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Abstract

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TEACHING NOTE

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Ordered tetrads from *fl* X *cys-3*

After experimenting with a number of crosses involving white or hyaline-spored mutants such as *asco* and *ws*, we have settled on the use of *cys-3* for production of microscopically visible ordered black/white tetrads of *N. crassa* in teaching classes of elementary genetics. As indicated previously (Perkins, 1979 Neurospora Newsletter 26: 10; Raju, 1980 Eur. J. Cell Biol. 23: 208-223), *cys-3* ascospores fail to pigment. In our experience, the lack of pigment persisted even after the optimum stage for scoring.

The specific combination used was *fl a* X *cys-3 A*. The *fl* isolate (No. H579) was obtained from Prof. Ho Coy Choke, Genetics Dept., University of Malaya and the *cys-3* isolate was FGSC #2292. The former is a good protoperithecial strain and the latter produces limited mycelium but an adequate quantity of conidia even on unsupplemented Neurospora medium. An alternative *fl a* strain (FGSC #4347) may be used.

The *fl a* parent was first inoculated onto Neurospora crossing medium (Westergaard and Mitchell, 1947 Am. J. Botany 34:573) in plastic petri dishes. After 5-6 days at laboratory temperature (23-25° C) protoperithecia and trichogynes were usually abundant. Then a conidial suspension of *cys-3 A* from l-week old cultures was prepared in tubes or flasks of sterile distilled water. Each dish of *fl a* was fertilized with about 2 ml of the conidial suspension. Excess water was decanted after agitating the dishes to ensure even coverage. It was not necessary to standardize the number of conidia in the suspension. Dishes were ready for class work 8-10 days after fertilization. By this time, black and white ascospores were seen on the inside of the petri dish lids.

Students were instructed to scrape 15-20 perithecia from the agar surface with a flattened needle, transfer them to 2-3 drops of water on a slide and to crush them en masse with sharp forceps. The perithecial debris was then discarded. A coverslip was placed over the water and perithecial contents. An index card was laid on top of the coverslip and slide and then pressed down firmly with the thumb to spread the asci. This method of slide preparation took only 1-2 minutes and obviated the tedious extraction and crushing of single perithecia from tube cultures. Usually each student needed to prepare only one slide to score 30 or more asci, with clear Division I and II patterns.

This method has been in use for the past 4 years. The centromere → *cys-3* distance has been scored consistently at 31-33 units by classes of 85-100 students. It has the added advantaae that excessive contamination of 1aboratories is avoided by the use of the nonconidiating *fluffy* isolate.

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