Lacy, A. M. Attempts to increase fertility in interallelic crosses.

Initial attempts at fine structure analysis of the td (tryp-3) locus were frustrated by the almost complete lack of spore production from crosses between td mutants. A number of more or less rationally based methods for increasing fertility have been tried and have met with varying degrees of success (Lacy 1959 Ph. D. Thesis. Yale University). Some of them are presented here for the possible benefit of those with

Neurospora fertility problems. (Not all of these methods were original in our laboratory; some of them were worked out by other people studying interallelic crosses at other loci.)

The obvious remedy of adding high concentrations of L-tryptophan (up to 500γ/ml.) to the Westergaard and Mitchell synthetic crossing medium (Sx) was not successful in promoting crossing between the original td mutant stocks. The excess tryptophan proved extremely inhibitory to the growth of these strains.

The use of suppressed td mutants as female parents further decreased, rather than increased, fertility. The same technique had been used without success in crosses between pyrimidine-3 mutants (Mitchell and Mitchell 1956 Genetics 41:319).

Attempts to remedy the situation by raising the general level of Neurospora fertility led to experiments on the effect of day length on crossing. While crosses seemed to progress more satisfactorily under conditions of alternating light and dark periods than under conditions of continuous light or continuous darkness, the effects were not dramatic enough to be of practical use.

A number of modifications of the basic Sx medium were tried: (1) Tryptophan-containing peptides (such as glycyl-tryptophan, leucyl-tryptophan) were added to Sx in concentrations such that the tryptophan moiety equalled 100-200γ/ml. Some tubes were further supplemented with free L-tryptophan. No increase in fertility was observed. (2) Small amounts (0.25%) of tryptone, tryptose, proteose-peptone, or N-Z case were added to Sx. No increase in fertility was observed. (3) Low sulfate Sx (supplemented with 200γ L-tryptophan/ml.), in which the $MgSO_4$ was replaced by $MgCl_2$, did not promote crossing although it had proved useful in obtaining complete asci from crosses between pan-2 mutants (Case 1957 Ph. D. Thesis, Yale University). (4) Lowering the sucrose concentration in Sx from 2.0% to 0.2% promoted fertility in crosses between pyrimidine mutants (Suyama, Woodward and Sarachek 1958 Microbial Genet. Bull. 16:29) but did not increase the fertility of td mutant crosses. (5) Switching from Sx to Difco corn meal agar (at pH 4-5 and also at pH 6.5-7), with and without added tryptophan, did not lead to improved fertility of td crosses.

Incubation of "male" conidia in a solution of 400γ L-tryptophan/ml. for a few hours at 30° C before crossing gave inconclusive results. However, addition of L-tryptophan solution directly to the slants at the time of crossing (giving a total increase of 80γ/ml. of medium) promoted spore production in crosses between some of the td mutants. A variation of this technique gave some increase in spore production (although not in spore viability) from crosses between arginine mutants (Newmeyer 1957 J. Gen. Microbiol. 16:449).

The best results in promoting fertility were obtained by the more laborious method of crossing the mutants to wild type ST74A and selecting the most fertile mutant isolates, recrossing and again selecting the most fertile isolates, and so forth. These more fertile strains also proved to be more tolerant to high concentrations of tryptophan, and their fertility was further increased by the addition of up to 600γ or more of L-tryptophan/ml. The optimal concentration varied between 300-600γ/ml. depending on the particular strains. For the fertility of these tryptophan tolerant strains, the total tryptophan concentration was important, but not the time of addition. For example, in a cross of td7 x td1, a total concentration of 400γ L-tryptophan/ml. was optimal, regardless of what proportion of the tryptophan was added during the preparation of Sx and what proportion at the time of crossing. Concentrations of 200γ/ml. + 200γ/ml., 300γ/ml. + 100γ/ml., and 400γ/ml. + 0γ/ml. all resulted in nearly wild type levels of spore production, while other combinations giving higher or lower total tryptophan concentrations resulted in lower spore production.

It is encouraging to note that some td mutants recently induced in derivatives of the original ST74A strain are quite fertile without extensive back-crossing even when crossed on Sx containing as little as 200γ L-tryptophan/ml. - - - Department of Biological Sciences, Goucher College, Towson, Maryland.