A clue to the cause of acquired female sterility in fluffy mutant strains of Neurospora crassa.

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Abstract
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1. We have omitted the DEPC in step 4 on occasion with no ill effect.
2. Smaller quantities of log phase mycelia may also be processed by this protocol. It is not necessary to scale down solution volumes for pads half the size used here. In general, we no longer bother to accurately measure the conidial inoculum and simply harvest one 14 x 150 Vogel's slant tube to inoculate.

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A clue to the cause of acquired female sterility in fluffy mutant strains of *Neurospora crassa*

Mutants at the *fl* locus produce no macroconidia and some isolates, attributed with high female fertility, are for this reason recommended as mating type testers in plates (e.g. Perkins et al. 1962 Can J. Genet. Cytol. 4:187-205; Davis and de Serres 1970 Meth. Enz. 17A:79-143). It is not mentioned that such strains may suddenly lose their fertility. Sudden female sterility of *fl* strains has, however also been a problem in many *Neurospora* laboratories. It should, however, also be noted that part of the difficulties may be avoided by adopting certain improvements in the stockkeeping procedures. By keeping fluffy stocks in suspended animation on anhydrous silica gel and renewing working stocks from this source, David Perkins (personal communication) has used fluffy strains for many years without experiencing loss of fertility.

We have reported on some *fl* alleles, *fl*(blo) ("bleak orange") which arose spontaneously in the wild type 74-0R23-1A (Kolmark and Veenhuizen 1984 Hereditas 101:277). The *fl*(blo) strains were found very satisfactory as mating type testers. We have re-isolated these strains through several backcrosses to the wild types 74-ORS A and a (FGSC 2489 and 4200, resp.) and improved their usability as testers by selecting phenotypes with flat growth and no "creeping" under the lid at the plate edge, i.e. reduced aerial hyphae on crossing medium (Westergaard and Mitchell 1947 Am. J. Bot. 34:573-577). For the testings we find that it is convenient to use square plastic plates, 10 x 10 x 2 cm. Conidialess strains are tested by direct transfer. Conidiating strains can be tested using a drop of suspension, but it is labor saving to transfer the conidia directly with a piece of wetted filter paper. We are using circular discs, 5 mm diameter (made with a page puncher) for that purpose. When the tests are made on 5 days prestarted testers the readings can in most cases be made after 1 or 2 days, all at 25°C.

We have in some cases found that female fertility of the *fl*(blo) isolates deteriorated similarly as experienced with other *fl* alleles received from the Fungal Genetics Stock Center. More recently we have found a genetic segregation into high and low female fertility among the offspring from one *fl*(blo) strain: Cross no. 1.184: 1.126-2 A *fl*(blo) X 74-ORS a wild type.

The isolates from 2 dissected asci, each with 8 germinated spores, were tested for fertility using 15 x 2 cm test tubes. The isolates, previously mt-tested, were prestarted and suspensions of equal densities of conidia from crisp mutant strains were added at day 6. In ascus no. 2 was found a Mendelian segregation for "high" and "low" fertility independently of whether *fl*(blo) were present as + or -, while in ascus no. 9 all spore pairs were of the "high" female fertility type. Replicability was excellent within spore pairs.

| Spore-pair | 1,2 | 3,4 | 5,6 | 7,8 |
|------------|-----|-----|-----|-----|
| Ascus no.  | A/a blo fert | A/a blo fert | A/a blo fert | A/a blo fert |
| 1.184-2    | a + low | a + high | A - low | A - high |
| -9         | A + high | A - high | a + high | a - high |

Although all isolates in ascus no.9 were of the "high" fertility type, it was noticed that the time of appearance of the protoperithecia was unequal, e.g. there was a considerable delay in the blo^+ spore pair 1,2.
Since we have not noticed any "low" fertility segregants among offspring from 74-ORS (A,a) in other crosses, this type most likely comes from the (blo) parent. Possibly it was present as a mutant from "high" to "low" fertility at the time when cross 1-184 was made (prestarted with 1.126-2 A fl (blo)). This assumption is supported since it was found shortly later that the strain had deteriorated in its female fertility.

We assumed that a cross between the high fertility isolates from ascus 1-184-2 might eliminate the low fertility gene. This was realized in cross no. 1.190. One isolate from each of 8 different fl(blo) spore pairs (obtained in 7 dissected asci) were tested for female fertility in plates, and all were found to be of the "high" fertility type.

According to these findings it may be possible to rescue other fl alleles to their former high fertility through crosses with a standard wild type strain.

We are using another precautionary measure by keeping a cross between two highly fertile fl(blo) strains (selected with 74-ORS background) in the refrigerator. So far all ascospores isolated from this cross have been of the high fertility type.

It is realized that these methods do not keep the strains absolutely isogenic if this is very important.

Since female fertility/sterility depends on a one-gene difference in this system it can be studied by comparing + and - fertile strains by cytological, biochemical or molecular methods. (Supported by grants from Swedish Natural Science Research Council).

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Wilson, C.
FGSC has collected and maintained stocks of Neurospora sp., Aspergillus nidulans and other filamentous fungi for nearly 25 years. Accounts of methods employed by the Stock Center appeared in early volumes of Neurospora Newsletter, but it has been suggested that many current workers are unaware of how strains are processed and preserved. Since the fungal genetics community is asked to trust the Stock Center to maintain materials which may be irreplaceable, it seems proper to explain what is done with these materials. Some methods may be new to some readers, and possibly useful to them in day to day operations. While I report the methods, I wish to make clear that they have been developed over the years by the Stock Center, and most were passed along to me by Ruth Rimbey and Ray Barratt. I have made certain adjustments to satisfy my own whims.

Most strains of Neurospora and A. nidulans produce large numbers of conidia, and can be routinely stored in silica gel and in lyophil. Both methods are employed for such strains. Upon receipt of a new culture, it is transferred to a fresh slant and grown at 25°. An adequate amount of conidiating material is generally produced within 4 days. The strain is then processed. In the case of sparsely conidiating cultures, the amount of fresh material can be increased by growing more than one slant. The aim is to maximize the number of viable conidia in the suspension fluid used for preservation. Some colonial strains require a longer period before finally producing abundant conidia. Recognition of this character comes through trial and error – with such strains the culture tube can be sealed with a permeable membrane cap to retard drying. fl mutants have long been difficult to preserve, but I have found a medium described by Turian (Nature 202:1240, 1964) to be very helpful. This is Westergaard-Mitchell (synthetic cross) medium supplemented with 10 mM Na-citrate. fl makes a spreading growth with few aerial hyphae on this medium, but I found that such preparations were qualitatively easier to lyophilize. I later measured the number of conidia (presumably microconidia) produced and found greatly increased numbers on this "conidiogenic" medium (Neurospora Newsl. 32:18, 1985).

When an adequate amount of material has grown, preservation on silica gel and in lyophil can be accomplished at the same time. The suspension fluid used is nonfat dry milk (7% w/v), autoclaved in 13 X 100 mm tubes for 10 minutes. 2.0 ml is an adequate volume. The mycelial-conidial mass is loaded into the milk with a sterile loop or needle. Normal concentrations of conidia can usually be suspended by agitation with the tip of a Pasteur pipette, but cultures that are largely aconidial will require grinding with a stouter implement. I use a small aluminum rod (4 mm diam) which can be flamed...