How to obtain asci as unordered groups of ascospores ejected from the perithecium.

Background

The ascospores of each individual ascus represent the tetrad of products resulting from a single meiosis. Using data from all four products (tetrad analysis) provides information on crossing over, gene conversion, segregation, and meiotic drive, including information that cannot be obtained (or cannot be obtained readily) using random meiotic products. In Neurospora, the position of products in the intact linear ascus may be noted (ordered tetrads) or data may be obtained from ejected groups or eight ascospores in which the original order is unknown (unordered tetrads).

The constitution of spores in intact, ordered asci can reveal whether markers segregated at the first or second meiotic division, and whether sister ascospores are identical. This information is lost when unordered asci are used. However,, knowing the linear order of spores in the ascus is not necessary for most purposes. Essential information on linkage and crossing over can be obtained far less laboriously if unordered shot asci are used. Unordered shot asci are, in fact, more reliable than ordered asci for diagnosing chromosome rearrangements, because ejection of ascosores from the perithecium does not occur until viable ascospores have matured enough to become pigmented (Perkins 1974). (Ascus development is asynchronous in Neurospora. When perithecia are opened to observe patterns of ascospore abortion, asci with eight spores that are unpigmented and inviable because of meiotically generated segmental deficiencies cannot usually be distinguished from asci with eight potentially viable spores that are unpigmented only because they are not yet mature.)

Shot asci were used by Shear and Dodge (1927) to establish that mating-type genes of *N. crassa* show Mendelian segregation. However, when Beadle and Tatum obtained the first biochemical mutants in 1941, they employed ordered asci which, in addtition to providing a direct demonstration of Mendelian segregation, allowed them to determine centromere distances Ejected, unordered asci were not used again by Neurospora workers until 30 years after Shear and Dodge, when Strickland (1960) demonstrated their usefulness. Subsequently, shot asci proved to be indispensable for large-scale studies of meiotic recombination (e.g., Perkins 1962, Bole-Gowda *et al.* 1962) and chromosome rearrangements (Perkins 1974). In some situations, they are also useful for genetic mapping (Perkins 1986). When shot asci are used in combination with a marker that is known to segregate at the first meiotic division, unordered asci become nearly as informative for tetrad analysis as ordered asci, although they require only a fraction of the effort (Perkins *et al.* 1986).

Procedure for using unordered asci

Crosses are made in petri dishes, which are kept inverted throughout incubation and during the collection of asci. Best results are obtained if perithecial beaks are not bent. Since beaks are directed toward a light source, incident light during incubation should come from directly below the inverted plates rather than from one side. Plates are placed in the dark for 12 to 24 hours before asci are collected. Ascus shooting is slow when dark-incubated plates are first brought into the light. Shooting accelerates for the next hour or two, then falls off rapidly. Shooting resumes and asci can be collected again after plates have been incubated overnight in the dark.

Groups of eight ascospores are collected on a 4% water-agar slab placed on a microscope slide that is raised on an underlying stack of slides so that the collecting surface is within 1 mm of the ostioles. Unlike asci of *Ascobolus immersus*, the eight ascospores of Neurospora asci are not held together as a unit after being ejected Ascospore-scatter is a function of distance from the ostiole. Distance from beak to collecting surface is thus important. Each slab is exposed for a period ranging from a few seconds to several minutes, depending on the rate of shooting.

If the component ascospores are to be germinated to obtain progeny, well separated groups of eight are removed from the collecting slab under 40-60× magnification. A flattened platinum-iridium blade is used to lift out a piece of agar that bears the entire group. (See *How to make tapered platinum-iridium needles and use them for isolating ascospores*.) Each octad-bearing agar piece is placed face-up on the surface of the storage medium. The 4% agar storage medium (Metzenberg 1988) is designed to prevent overgrowth by hyphae from a spontaneously germinating ascospore or from contaminating parental vegetative cells or airborne contaminants. Fifty or more groups can be aligned in each storage plate. Storage plates are incubated 7 to 10 days at 25° or 30°C (not higher). The eight component ascospores are then separated, isolated to individual 10 × 75 mm tubes, and heat-shocked 30 minutes in a 60°C water both. With at least some genotypes, improved germination is obtained after longer incubation. Storage plates can be kept at 30° for a month if wrapped in plastic to prevent evaporation.

Ascospores for tubing are separated by using the platinum-iridium blade to lift the octad-bearing piece of agar and place it face-down on a 4% agar surface. The blade is then used to slide the inverted agar block to one side, leaving the ascospores stranded behind it on the flat underlying surface. This is best achieved if the underlying agar is not too moist. Trial and error will reveal whether drying of the surface is optimal. Individual ascospores are then teased to positions that are widely enough spaced so that pieces of agar each bearing a single ascospore can be picked up and tubed, just as is done when ascospores are isolated at random. The tubes are then ready for heatshock.

A preliminary diagnosis of chromosome rearrangements can be achieved simply by classifying shot asci in situ for the numbers of aborted ascospores, without any need to isolate or germinate ascospores. Shot octads are observed under 60× magnification, with light transmitted from above and below and intensities optimally balanced for distinguishing defective ascospore pigmentation. Asci are classified according to the number of ascospores that are defective in pigmentation (8 Black:0 White, 6B:2W, 4B:4W, 2B:6W, or 0B:8W). Because ascospores containing deficiencies are inviable and fail to become pigmented, crosses heterozygous for reciprocal translocations result in unordered-ascus frequency distributions that are symmetrical around 4B:4W, while insertional and terminal or quasiterminal translocations result in distributions that are symmetrical around 6B:2W (Perkins 1974, 1986). See *How to recognize and diagnose chromosome rearrangements*.

If the objective of a cross is only to score asci for patterns of ascus abortion, it is advantageous to use a *fluffy* strain as protoperithecial parent, ensuring that conidia do not block the path of ejected asci. The *fluffy* mutation is disadvantageous, however, if the intention is to age and germinate shot ascospores and obtain progeny for analysis. Ascospores containing *fluffy* tend to germinate spontaneously, and if this occurs during ageing, they will be killed when exposed to heat shock. If the genetic background of the strain being tested is Oak Ridge, a *fluffy* tester that is RL genetic background should be used, to minimize the number of bubble asci. (See *How to use fluffy testers for determining mating type and for other applications.* See *How to minimize "bubble-ascus" abortion in crosses for cytology***.**

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