How to determine the species of a wild-collected isolate.

Background

Although Shear and Dodge (1927) based their formal taxonomic descriptions of *N. crassa*, *N. sitophila*, and *N. tetrasperma* on conventional morphological and culture characteristics, strains of the three species had been intercrossed in all combinations before the descriptions were published (Table 6 of their paper), showing that fertility was reduced in intercrosses. Reproductive isolation thus influenced their taxonomic judgment, as was true also when Tai (1935) described *N. intermedia*. Because morphological differences between the heterothallic spedies are tenuous and difficult to determine with confidence, Perkins *et al.* (1976) advocated using crossing criteria as the most practical, convenient, and reliable basis for assigning heterothallic strains to species. Species tester strains were established that were derived from authenticated representatives of each species. Strains that produce fertile perithecia and abundant asci with viable black ascospore with one tester but not with the others were assigned to the same species. (Species defined by crossing ability are called *biological species*.) All strains producing asci with four self-fertile spores were called *N. tetrasperma*. One new heterothallic species, *N. discreta*, was described solely on the basis of crossing criteria (Perkins and Raju 1986).

Phylogenies based on DNA sequence are now available indicating that the conventional biological species correspond well with phylogenetic species, although molecular divergence appears in some instances to precede reproductice isolation. Isolates assigned to the same biological species may thus comprise more than one phylogenetic species (Dettman *et al.* 2003a, b). Molecular comparisons also suggest that several of the homothallic isolates that were originally described as different species on the basis of ascospore morphology may in fact be conspecific (Dettman *et al.* 2001).

Procedure for conidiating species

If perithecia form in the original pure culture and if four-spored asci are produced, the species is called *N. tetrasperma*. If a strain is self-sterile and no perithecia are formed in pure culture, it is crossed to standard species-tester strains, beginning with *mat A* and *mat a N. crassa* strains that contain the *fluffy* (*fl*) mutation. Mutant *fluffy* strains produce no conidia and are highly fertile in conspecific crosses. This test usually reveals mating type by initiating perithecial development even when species are different and the perithecia do not develop fully. If ascospores from this cross with the *N.crassa* testers are abundant and mostly black, the species is *N. crassa*. If ascospores are abundant but almost all are unpigmented, with at most a few that are black the strain is probably *N. intermedia*. This diagnosis is confirmed by crossing to an *N. intermedia* tester. If perithecia are rudimentary or if they fail to form beaks and eject no or few ascospores, the strain being tested probably belongs to one of the other species. Crosses are then made to *N. sitophila*, *N. tetrasperma*, and *N. discreta* testers.

Tests are conveniently made by spotting a wet loopful of conidia on a lawn of the tester strain. Plates for testing are prepared by inoculating the testers to crossing medium in petri dishes and incubated 5 days at 25º C to allow protoperithecia to form. At least 20 strains can be spotted at marked positions in each petri plate. If all crosses fail when sucrose is used as the carbon source, filter paper can be substituted for sucrose (Fairfield and Turner 1993). Tests can also be carried out using testers that have been grown up on slants in 12 × 75 mm tubes (for *fluffy* testers) or in 15 × 100 mm tubes (if the tester is a conidiating strain). Tests are read after 10 days at 25º C. The presence and types of ascospores shot to the lid of the plate or the wall of the tube is determined at ~60× magnification using transmitted light from a plain mirror. Detailed instructions for handling ambiguous tests and for the use of alternative testers are given by Turner *et al.* (2001). Testers currently in use are given in Table 1, which was copied from that reference.

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TABLE 1

Strains Used as Species Testers in Diagnostic Crosses

Strain designation	FGSC No.	Origin and characteristics
A. Preferred testers (species reference strains)		
Neurospora crassa		
fl^{P} *(OR) A*	4317	Highly fertile, aconidiate *fluffy* strains essentially coisogenic with the standard Oak Ridge (OR) laboratory wild types. *fl*^{P} originated spontaneously from 74A × 73A, and was backcrossed recurrently to OR or OR-related strains (Newmeyer *et al*., 1987).
fl^{P} *(OR) a*	4347	
Neurospora intermedia		
Shp-1A	3416	Conidiating f_5 isolates from *N. intermedia* P13A (FGSC 1766) x P17a (FGSC 1767) from Taiwan, selected for fertility and uniform growth (Shew, 1978).
Shp-1a	3417	
Neurospora sitophila		
fl A	4762	Nonconidiating *Sk-1* strains. *fluffy* allele P1012 arose spontaneously in *N. sitophila.* From third recurrent backcross to the conidiating *N. sitophila* standards P8085A and P8086a.
fl a	4763	
Neurospora discreta		
P851A	3228	P851 collected near Kirbyville, Texas. P8127 is from the 4th recurrent backcross to P851 of progeny from P851A × Kirbyville-1 a (P846) (Perkins and Raju, 1986).
P8127a	4378	
Neurospora tetrasperma		
85A	1270	Homokaryotic f_{12} progeny from (*A* + *a*) strain 87 of Dodge (Howe, 1963).
85a	1271	
B. Strains that might be used as alternative species testers		
Neurospora crassa		
OR23-1VA	2489	Conidiating standard laboratory wild types.
ORS-6a	4200	

Neurospora intermedia		
P420A	2316	Wild-collected *N. intermedia* strain from Florida.
P405a	1940	Wild-collected *N. intermedia* strain from Florida.
fl^{P}A	5798	*fluffy* (allele P) introgressed from *N. crassa* *fl*^{P}a *by*
fl^{P}a	5799	seven recurrent backcrosses to Shp-1A or Shp-1a.
P675a	2500	From Vehar, India.
Neurospora sitophila		
P8085A	2216	Conidiating *Sk-1* killer strains previously used as reference
P8086a	2217	testers (Perkins *et al*., 1976).
fl^{P}A	4887	*fluffy* (allele *P*) introgressed from *N. crassa* by five recurrent
fl^{P}a	4888	backcrosses to *N. sitophila*. Sensitive to killing by *Sk-1*.
P2443A	5940	Conidiating strain from Tahiti, sensitive to killing by *Sk-1*.
P2444a	5941	Conidiating strain from Tahiti, sensitive to killing by *Sk-1*.

Note. For further information, see Perkins *et al*. (1976) and Perkins and Raju (1986). The supplementary strains were authenticated in crosses to the species reference strains. They are used for areas where some strains are infertile with the standard reference strains or produce a high percentage of immature ascospores with them. *N. intermedia* testers P420 and P405 are sometimes more fertile than the Shp testers when crossed with *N. intermedia* isolates from the Western Hemisphere. P675 and P680 (FGSC 2499) were found to be best for identifying *N. intermedia* from India, Thailand and Malaysia. P680 was used extensively but is no longer recommended because it carries a senescence plasmid. The *N. crassa* and *N. intermedia* strains listed here as testers are all sensitive to killing by *Spore killer-2* and *Spore killer-3*. Spore killer strains are rare in these species. In *N. sitophila*, killer strains and strains sensitive to killing are both common. Testers of both types are therefore listed.