Metzenberg, R.L. ond S.K. Ahlgren. Hybrid strains useful in transferring genes from one species of Neurospora to another.

The investigation of natural variation in the genus Neurospora has been limited by the absolute or relative infertility of interspecific crosses. especially where it is desirable to move moderately deleterious genes, such as auxotrophic markers, from one species to another. A number of workers hove done this successfully, but we have found only sporadic and haphazard fertility in such crosses, To circumvent this difficulty, we hove developed a "transfer kit" - a

series of interspecific hybrids that allow one to move a given trait quite easily from one species to another in two or more small phylcgenetic steps, rather than one large one. The kit is of purely utilitarian value, and we have made no attempt to determine which chromosomes, or how many, are derived from a particular species. For example, in the crassa-tetrasperma gradient, the strain designation C1, T3 means only that the strain had one N. crassa grandparent and three N. tetrasperma grandparents, and does not imply that it contains N. crassa and N. tetrasperma genes in precisely that ratio. Some miscellaneous exotic strains cross reasonably well with one or more members of this gradient even though they arc infertile with both of the parent species.

The nomenclature we have adopted for these interspecific hybrids is as follows. Each hybrid is identified by letters and numbers that refer to the most recent cohort of ancestors that were not laboratory hybrids. For example, C17, S15-a had 17 N. crassa great-great-great-grandparents and 15 N. sitophilo great-great-great-grandparents. Similarly, C4, T4-a had 4 N. crassa great-grandparents and 4 N. tetrasperma great-grandparents, but some of its grandparents were hybrids.

In establishing the N. tetrasperma - N. crassa hybrid line, we plated 343.6AE (FGSC#606, actually found to be mating type a) on Westergaard-Mitchell medium (1947 Am. J. Botany 34:573). In our hands this isolate shows the highest fertility of any N. tetrasperma strain with N. crassa, and is also quite fertile with several N. intermedia strains. (Crosses of the "type" strains of N. tetrasperma, 85A and 85a (FGSC#1270 and #1271, respectively) to N. crassa did not, in our hands, give any viable spores). After 4 days at 25°C, the plate was treated with a suspension of N. crassa 74-OR23-1A (FGSC#987) conidia. After 3 weeks, a modest number of spores had collected on the lid of the Petri plate. These were suspended in water and heated at 55-60°C for 30 minutes to induce germination. Of 39 germinated spores, 37 grew into cultures of mating type A, and 2 were of mating type a. A similar assymetry has been described previously (H&e and Haysman 1966 Genetics 54: 292). The two a strains ware suspected of having a very atypical chromosome complement, and were discarded; an a strain with roughly the-desired ancestry (C4, T4-a ) was derived as described in the pedigree below. All other crosses were made by simultaneous inoculation on Westergaard-Mitchell medium. All of the N. crassa - N. tetrasperma hybrids chosen for this kit were aryl sulfataseless. The strains comprising the kit ore being placed in the collection of the Fungal Genetics Stock Center.

Many of the strains used by Dodge were retrieved from his laboratory after his death, and there was some uncertainty about their identity. As noted above, 343.6AE is actually mating type a. Earlier, H. B. Howe found this strain to contain the e allele (unpublished data). 394.5ae (FGSC#609) is a self-fertile heterocaryon. Last of all, the strain designated "N. intermedia, no #, secondarily homothallic" (FGSC#688) should be listed as Neurospora tetroi and is identical with the Centraalbureau voor Schimmelculture (CBS) stock #25935 (Barratt, personal communication) (See Tai 1935 Mycologia 27: 328 ). FGSC#688 is extremely fertile with N. tetrasperma testers, and gives the appearance of being N. tetrasperma.

We gratefully acknowledge advice from D. Novak and A. Srb, and also thank them for furnishing their strain of N. intermedia, NIT-A, (FGSC#1755) and other useful strains. (It should be noted that NIT-A does have some N. crassa ancestry; Srb prepared this strain by "carrying the mating type allele from N. crassa, conveyed by 10 generations of backcross, to N. intermedia". The original hybrid was between N. crassa and NIT-a (FGSC#1754). Hence, in our nomenclature, NIT-A would be C1, 12047-A).

We are likewise indebted to R. H. Davis for N. sitophila 3A. He and M. Grindle have described its origin as follows. "N. sitophilo 2a and 3A were kindly provided by J. Fincham. Strain 2a was pure N. sitophilo, while 3A was a third-generation backcross of an N. crassa x N. sitophilo hybrid to N. sitophila 2a". Fincham obtained the latter strain from H. L. K. Whitehouse (1942 New Phytologist 41: 23). Whitehouse obtained it from J. Ramsbottom and F. L. Stephens (1935 Trans. Brit. Mycol. Soc, 19: 215), who, in turn, got it from W. H. Wilkins, who found it growing on beech battens in a lumberyard kiln in Chichester, Great Britain, in 1933. In our nomenclature, N. sitophila 3A would be called C1, S15-A.

The origin of the new hybrid stocks is as follows:

N. tetrasperma 343.6AE x N. crassa 74-OR24-1A → C1, T1-A.

N. tetrasperma 343.6AE x C1, T1-A → C1, T3-A and C1, T3-a

C1, T3A x C3, T1-a → C4, T4-a.

N. crassa 74-OR8-1a x N. intermedia NIT-A → C2049, 12047-a and C2049, 12047-A.

N. crassa 74-ORE-1a x N. sitophila 3A → C17, S15-a.

The new stocks which have been deposited in the Fungal Genetics Stock Center collection have been assigned the following numbers: N. sitophila 3A (FGSC#1769); C1, T1-A (FGSC#1770); C1, T3-A (FGSC#1771); C1, T3-a (FGSC#1772); C3, T1-A (FGSC#1773); C3, T1-a (FGSC#1774); C2049, 12047-A (FGSC#1775); C2049, 12047-a (FGSC#1776); C17, S15-a (FGSC#1777); C4, T4-a (FGSC#1778); N. sitophila-2a (FGSC#1779).

- - - Department of Physiological Chemistry, University of Wisconsin, School of Medicine, Madison, Wisconsin 53706.