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Substitution of paper for sucrose can reverse apparent male sterility in Neurospora

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Abstract

For many years we ascertained the species of newly collected Neurospora cultures by using them to fertilize standard species testers which had grown for five days on crossing medium with sucrose as the carbon source.

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For many years we ascertained the species of newly collected Neurospora cultures by using them to fertilize standard species testers which had grown for five days on crossing medium with sucrose as the carbon source. When new cultures would not cross to any of the testers, we tried to cross them to each other, reasoning that if any pair belonged to a previously unknown new species, it would cross successfully. This led to the discovery of *N. discreta* (Perkins and Raju 1986 Exp. Mycol. 10:323-338).

For each species, optimum tester strains were either developed or sought out among those collected. While developing testers for *N. discreta*, we turned to the use of paper instead of sucrose as the carbon source in order to increase female fertility. (The use of filter paper is described by Davis and de Serres 1970 Methods Enzymology 17A: p. 131. Some laboratories routinely use filter paper for crosses, e.g. Kinsey et al. 1980 Genetics 95:305-316, either because of fertility effects or because conidiation is greatly reduced.) We use Westergaard and Mitchell synthetic crossing medium with 1.5% agar. A piece of paper (we use Whatman chromatography paper, which comes in convenient rolls) is placed in each tube. After autoclaving, each tube must be slanted individually to orient the paper approximately along the top of the slant. Some of the cultures in the *N. discreta* group that had been female sterile on medium with sucrose made protoperithecia on the paper medium and made fertile crosses with viable ascospores when another culture in the group was used as male parent.

Some isolates from nature had not crossed to any of the species testers and had not crossed among themselves on medium with sucrose. With the use of the new *N. discreta* testers on medium with paper, some of these were identified as *N. discreta*. The others were crossed among themselves, using the medium with paper. It was found that some of the cultures that did not produce protoperithecia on sucrose medium produced abundant protoperithecia on medium with paper. From a mixed culture that produced ascospores, pure strains of each mating type were isolated and used as reference strains. Cultures that crossed to them were tentatively designated as a new species, *N. celata*, (Turner and Fairfield, Fungal Genet. Newsl. 37:46).

We assumed that the medium with paper was affecting only the female parent, particularly after observing the profusion of protoperithecia produced on paper by some strains that apparently did not produce them on sucrose; so when we retested all the strains of the putative new species using the standard testers of other species, the more easily dispensed sucrose medium was used. We have now discovered that sucrose medium does not just affect female fertility -- it will not support male fertility of certain Neurospora strains, even when the female parent is of the same species and is fully capable of crossing on sucrose to strains that do not have this conditional male sterility.

In the course of developing a revised protocol for determining the species of newly collected cultures, an unidentified strain was used as a protoperithecial parent on medium with paper. Both

the *N. sitophila* tester strain and the putative *N. celata* tester successfully fertilized this strain and produced fertile perithecia with abundant ascospores. Following this observation, a set of strains previously identified as *N. celata* were crossed to *N. sitophila*. Each cross was made four times: the *N. celata* parent was used as female on both sucrose and paper, and the *N. sitophila* strain was used as female on both media. The crosses on sucrose medium were sterile, regardless of which strain was used as female. In contrast, all of the crosses on paper medium resulted in fully fertile perithecia with abundant black ascospores. Thus, the strains tentatively called *N. celata* are actually a group of *N. sitophila* that are conditionally sterile on sucrose medium, even when used as male parent.

Another project to test whether use of paper instead of sucrose would enhance male fertility was carried out using isolates that had given anomalous results on medium with sucrose. They had crossed and produced defective ascospores with *N. crassa fluffy* (this result is typical of *N. intermedia*) but had shown no crossing reaction to conidiated *N. intermedia* testers. Using medium with paper, the previously used *N. intermedia* testers were fertilized with the anomalous isolates, which did cross and make black viable ascospores on the paper medium. These isolates clearly are fertile, but when they were crossed to a conidia- producing parent on sucrose medium, there was no observable mating reaction -- a phenocopy of male sterility or species incompatibility. The absence of macroconidia is the most obvious characteristic that the fluffy testers, which show enhanced fertility in many different contexts, share with the female parents grown on paper medium.

In summary, the phenomenon of conditional male sterility has been found for a subgroup of *N. sitophila* and a subgroup of *N. intermedia*. We have not had time to explore possible mechanisms for this effect.