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

Certain morphological and biochemical mutants ore female sterile, i.e., fail to produce perithecio and spores, or cross poorly when used as female parents. Reciprocal crosses between two such mutants moy either foil completely or moy be extremely slow. Our work with female sterile mutants suggests that heterokoryonr moy be effective in overcoming these difficulties in many instances. Horowitz et al. (1960 J. Mol. Biol. 2: 96) were successful in making a cross between the female sterile tyrosinase mutants ty-1 ond ty-2 when ty-1 was in a heterokaryon used as a female parent ond ty-2 was the mole parent.

In our laboratory heterokoryonr having all pairwise combinations, except one, of seven or eight different female sterile mutants were tested for their ability to produce perithecio and spores when the heterokoryonr were used as female parents. Only one combination foiled repeatedly, and it has not been established that the mutants ore non-allelic. All other combinations produced on abundance of perithecio and ascospores, with over 95% of the crosses shooting ascospores within two weeks after fertilization. Only rarely did a cross involving on effective combination of mutants foil, probably due to a distorted nuclear ratio in the heterokaryon; further attempts in each case produced substantial numbers of perithecio 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, ore subtly different from the wild type. They grow more slowly, ore less pigmented, and form a more continuous culture than the wild type (the wild types 74-OR23-1A and 74-ORB-lo used for comparison form a dense bond of conidio above a gop of relatively sparse aerial growth in a 10 x 75 mm tube.)

The mutants pan-2 (B3) ond nit-3 (Y31881) were used as heterokaryon forcing markers, since they ore non-leaky and do not seem to affect growth characteristics or fertility. The heterokaryons were grown on a Westergaard ond Mitchell crossing medium having 2% sucrose ond 1.5% agar. After seven days they were fertilized with a conidiol 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 orcosporer, suggesting that most female sterile mutants can be transmitted through crosses when used as female parents in heterokoryonr. 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 hod 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 nic-3 mutant was recovered among the progeny, demonstrating that the fs x fs component of the cross hod token place.

The success with which the use of heterokaryons in the above crosses resulted in the production of perithecio and ascospores suggests that progeny moy be recovered from crosses between various other morphological or biochemical mutants which have reduced female fertility if heterokoryonr ore similarly used. In making such crosses, for instance between the mutants mut-1 and mut-2, the following format using pan-2 as on ascospore color marker is convenient: (pan-2 A + nic-3 mut-1 A) ♀ x pan-2 mut-2 a ♂ Ascospores having pan-2lo not develop pigment in crosses mode on a medium lacking pantothenic acid (Threlkeld 965 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 crosser are mode on minimal medium. All of the dork spores will represent the mut-1 x mot-2 component, which is desired. The dark and pole spores con 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 con be added too cross tube at the time of fertilization. This is done by preparing the conidiol suspension of the mole 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 con be added. This will cause pon ascospores to darken so they will be indistinguishable from pan+, but isns necessary since pole ascospores usually show reduced germination. We ore depositing pan-2 and nit-3 mutants crossed into a St. Lawrence (Oak Ridge) genetic background with the Fungal Genetics Stock Center. - - - Deportment of Biology, McMaster University, Homilton, Ontario, Canada. (Present address of OMM: Deportment of Biological Sciences, Stanford University, Stanford, California 94305).