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Abstract

Further information on the origin of the Yale and Oak Ridge wild-type strains of *Neurospora crassa*

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Studier on series of alleles in N. crassa before 1953 were usually made on mutants induced in many different wild-type strains. The development of the filtration-concentration technique (Fries 1947 Nature 159: 199; Woodward, de Zeeuw and Srb 1954 Proc. Natl. Acad. Sci. U. S. 40: 192; Cotcheside 1954 J. Gen. Microbial. 11: 34) made it possible to avoid the complication of differences in genetic background and to obtain series of allelic mutants induced in the same genetic background for genetic analysis. The cytological studier of St. Lawrence (1953 Ph. D. Thesis, Columbia University) and Singleton (1948 Ph. D. Thesis, California Institute of Technology) reported various meiotic abnormalities in crosses of the standard Yale wild-type strains (SY 7A x SY 4a) and the Abbott wild-type strains (Abbott 4A x Abbott 12a), respectively. McClintock's studier on the Cal. Tech. wild-type strain 2292-2A (McClintock 1955 Carnegie Inst. Washington Yearbook 53: 254) showed that it carried a structurally altered chromosome 5, resulting from the addition or insertion of a segment of uncertain origin into one of the arms so that it was much larger than its normal homologue and comparable in total length to chromosome 3.

There studier demonstrated a need for a new wild-type strain as a starting point in filtration-concentration experiments to avoid the complications in genetic analysis brought about by the presence of undetected structural modifications of chromosomes presumed to be normal in their chromosome constitution. The development of new wild-type strains was undertaken by St. Lawrence (op. cit.) and at Yale University two new wild-type strains ST 74A (St. Lawrence A standard) and ST 73a (St. Lawrence a standard) (that she derived by morphological and cytological selection of progeny from intercrosses of Emerson's wild-type strains E 5256A and E 5297a (see Barratt 1962 NN#2: 24)) were obtained for use in the mutant screening programs that were started early in 1953. In these experiments mutants were induced in ST 74A and f_1 progeny were obtained from a cross to ST 73a.

In the heterocaryon tests mode to distinguish mutants with identical biochemical requirements, however, problems developed with the use of the f_1 progeny from crosses to ST 73a because of the segregation of heterocoryon-incompatibility genes. Many f_1 progeny would not form heterocaryons with standard tester strains of mating type A that originated in ST 74A. To avoid this difficulty, an inbreeding program was initiated to replace ST 73a with a mating type a wild-type strain which was as nearly like ST 74A as possible with respect to genes controlling heterocaryon formation and growth. A spontaneous pan-2 mutant (74A-Y153-M96) from ST 74A was crossed to ST 73a. The inbreeding program consisted of two successive backcrosses of a mating type a, pan-2 isolate with ST 74A (Fig. 1). A pan-2 mutant of spontaneous origin, rather than one induced, in ST 74A was chosen for the backcrossing program on the assumption that such a mutant was less likely to possess changer at other loci in the genome. The heterocaryon reactions of pan-2 isolates were checked in each generation with different biochemical mutants induced in ST 74A until no further segregation of heterocaryon-incompatibility genes was indicated.

In the f_1 generation pan-2+ isolates were tested with an ad-8 mutant of mating type A that originated in ST 74A (74A-Y152-M47) and one isolate (74-YU371-11.7a) which responded to give a slow-growing bisexual heterocaryon was chosen for further bockcrossing to ST 74A. The segregation of heterocoryon-incompatibility genes in this generation was clearly indicated since some of the pan-2 isolates did not respond in heterocaryon tests with two other testers: (1) a pan-1 mutant of mating type A that originated in ST 74A (74A-Y164-M65) and (2) an ad-4 mutant of mating type a (74-YU390-9a) that resulted from a cross of a mating type A mutant that originated in ST 74A with ST 73a. All f2 progeny from the backcross of 74-YU371-11.7a to ST 74A formed heterocaryons with the pan-1 and ad-4 testers, but differences in the linear growth rates of the heterocaryons were found. The response of bisexual heterocaryons, however, was more uniform between the a isolates and the mating type A pan-1 tester than between the A isolates and the mating type a ad-4 tester (perhaps indicating that 74-YU390-9a was not completely isogenic with ST 74A). One pan-2 isolate (74-YU387-1.7a) which gave wild-type growth rate with the ad-4 tester and a slow-growing bisexual heterocaryon with the pan-1 tester was backcrossed to ST 74A. In the f_3 generation, all heterocaryon tests of the progeny with the pan-1 and ad-4 testers were uniform showing that there was no further segregation of heterocaryon-incompatibility genes in this generation. To verify this conclusion progeny from the f_3 generation were crossed to other biochemical mutants of spontaneous origin recovered in filtration-concentration experiments an ST 74A. In all cases the heterocaryon responses of the progeny from the f_4 generation were like the responses of the f3 generation. Since there was no evidence of further segregation of heterocaryon-incompatibility genes in either generation, two wild-type strains (pan-2+) were selected from those asci in the f3 generation where the pan-2 segregants hod been tested. For convenience, these strains, 74-YU392-3.1a and 74-YU392-5.5A have been referred to as 3.1a and 5.5A , respectively.

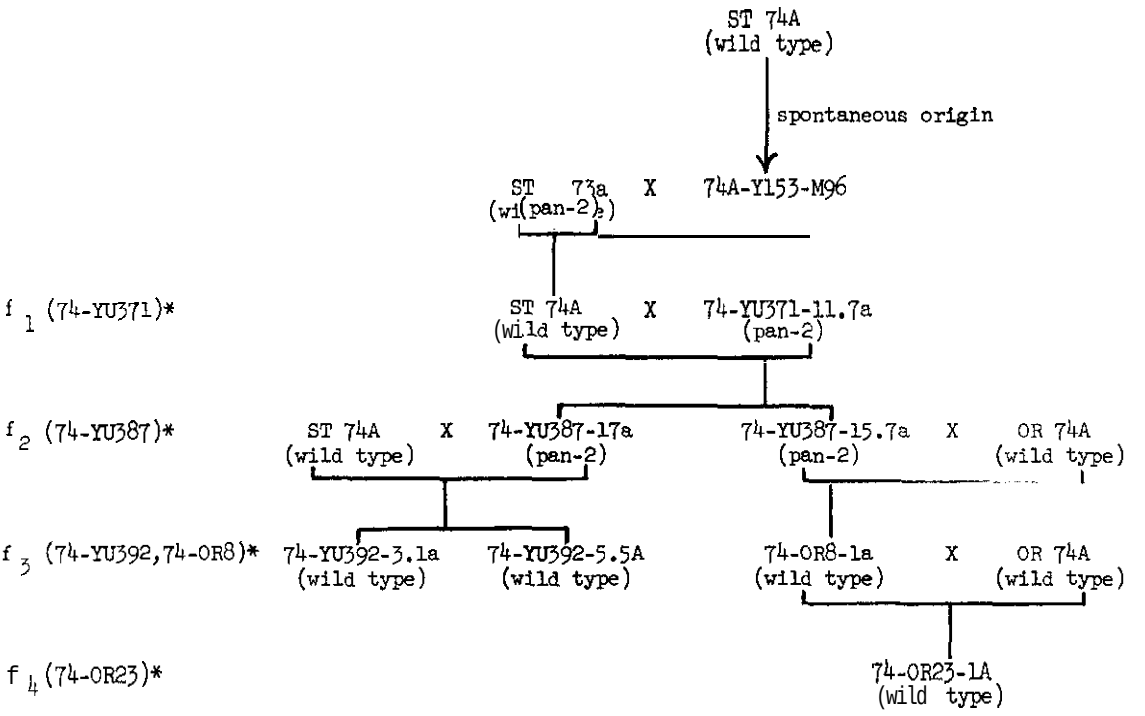
A parallel analysis of f3 progeny from a backcross of a pan-2 strain (74-YU387-15.7a) with the Oak Ridge conidial isolate of ST 74A (OR 74A) gave similar results. In this case no segregation of heterocaryon-compatibility genes was found among the pan-2 progeny in heterocaryon tests with mutants induced in OR 74A or other inbred mating type a testers. A wild-type strain was selected from the f_3 progeny of this cross to provide a new mating type a wild-type standard at C. Ik Ridge (74-OR8-1a).

Early in 1960 we noticed that there was an unusually high percentage of tan ond white ascospores (ca. 11%) and lower ascospore germination (ca. 60%) in backcrosses of mutants induced in OR 74A, or in wild-type OR 74A, with 74-OR8-1a. These and other data clearly implicated the Oak Ridge isolate of ST 74A which hod somehow changed during the course of vegetative transfer sometime prior to 1959. To obtain a replacement for OR 74A a backcross was made to 74-OR8-1a to obtain a mating type A wild-type strain that would give high fertility and a high percentage of black ascospores. f_4 progeny (Fig. 1) were backcrossed to 74-OR8-1a, and the percentages of white, tan and block ascospores in each cross were determined by making counts on suspensions in a hemocytometer with transmitted light at 90x magnification. Several mating type A isolates were obtained from this cross that gave very low percentages of tan or white ascospores, and one was chosen (74-OR23-1A) that gave 398 black, 2 ton and 0 white ascospores in a total of 400 counted.

The following pedigree of the Yale ond Oak Ridge derivatives of the original St. Lawrence wild-type strains corrects and extends that given by Barratt in Neurospora Newsletter #2.

Figure 1

Pedigree of Yale (YU) and Oak Ridge (OR) wild-type strains of *N. crassa*. (* = cross numbers in each generation.)



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