How to use Spore killer for distinguishing 1st- and 2nd-division segregation with unordered asci.

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Background

Genetic mapping of centromeres and determining the genetic distance of markers from the centromere have from linear asci traditionally been accomplished in Neurospora by isolating ascospores from linear asci in serial order to determine the frequency of second-division segregation. In organisms with unordered tetrads, such as Saccharomyces and Chlamydomonas, a single known centromere marker is used to reveal whether alleles at another locus segregate at first or second division. The same method can be used in Neurospora, where asci are readily collected as unordered groups of eight ascospores shot from the perithecium -- a much less laborious procedure than isolating spores in order from intact asci. A centromere marker that is expressed autonomously in ungerminated ascospores would be ideal for the purpose, but the available ascospore-color mutants in Neurospora map too far from a centromere to be useful. The need is filled by *Spore killer* haplotypes. The known Spore killer complexes in *N. crassa* are completely centromere-linked, and in each ascus they result in failure of pigmentation of the four ascospores that do not carry the killer element (Turner and Perkins 1979). In crosses that are heterozygous for *Spore killer* and for a mutant marker being tested, first-division segregation of the marker produces asci in which all four surviving ascospores carry either the mutant gene or its wild type allele, while second-division segregation produces asci in which two of the survivors are mutant and two are wild type. Use of *Spore killer* thus reduces many-fold the labor involved in obtaining tetrad data that would otherwise require ordered dissection of intact asci: Obtaining ejected groups of eight ascospores is much faster and easier than isolating intact linear asci, while at the same time *Spore killer* reduces by half the number of ascospores to be tested in each ascus. Separating the four surviving ascospores from each ejected group is nearly as quick as picking up random ascospores (Perkins *et al.* 1986).

Procedure

Spore killer strains in four Neurospora species are available from the Fungal Genetics Stock Center (FGSC Catalog, section headed *Spore killer tester strains*). *Sk-2* A (FGSC 3114) and *Sk-2* a (FGSC 3115) or *fl; Sk-2* A (FGSC 3297) and *fl; Sk-2* a (FGSC 3298) are recommended for use in *N. crassa*.

Crosses are made in petri dishes. Shot asci are collected on an agar surface and progeny are obtained from viable ascospores as described by Perkins *et al.* (1986) or in *How to obtain asci as unordered groups of ascospores ejected from the perithecium*. In addition to octets with all four black ascospores viable, asci in which only three spores germinate can be used in calculating second division segregation frequencies.

The number of viable ascospores in crosses heterozygous for *Spore killer* can be reduced from four to two by using the *Four spore* mutants *Fsp-1* and *Fsp-2*, which omit a postmeiotic mitosis and form ascospores that contain the four immediate products of meiosis (Raju 1986; see example in Perkins *et al.* 1986). Double-mutant *Fsp-2; Fsp-1* strains are available in both mating types (FGSC 5069 A, 5070 a). Although use of the *Fsp* mutants potentially cuts labor in half, redundancy in eight-spored asci may be an advantage if germination is low and asci are preferred that have four nonaborted ascospores rather than the two that are obtained when the mutant *Fsp* alleles are present.

References

Perkins, D. D., N. B. Raju, V. C. Pollard, J. L. Campbell, and A. M. Richman. 1986. Use of Neurospora *Spore killer* strains to obtain centromere linkage data without dissecting asci. Can. J. Genet. Cytol. 28: 971-981.

Raju, N. B. 1986. Ascus development in two temperature sensitive *Four-spore* mutants of *Neurospora crassa*. Can. J. Genet. Cytol. 28. 982-990.

Turner, B. C., and D. D. Perkins. 1979. *Spore killer*, a chromosomal factor in Neurospora that kills meiotic products not containing it. Genetics 93: 587-606.

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