How to enhance the formation of perithecia and the production of progeny by poorly fertile crosses.

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Background

Fertility and fecundity can be impaired in a multitude of ways, both, environmental and , genetic. Temperature, light, humidity, and substrate are among the physical factors that may affect fertility. Media constituents and pH are important. Aging and dessication impair ascospore germination.

Even with ideal conditions, genetic constitution may be of overriding importance and may involve either recessive or dominant factors.

Genetically based infertility may be due to mutant genes or to segmental duplication. Fertility is impaired ininterspecific crosses. Infertility may entail defective formation or function of protoperithecia and perithecia ("female sterility"). It may result fromdefects in the pheromone system or functioning of the mating type idiomorphs; Various developmental stages may be blocked, from crozier formation through karyogamy, meiosis, and ascus development. The formation and viability of ascospores may be impaired. When infertility is being discussed orpossible remedies are being considered, it is important to recognize and distinguish between possible alternatives (Perkins 1994).

Suggestions for overcoming difficulties in crossing or obtaining viable ascospores

Crosses are commonly made on synthetic cross medium (SC) (Westergaard and Mitchell 1947) with 1% or 1.5% sucrose at 25°C. (See *How to make a cross*. If difficulty is experienced when standard conditions are used, the following precautions and variations may help:

Physical and physiological factors

Once ascospores have been formed, make sure that they are mature before subjecting them to heat shock. For good germination, ascospores should not be heat shocked until they have been aged by holding them at 25°–30°C for a week or longer after they become black or after black spores have been ejected from the perithecium. (Temperatures above 30°C should be avoided.)

Make sure that ascospores are not dehydrated, as occurs if crosses are allowed to dry down. Suspending dehydrated ascospores in water for several hours before heat shock restores high germination (Strickland and Perkins 1973).

The temperature at which crosses are incubated should not exceed 30°C.

A second, local fertilization of a protoperithecial lawn should not be attempted if perithecia are already forming as the result of previous fertilization at another location in the same plate or tube. Development of perithecia at one location inhibits development of new perithecia elsewhere on the same lawn (Howe and Prakash 1969, Calhoun and Howe 1972, Metzenberg

1993, Peleg *et al.* 1996). (This is manifested when the formation of perithecia high in a slant inhibits development of those lower in the slant.)

Although the pH of standard SC is ~6.5, Metzenberg found ascospore production with homothallic species to be better at pH 4.5 than on medium of higher pH (Glass *et al.* 1990).

Several homothallic species fail to produce perithecia in the dark or in constant light, although crosses develop normally under an alternating light-dark regime (Raju 1981). (The action spectrum has not been determined.) So far as is known, light has little or no effect on crossing in *N. crassa* and other conidiating species. However, critical testing for light effects may not have been done, especially with strains showing poor fertility. Using a 12L:12D cycle for difficult crosses of *N. crassa* shouldn't hurt and might help to promote fertility.

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Medium and substrate

Production of protoperithecia and perithecia is inhibited by reduced nitrogen in the crossing medium. If there is a choice, wild type should be used as the protoperithecial parent. If both parents are mutant, use the strain with the simplest requirement or least altered morphooogy as female. When possible, use amino acid, purine, or pyrimidine auxotrophs as fertilizing parents rather than as protoperithecial parents. If amino acid supplementation of the crossing medium cannot be avoided, keep the concentration of amino acids, purines, and pyrimidines below 0.3 mg/ml. Better yet, avoid the need for supplementation by combining a mutant parent with *helper-1* (or another complementing inactive-mating-type helper strain) to form a wild-type heterokaryon for use as protoperithecial parent on minimal SC. (See *How to use helper strains for maintaining and crossing handicapped recessive mutants.*)

Reducing sucrose concentration in SC to 0.5% or less reduces conidiation and may result in larger (though perhaps fewer) perithecia. With some strains, substituting filter paper for sugar may have a marked effect in overcoming infertility (Fairfield and Turner 1993). Filter paper rather than sucrose is used routinely by some labs (e.g., Kinsey *et al.* 1980, Catcheside and Austin 1971).

Cornmeal agar rather than SC may be worth trying.

Pandit *et al.* (2000) have found that yellow-ecotype strains of *N. intermedia* cross best when pieces of corncob provide the physical substrate.

Productivity of difficult crosses may be enhanced by using a filter-paper cone with its base set in liquid crossing medium (Murray 1969).

Nonabsorbant cotton partially submerged in liquid crossing medium can result in luxuriant fruiting of some crosses that produce few or no perithecia on conventional agar slants (Prakash 1963).

In *N. tetrasperma* (but not in *N. crassa*, Viswanath-Reddy *et al.* 1977), substituting a polyol for sucrose can induce the formation of protoperithecia and perithecia at temperatures as high as 37°C (Viswanath-Reddy and Turian 1975). Ribitol is especially effective.

Genetic and cytogenetic factors

If difficulty is experienced in using a recessive mutant strain as protoperithecial parent, the mutant can be combined with *helper-1* or another inactive-mating-type helper strain to form a phenotypically wild-type heterokaryon. (See *How to use helper strains for maintaining and crossing handicapped recessive mutants*.) This requires that the mutant be heterokaryon-compatible with Oak Ridge strains. For possible partners to form heterokaryons with strains that are not OR-compatible, see strains listed in FGSC Catalog Part VI. D. 1, "Normal sequence testers for *het-c, het-d, het-e*".

Overt or cryptic genetic differences may affect female fertility. If production of perithecia is poor when one parent is used as protoperithecial parent, try the reciprocal cross using the other parent as female, or inoculate both parents together.

The nonconidiating mutant *fluffy* (*fl*) is exceptionally fertile, and crosses using it as protoperithecial parent may be more productive than when wild type is used. See Perkins *et al.* (1989)

When crossing a mutant × wild type, use wild type as the protoperithecial parent. If both parents are auxotrophs, use the strain with the simplest requirement as female. When possible, use amino acid auxotrophs as fertilizing rather than as protoperithecial parents. If amino acid supplementation of the crossing medium cannot be avoided, keep the concentration of amino acids, purines, and pyrimidines below 0.3 mg/ml. Better yet, avoid the need for supplementation by combining one mutant parent with *helper-1* to form a wild-type heterokaryon.

If mating ability or fertility is impaired in crosses using a newly induced mutation, consider the possibility that the mating defect may be due to cryptic genetic changes (gene mutations, rearrangements) other than the mutation of interest. Cleaning up the mutation by backcrossing to a standard wild type or *fluffy* tester may provide fertile mutant progeny that are freed of the deleterious factors. (To minimize the number of deleterious additional mutations in mutant hunts, keep the mutagen dose low. A survival level of at least 70% is advantageous (see, for example, Bos, 1987).

Segmental duplications are typically barren in crosses, making perithecia but producing few asci or ascospores. The barren condition is dominant. It is probably due in large part to meiotic silencing of unpaired DNA (MSUD). Fertility can often be increased (though it is not restored completely) by crossing the duplication strain with a suppressor of MSUD called *Sad-1* ('*Suppressor of ascus diminance*') (Shiu *et al.* 2001).

Interspecific crosses between different Neurospora species are typically barren, although a few ascospores can usually be obtained in intercrosses. Once again, fertility is increased if a *Sad* suppressor is present in the cross, indicating that the fertility barrier may be due to failure of meiotic pairing (Shiu *et al.* 2001).

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