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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-OR23-1A (Kolmark and Veenhuizen 1984 Hereditas 101:277). The fl(blo) strains were found very satisfactory as mating type testers. We have reisolated 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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