

Mylyk, O. M. and S. F. H. Threlkeld. The use of heterokaryon in facilitating crosses between *Neurospora* strains having reduced female fertility.

successful in making a cross between the female sterile tyrosinase mutants ty-1 and 'y-2 when ty-1 was in a heterokaryon used as a female parent and ty-2 was the male parent.

In our laboratory heterokaryon having all pairwise combinations, except one, of seven or eight different female sterile mutants were tested for their ability to produce perithecia and spores when the heterokaryon were used as female parents. Only one combination failed repeatedly, and it has not been established that the mutants are non-allelic. All other combinations produced on abundance of perithecia and ascospores, with over 95% of the crosses shooting ascospores within two weeks after fertilization. Only rarely did a cross involving an effective combination of mutants fail, probably due to a distorted nuclear ratio in the heterokaryon; further attempts in each case produced substantial numbers of perithecia and ascospores.

The female sterile mutants used are all morphologically different from the wild type. One is the mutant leu-1 (33757), in which female sterility and abnormal morphology seem to be inseparable from the biochemical requirement. One mutant has a somewhat colonial morphology at 25°C and does not grow at all at 34°C. The others, including leu-1, are subtly different from the wild type. They grow more slowly, are less pigmented, and form a more continuous culture than the wild type (the wild types 74-OR23-1A and 74-ORB-10 used for comparison form a dense band of conidia above a gap of relatively sparse aerial growth in a 10 x 75 mm tube.)

The mutants pan-2 (B3) and nit-3 (Y31881) were used as heterokaryon forcing markers, since they are non-leaky and do not seem to affect growth characteristics or fertility. The heterokaryons were grown on a Westergaard and Mitchell crossing medium having 2% sucrose and 1.5% agar. After seven days they were fertilized with a conidial suspension of one female sterile mutant or, in separate tests, with al-2 (15300). A typical cross was: (nit-3 fs-m A + pan-2 fs-n A) ♀ x fs-p a (or al-2 a) ♂ in which fs-m, fs-n and fs-p represent different female sterile mutants. Most crosses produced on abundance of ascospores, suggesting that most female sterile mutants can be transmitted through crosses when used as female parents in heterokaryon. This is supported by the fact that most of our female sterile mutants were originally detected as a result of segregation in crosses where strains used as female parents had accumulated the mutants. We tested further to see if one of the female sterile mutants could be recovered from a cross where it was present in both parent nuclei. The following was attempted: (pan-2 A + nit-3 fs A) ♀ x pan-2 fs a ♂. The nit-3 mutant was recovered among the progeny, demonstrating that the fs x fs component of the cross had taken place.

The success with which the use of heterokaryons in the above crosses resulted in the production of perithecia and ascospores suggests that progeny may be recovered from crosses between various other morphological or biochemical mutants which have reduced female fertility if heterokaryon are similarly used. In making such crosses, for instance between the mutants mut-1 and mut-2, the following format using pan-2 as an ascospore color marker is convenient: (pan-2 A + nit-3 mut-1 A) ♀ x pan-2 mut-2 a ♂. Ascospores having pan-2 do not develop pigment in crosses made on a medium lacking pantothenic acid (Threlkeld 1965 Can. J. Genet. Cytol. 7: 1). Hence all ascospores from the pan x pan component and half of those from the nit-3 mut-1 x pan-2 mut-2 component will be pole if the crosses are made on minimal medium. All of the dark spores will represent the mut-1 x mut-2 component, which is desired. The dark and pole spores can be easily distinguished on a block of agar under a dissecting microscope when illuminated from above.

To enhance the germination of nit-3 ascospores, nicotinamide can be added to cross tube at the time of fertilization. This is done by preparing the conidial suspension of the male parent in a solution of 0.04 mg/ml nicotinamide, adding approximately 1.5 ml to a 15 x 150 mm cross tube containing 5 ml of medium, and spreading the suspension by shaking the tube. If pan-2 must be recovered from such a cross, a similar amount of pantothenic acid can be added. This will cause pan ascospores to darken so they will be indistinguishable from pan⁺, but is not necessary since pole ascospores usually show reduced germination. We are depositing pan-2 and nit-3 mutants crossed into a St. Lawrence (Oak Ridge) genetic background with the Fungal Genetics Stock Center. - - - Department of Biology, McMaster University, Hamilton, Ontario, Canada. (Present address of OMM: Department of Biological Sciences, Stanford University, Stanford, California 94305).