (1993).

Strains containing the aconidiate mutation *fluffy* (*fl*) are conveniently used a female parents in test crosses for scoring killer vs. sensitive. The *fl* testers are highly fertile, and because conidia are absent, ascospores ejected to the sides of the tube can be seen clearly. With *N. crassa*, tests are made by fertilizing the testers on 10 ×75 mm slants of synthetic cross medium with 1% sucrose and examining shot ascospores after 10 days at 25ºC. With *N. intermedia*, *N. sitophila*, and *N. discreta*, tests are best made on 13 × 100 mm slants using synthetic cross medium with filter paper as sole carbon source. If this medium is employed, stocks without the *fluffy* mutation can be used, because few conidia are produced. Standard stocks of *N. tetrasperma* are also satisfactory as testers because they make few conidia at 25ºC, even on sucrose medium.

References

Perkins, D. D. 1994. Corrections to Turner and Perkins "Strains for studying Spore killer in four Neurospora species". (FGN 40:76-78, 1993). Fungal Genet. Newslett. 41:14.

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Turner, B. C., and D. D. Perkins. 1979. Spore killer, a chromosomal factor in Neurospora that kills meiotic products riot containing it. Genetics 93: 587-606.

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Table 1. Strains for identification and study of Spore-killers in Neurospora

Species and genotype	Origin of allele*	FGSC No. *A*	FGSC No. *a*	Comment†
Neurospora crassa				
Sk-2^K	B	6648	6647	10th backcross to *N. crassa*, mixed background
Sk-2^K	B	3114	3115	10th backcross to *N. crassa*, inbred to OR wild type
cum Sk-2^K acr-7	B	-	7432	
Sk-2^K acr-7	B	6930	-	10th backcross to *N. crassa*
Sk-2^K acr-7 leu-1 his-7	B	-	7373	
Sk-2^K acr-2 leu-1 his-7	B	7387	7388	
Sk-2^K acr-2 leu-1	B	7375	7374	
Sk-2^K acr-2 his-7	B	7376	-	
Sk-2^K leu-1	B	7371	-	
Sk-2^K his-7	B	7378	-	
Sk-2^K phe-2 dow	B	4538	4539	
Sk-2^K dow	B	4260	4261	
Sk-2^K; fl	B	3297	3298	9th backcross to *N. crassa*
Sk-2^K	P	7368	7367	12th backcross to *N. crassa*
Sk-2^K acr-2	P	7385	7386	
Sk-2^K	J	7369	7370	12th backcross to *N. crassa*
cum Sk-2^K acr-2	J	7383	7384	
Sk-2^K acr-2	J	6928	6929	15th backcross to *N. crassa*
Sk-2^K	J	7392	7393	Used for testing *N. crassa* from India
Sk-2^S Sk-3^S fl ‡		6682	6683	*fl^P* (RL) testers
r(Sk-2)-1	-	2222	-	Iowa-1, Louisiana (P527)
r(Sk-2)-1 cum		7379	7380	
cum r(Sk-2)-1 acr-7		-	7389	
r(Sk-2)-2		-	7398	Derived from *N. crassa* P2604, Georgetown, Malaya
Sk-3^K	P	3577	3578	10th backcross to *N. crassa*
cum Sk-3^K	P	7382	7381	
cum Sk-3^K his-7	P	7390	7391	
Sk-3^K acr-2	P	-	7077	
Sk-3^K acr-7	P	6931	6932	15th backcross to *N. crassa*
Sk-3^K fl	P	3579	3580	10th backcross to *N. crassa*
Sk-2^S Sk-3^S fl ‡		6682	6683	*fl^P* (RL) testers
r(Sk-3)		7395	-	6th backcross to *N. crassa*
cum r(Sk-3)		-	7396	6th backcross to *N. crassa*
cum r(Sk-3) leu-1		-	7394	9th backcross to *N. crassa*
r(Sk-3) acr-7 ser-1		7397	-	6th backcross to *N. crassa*
Neurospora intermedia				

Sk-2^K	B	7401 7402	3rd and 4th backcross to Taipei background
Sk-2^K	P	7429 -	3rd backcross to Taipei background
Sk-2^K	J	7399 7400	f_1 of Tjiawi-2d (P162) × Taipei-1c (P13)
Sk-2^K	SA	7426 -	Menggatal, Sabah (P3126)
r(Sk-2)		1832 1833	Townsville-1b (P113), Townsville-1 (P112)
Sk-3^K	P	3193 3194	Derived from Rouna-1 (P32)
r(Sk-3)		6595 5123	Tahiti (P2427, P2421)
Sk-2^S *Sk-3*^{S} ‡		3416 3417	Shew wild types (Taipei background)
Sk-2^S *Sk-3*^{S} *fl* ‡		5798 5799	7th backcross of *fl*^{P} from *N. crassa* to Shew wild types
Neurospora sitophila			
Sk-1^K		2216 2217	Derived from Dodge's Arlington stocks
Sk-1^K*; fl*		4762 4763	*fl*^{P} (1012) from Whitehouse *N. sitophila*, 3rd backcross to Dodge stocks
Sk-1^S		5940 5941	Tahiti (P2443, P2444)
Sk-1^S*; fl*		4887 4888	5th backcross of *fl*^{P} from *N. crassa* to Panama VP203 or derivative
***Neurospora tetrasperma*#**			
Sk-2^K *acr-2*	J	6934 6935	8th-9th backcross to *N. tetrasperma*
Sk-2^K *acr-2*; *E*	J	6936 6937	4th backcross to *N. tetrasperma*
Sk-3^K *acr-7*	P	6938 6939	7th-8th backcross to *N. tetrasperma*
Sk-3^K *acr-7*; *E*	P	6940 6941	8th backcross to *N. tetrasperma*
Sk-2^S	-	1270 1271	Wild types 85A, 85a (also *Sk-3*^{S})
Sk-2$^{S;}$ *E*	-	5897 5901	85A, 85a background (also *Sk-3*^{S})

The ooriginal published version of this table contained errors which have been corrected here, incorporating changes given in Fungal Genet. Newslett. 41: 14, 1994.

* B: Brunei (Borneo); J: Java; P: Papua New Guinea; SA: Sabah (Borneo).

† "*n*th backcross" Indicates progeny from the *n*th backcross of *Sk*^{K} into the alien genetic background. Introgressed killer strains with markers, for which there is no comment, are all from well backcrossed parents. Stock numbers prefixed with P are given for strains that originated from nature. For origins of stocks designated by place names, see FGSC Neurospora Stock List, Part V.

‡ These strains are sensitive to killing both by *Sk-2*^{K} and by *Sk-3*^{K}. The double symbol is used to specify phenotype and does not imply that *Sk-2*^{K} and *Sk-3*^{K} necessarily represent two genes at separate loci. It has not been determined how many loci are involved in determining sensitivity vs. resistance to either or both Spore killers.

(See Raju and Perkins 1991 Genetics 129: 25-37. *E* = 8-spored ascus.)

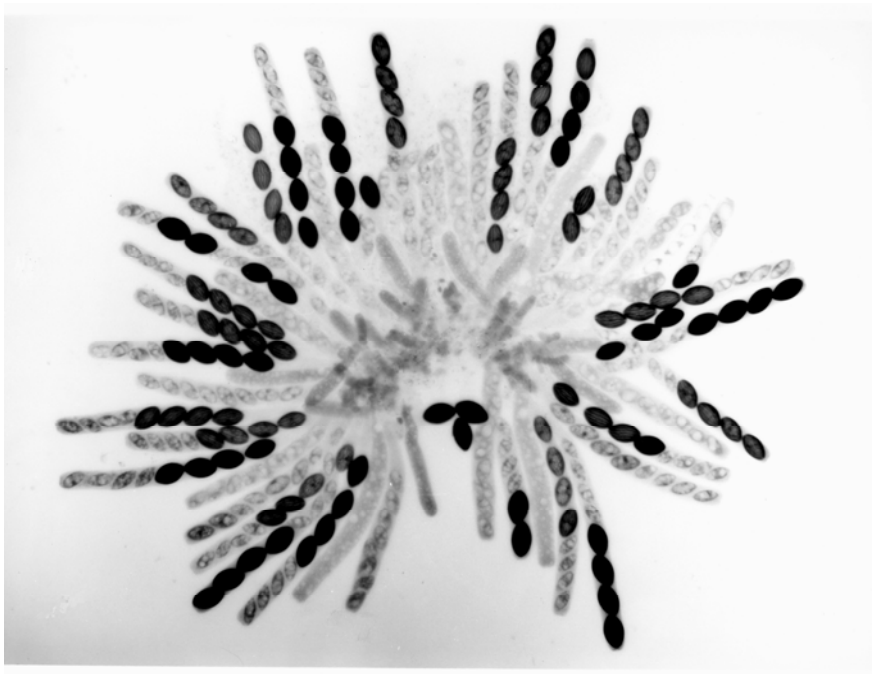

Fig 1. *N. sitophila*. *Spore killer-1* x Wild type. A rosette of asci at various stages of maturation. Most asci show four normal, larger ascospores (Killer) and four aborted, smaller ascospores (wild-type sensitive). The few asci that are not showing the 4:4 pattern are still immature. Photo credit: N.B. Raju.

Fig 2. *N. crassa*. Ascospore color-mutant (cys-3) x Wild type. Mature asci show four black (viable) and four white (inviable) ascospores. The white ascospores received the mutant allele and fail to pigment and mature. The asci with all white ascospores are still immature. Photo credit: N.B. Raju.

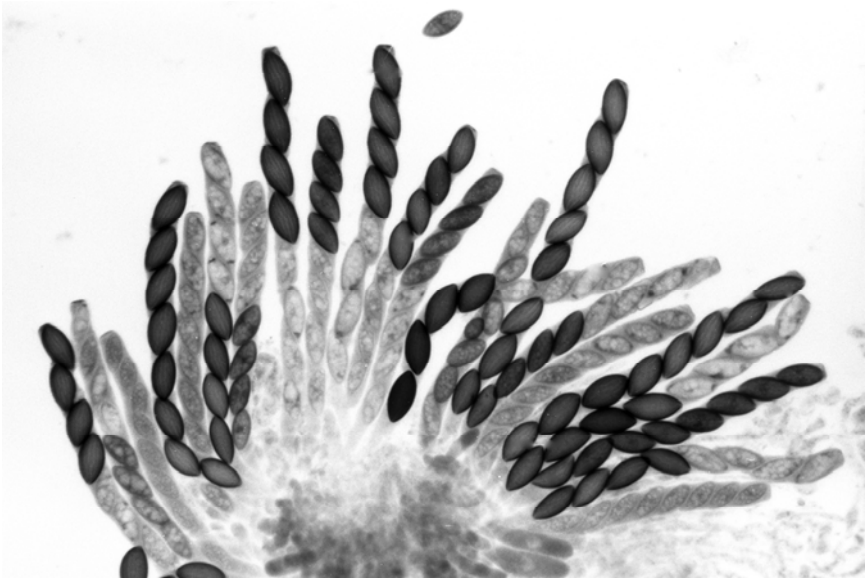

Fig 3. *N. crassa*. Reciprocal translocation x Normal. Asci containing four viable and inviable ascospores sometimes result from 3:1 segregation of the translocation quadrivalent. This segregation pattern is most prevalent in rearrangements where the translocation breakpoints are close to their respective centromeres. Photo credit: N.B. Raju.