How to prepare ascus rosettes for microscopic examination.

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Background

Direct observation of ascus rosettes from individual perithecia is useful for confirming that parental genomes in a cross do not differ by a gross rearrangement or other anomaly. First- and second-division segregation frequencies can be determined visually for genes that are expressed autonomously in the ascospores. Crosses heterozygous for Spore killers or ascospore color genes can be distinguished from chromosome rearrangements that produce 50% aborted ascospores (Raju 1979, 1994). The effect of mutations affecting meiosis and ascus development can be seen (Raju 1992). The frequency of bubble asci, characteristic of inbred crosses, can be determined (Raju *et al,* 1987). The effects of meiotic silencing by unpaired DNA (MSUD) and the expression of GFP-tagged genes in the developing asci can be examined (Shiu *et al,* 2001; Freitag *et al,* 2004). [However, because mature asci with aborted duplication/deficiency ascospores cannot be distinguished from immature normal asci, detection of heterozygous chromosome rearrangements and the preliminary diagnosis of the rearrangement type (*e.g.*, reciprocal *vs*. terminal or insertional translocation) can be accomplished more efficiently using unordered shot asci ejected rather than intact asci in rosettes (Perkins 1974).]

Procedure

For opening rosettes of maturing asci, use perithecia that have just ejected a few ascospores. At 25°C, this will be 8 to 10 days after fertilization. (Older perithecia will contain many empty ascus sacs.) Crosses between highly inbred strains such as OR23-1A and ORS6a produce a high proportion of aborted 'bubble' asci in which all eight ascospores vacuolate and degenerate (Raju *et al.* 1987). Although unaesthetic, such crosses can provide necessary information but outcrosses with low bubble-ascus frequencies are preferred. RL testers are available that give few bubble asci when crossed with strains in the OR background (Perkins and Pollard 1989).

Staining is helpful for resolving individual asci in the rosette. Place 8 -10 perithecia on a microscope slide in a drop of very dilute ferric acetate (mordant) solution (1/10th the concentration used for nuclear staining; Raju and Newmeyer 1977). Two short steel sewing

needles are used, mounted in short holders. One is sharp, the other blunt (chisel shaped from a sharpening stone). At 50× magnification under a dissection microscope, hold a perithecium in place with the blunt needle, cut it open along the length of the perithecial beak with the sharp needle, and press the contents out. Open 8-10 perithecia and remove the perithecial walls and other debris. The perithecial contents usually come out as a large rosette of 100 to 200 asci. Use both needles to divide each rosette into 2 to 4 pieces, then add 1-2 drops of dilute hematoxylin ($1/10^{th}$ the concentration used for nuclear staining) and immediately mix the rosettes into the stain. Collect the fragmented rosettes toward the center of the drop, place a cover glass, and gently blot out the excess stain under a filter paper, taking care not to let the cover glass slide on the rosettes. Seal the edges of the cover glass with dental wax (use heated bent paper clip) or nail polish. Examine the asci at 100-200×.

If hematoxylin or mordant is not available, an alternative is Lactophenol-Cotton Blue mounting medium, which is routinely used by mycologists for examination of colorless specimens. *Formula*: Phenol (crystals) 20 g, Poirrier's (Cotton) Blue 0.25 g, Lactic Acid 16 ml, Glycerol 15.5 ml, Distilled water 40-50 m. Dissect perithecia in a drop of Lacophenool-Cotton Blue and proceed as described above. Sealing is unnecessary if the specimens are to be examined within ~2 hours.

References

Freitag, M., P. C. Hickey, N. B. Raju, E. U. Selker, and N. D. Read. 2004. GFP as a tool to analyze the organization, dynamics and function of nuclei and microtubules in *Neurospora crassa*. Fung. Genet. Biol. 41: 897-910.

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. Newsl. 36:63-64.

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. Eur. 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. 1994. Ascomycete Spore killers: Chromosomal elements that distort genetic ratios among the products of meiosis. Mycologia 86: 461-473.

Raju, N. B., and D. Newmeyer. 1977. Giant ascospores and abnormal croziers in a mutant of *Neurospora crassa*. Exp. Mycol. 1: 152-165.

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

Shiu, P. K. T., N. B. Raju, D. Zickler, and R. L. Metzenberg. 2001. Meiotic silencing by unpaired DNA. Cell 107: 905-916.

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