How to minimize "bubble-ascus" abortion in crosses for cytology.

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Background

Because *Neurospora crassa* is an outbreeding species, recessive mutations of genes that are expressed only in the diploid or dikaryotic sexual phase are carried in heterozygous condition and rarely exposed to selection. Leslie and Raju (1985, Raju and Leslie 1992) have shown that vegetatively normal haploid isolates from natural populations carry on average one or more deleterious recessive mutations that can be detected when made homozygous by backcrossing.

In constructing the widely used Oak Ridge *N. crassa* wild type strains for use as standards, backcrosses were made repeatedly to the same recurrent parent in an effort to maximize isogenicity (see Newmeyer *et al*. 1987). In so doing, a recessive gene (or genes) must have been made homozygous that results in abortion of a majority of developing asci. The abortion follows a typically pattern. Development is normal until ascospore walls are laid down. Nuclei then become abnormal and the eight ascospores vacuolate and shrivel until they look like small bubbles in a shrunken ascus (Figure 1). Because new croziers and asci are formed continuously, the aborted 'bubble' asci are replaced and overall productivity of the perithecium is not greatly reduced. Ratios of segregating chromosomal genes are unaltered.

In contrast to crosses between the inbred standards, bubble-ascus frequencies are greatly reduced in outcrosses between Oak Ridge and unrelated strains, as well as between different wild-collected strains (Figure 2). Bubble asci are completely absent in homothallic species of *Neurospora* and in the inbreeding pseudohomothallic species *N. tetrasperma*.

Bubble asci do not impair the usefulness of Oak Ridge-background strains for mating type tests, for genetic analysis, or for cytological studies involving meiosis and early ascus development. Bubble asci are disadvantagious, however, in studies that depend on the production of mature asci with pigmented ascospores. This would include crosses carried out for the purpose of detecting and analyzing gross chromosome rearrangements (Perkins 1974), Spore killer meiotic drive elements (Raju 1979), or mutations that affect ascus development (Raju 1992), and also for crosses to determine whether GFP-tagged genes are expressed or silenced during ascus development (Freitag et al. 2004), or to document ascus development photographically (Raju 1980).

Procedure for crosses to avoid bubble asci

Rockefeller-Lindegren (RL) wild types are sufficiently different from the Oak Ridge (OR) standards so that crosses between the two are relatively free of bubble asci. For most purposes, it is convenient to use the highly fertile aconidiate *fluffy* RL strains as female parents Outcrosses of OR-background strains with other wild type strains would be equally effective in minimizing the occurrence of bubble asci. Strains *fl*(RL)*A* (FGSC

6682) and *fl*(RL)*a* (FGSC 6683) have been widely used as testers when it is desired to avoid bubble asci in crosses with strains of OR background (Perkins and Pollard 1989)..

The RL strains differ from OR in genes that determine heterokaryon incompatibility. *fl* testers in Oak Ridge background (FGSC 4317, 4347) (Perkins et al. 1989) should therefore be used in crosses where progeny are to be retained, to ensure that the progeny are of OR heterokaryon-incompatibility genotype.

References

Freitag, M., P. C. Hickey, N. B. Raju, E. U. Selker, and N D. Read. 2004. GFP as a tool to analyse cytoskeletal function and nuclear organization and dynamics in *Neurospora crassa*. (in preparation)

Leslie, J. F., and N. B. Raju. 1985. Recessive mutations from natural populations of Neurospora crassa that are expressed in the sexual diplophase. Genetics 111: 759-777.

Newmeyer, D., D. D. Perkins, and E. G. Barry 1987. An annotated pedigree of *Neurospora crassa* laboratory wild types. showing the probable origin of the nucleolus satellite and showing that certain stocks are not authentic. Fungal Genet. Newslett. 34: 46-51.

Perkins, D. D. 1974. The manifestation of chromosome rearrangements in unordered asci of Neurospora. Genetics 77: 459-489.

Perkins, D. D., and V. C. Pollard 1989. Alternate *fluffy* testers for detecting and diagnosing chromosome rearrangements in *Neurospora crassa*. Fungal Genet. Newslett. 36: 63-64.

Perkins, D. D., B. C. Turner, V. C. Pollard, and A. Fairfield 1989. Neurospora strains incorporating *fluffy*, and their use as testers. Fungal Genet. Newslett. 36: 64-67.

Raju, N. B. 1979. Cytogenetic behavior of Spore killer genes in Neurospora. Genetics 93: 607-623.

Raju N. B. 1980. Meiosis and ascospore genesis in Neurospora. European J. Cell Biol. 23: 208-223.

Raju, N. B. 1992. Genetic control of the sexual cycle in Neurospora. Mycol. Res. 96: 241-262.

Raju, N. B., and J. F. Leslie. 1992. Cytology of recessive sexual-phase mutants from wild strains of *Neurospora crassa*. Genome 35: 815-826.

Raju, N. B., D. D. Perkins, and D. Newmeyer 1987. Genetically determined nonselective abortion of asci in *Neurospora crassa*. Can. J. Bot. 65: 1539-1549.

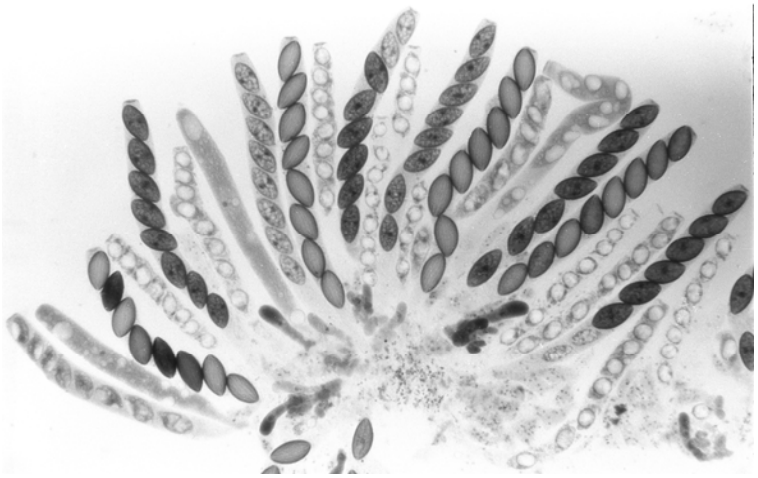

Fig 1. *N. crassa*. Oak Ridge x Oak Ridge. A rosette of maturing asci from a cross between two inbred strains. ~50% of asci have aborted after ascospore delimitation as “bubble asci”. The just-delimited ascospores in these asci shrivel and shrink. Photo credit: N.B. Raju.

Fig 2. *N. crassa*. Houma x Houma. A rosette of maturing asci from a cross of unrelated wild strains that have not been inbred. Note that all asci are developing and maturing normally – no bubble asci. Photo credit: N.B. Raju.