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Hybrid strains useful in transferring genes between species

Abstract

Hybrid strains useful in transferring genes between species

Metzenberg, R.L. and S.K. Ahlgren. Hybrid strains useful in transferring genes from one species of *Neurospora* to another.

only sporadic and haphazard fertility in such crosses,

The investigation of natural variation in the genus *Neurospora* has been limited by the absolute or relative infertility of interspecific crosses, especially where it is desirable to move moderately deleterious genes, such as auxotrophic markers, from one species to another.

A number of workers have done this successfully, but we have found To circumvent this difficulty, we have developed a "transfer kit" a

series of interspecific hybrids that **allow** one to move a given trait quite easily from one species to another in two or more small phyllogenetic steps, rather than one large one. The kit is of purely **utilitarian value**, and we have made **no attempt to determine** which chromosomes, or **how many, are** derived from a particular species. For example, in the **crassa-tetrasperma gradient**, the strain designation **C1, T3** means only that the strain had **one N. crassa grandparent and three N. tetrasperma grandparents**, and does not imply that it **contains N. crassa and N. tetrasperma genes in precisely that ratio**. **Some miscellaneous exotic strains** cross reasonably well with one or more **members of this gradient** even though they are infertile with both of the **parent species**.

The nomenclature we have adopted for these interspecific hybrids is as follows. Each hybrid is identified by letters and numbers that refer to the most recent cohort of ancestors that were **not laboratory hybrids**. For example, **C17, S15-a** had 17 **N. crassa** great-great-grandparents and 15 **N. sitophilo** great-great-grandparents. Similarly, **C4, T4-a** had 4 **N. crassa** great-grandparents and 4 **N. tetrasperma** great-grandparents, but some of its grandparents were hybrids.

In establishing the **N. tetrasperma** x **N. crassa** hybrid line, we plated **343.6AE** (FGSC#606, actually found to be mating type a) on **Westergaard-Mitchell medium** (1947 *Am. J. Botany* 34:573). In our hands this isolate shows the highest fertility of any **N. tetrasperma** strain with **N. crassa**, and is also quite fertile with several **N. intermedia** strains. (Crosses of the "type" strains of **N. tetrasperma**, **85A** and **85a** (FGSC#1270 and #1271, respectively) to **N. crassa** did not, in our hands, give any viable spores). After 4 days at **25°C**, the plate was treated with a suspension of **N. crassa 74-OR23-1A** (FGSC#987) conidia. After 3 weeks, a modest number of spores had collected on the lid of the **Petri plate**. These were suspended in water and heated at **55-60°C** for 30 minutes to induce germination. Of 39 germinated spores, 37 grew into cultures of mating type A, and 2 were of mating type a. A similar **assymetry** has been described previously (H&e and Haysman 1966 *Genetics* 54:292). The two a strains were suspected of having a very atypical chromosome complement, and were discarded; an a strain with roughly the desired ancestry (C4, T4-a) was derived as described in the pedigree below. All other crosses were made by simultaneous inoculation on **Westergaard-Mitchell medium**. All of the **N. crassa** - **N. tetrasperma** hybrids chosen for this kit were **aryl sulfataseless**. The strains comprising the kit are being placed in the collection of the **Fungal Genetics Stock Center**.

Many of the strains used by Dodge were retrieved from his laboratory after his death, and there was some uncertainty about their identity. As noted above, **343.6AE** is actually mating type a. Earlier, **H. B. Howe** found this strain to contain the **e** allele (unpublished data). **394.5ae** (FGSC#609) is a self-fertile heterocaryon. Last of all, the strain designated "**N. intermedia**, no #, secondarily homothallic" (FGSC#688) should be listed as **Neurospora torii** and is identical with the **Centraal-bureau voor Schimmelcultuur** (CBS) stock #25935 (Barratt, personal communication) (See Taj 1935 *Mycologia* 27: 328). **FGSC#688** is extremely fertile with **N. tetrasperma** testers, and gives the appearance of being **N. tetrasperma**.

We gratefully acknowledge advice from **D. Novak** and **A. Srb**, and also thank them for furnishing their strain of **N. intermedia**, **NIT-A**, (FGSC#1755) and other useful strains. (It should be noted that **NIT-A** does have some **N. crassa** ancestry; **Srb** prepared this strain by "carrying the mating type allele from **N. crassa**, conveyed by 10 generations backcross, to **N. intermedia**". The original hybrid was between **N. crassa** and **NIT-a** (FGSC#1754). Hence, in our nomenclature, **NIT-A** would be **C1, 12047-A**.)

We are likewise indebted to **R. H. Davis** for **N. sitophila 3A**. He and **M. Grindle** have described its origin as follows. "**N. sitophilo 2a** and **3A** were kindly provided by **J. Fincham**. Strain **2a** was pure **N. sitophilo**, while **3A** was a third-generation backcross of an **N. crassa** x **N. sitophilo** hybrid to **N. sitophila 2a**". **Fincham** obtained the latter strain from **H. L. K. Whitehouse** (1942 *New Phytologist* 41:23). **Whitehouse** obtained it from **J. Ramsbottom** and **F. L. Stephens** (1935 *Trans. Brit. Mycol. Soc.*, 19: 215), who, in turn, got it from **W. H. Wilkins**, who found it growing on beech battens in a lumberyard kiln in **Chichester, Great Britain**, in 1933. In our nomenclature, **N. sitophila 3A** would be called **C1, S15-A**.

The origin of the new hybrid stocks is as follows:

N. tetrasperma 343.6AE x **N. crassa 74-OR24-1A** → **C1, T1-A**.

N. tetrasperma 343.6AE x **C1, T1-A** → **C1, T3-A** and **C1, T3-a**

C1, T3A x **C3, T1-a** → **C4, T4-a**.

N. crassa 74-OR8-1a x **N. intermedia NIT-A** → **C2049, 12047-a** and **C2049, 12047-A**.

N. crassa 74-ORE-1a x **N. sitophila 3A** → **C17, S15-a**.

The new stocks which have been deposited in the **Fungal Genetics Stock Center** collection have been assigned the following numts: **N. sitophila 3A** (FGSC#1769); **C1, T1-A** (FGSC#1770); **C1, T3-A** (FGSC#1771); **C1, T3-a** (FGSC#1772); **C3, T1-A** (FGSC#1773); **C3, T1-a** (FGSC#1774); **C2049, 12047-A** (FGSC#1775); **C2049, 12047-a** (FGSC#1776); **C17, S15-a** (FGSC#1777); **C4, T4-a** (FGSC#1778); **N. sitophila-2a** (FGSC#1779).

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