How to introgress genes from one species to another.

Background

Different species combinations differ in the degree of reproductive isolation. Crosses of *N. intermedia* × *N. crassa* are relatively fertile, producing up to several percent viable black ascospores. At the other extreme, *N. discreta*, which was described as a new species on the basis of reproductive isolation (Perkins and Raju 1986), has so far failed to produce black ascospores and viable progeny in crosses with any other species. Nor have homothallic Neurospora species been crossed successfully among themselves or with heterothallic species.

Transfer of markers from one species to another may be desired for a variety of objectives. For example, to determine whether mating type idiomorphs are functional in heterospecific combinations (Metzenberg and Ahlgren 1969, Perkins 1977), to compare linkage maps and test mutants in one species for allelism with possible counterparts in another (e.g., Fincham 1951, Howe and Haysman 1963), or to examine the behavior of *Spore killer-2* and *Spore killer-3* (which originated in *N intermedia*) after transfer to *N. crassa* or *N. tetrasperma* (Turner and Perkins 1979, Raju and Perkins 1991).

When different species are intercrossed and the participants are of opposite mating type, perithecia are usually produced. These are typically either rudimentary or barren, but they nearly always progress far enough to reveal the mating type of individuals that are tested..The mating type of an isolate is indicated when perithecia begin to develop in tests with one mating-type tester (*mat A* or *mat a*), but not with the other, even though the perithecia may not progress far enough to produce ascospores. (This is true for most of the isolates in intercrosses that involve pairwise combinations of all the conidiating species, including *N. discreta*.) A few viable ascospores are produced by most species combinations, at least between certain isolates. Once the initial bottleneck is passed and f_1 progeny have been obtained, recurrent backcrosses typically show progressively increased fertility.

Procedure

If large-scale crosses between opposite mating-type strains of the two species fail to produce viable ascospores, the crossing medium may be at fault and should be varied. Substituting filter paper for sucrose in the crossing medium has been found helpful for some combinations and isolates (Fairfield and Turner 1993). Cornmeal agar provides another alternative. *N. intermedia* strains of the yellow ecotype cross best on fragmented corncobs (Pandit *et al.* 2000). If manipulation of the medium fails to promote crossing, bridging strains may be used, as described below, or representative strains from other populations may be tested. See *How to make a cross*.

Metzenberg and Ahlgren (1969) developed a set of hybrid strains for introgressing genes between pairs of species:: *N. crassa* with *N. intermedia, N. crassa* with *N. sitophila,* and *N. crassa wit h N. tetrasperma*. The strains they describe are available from FGSC (2004). Of these, C_4T_4 *mat a* has been most widely used to exchange genes between *N. crassa* and *N. tetrasperma* (Perkins 1991, Jacobson 1992).

The fertility and fecundity of interspecific crosses with *N. crassa* is reportedly increased by presence of the mutant gene *Sad-1*, which suppresses meiotic silencing by unpaired DNA (MSUD) (Shiu *et al.* 2001). (This would suggest that reproductive isolation may be due in part to interspecific differences in chromosome structure.)

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