

Inter-strain cross-fertility tests on cultures from Israel, America and Canada in the homothallic fungus, *Sordaria fimicola*
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Inter-strain cross-fertility was studied in relation to geographical origin in a homothallic, self-fertile fungus, by looking for hybrid perithecia in wild-type x ascospore colour mutant crosses. Strains from opposite slopes in 'Evolution Canyon', Israel, showed no cross-fertility with American or Canadian strains; there was excellent cross-fertility with other strains from the same slope, but an occasional lack of cross-fertility with strains from the other slope.

Genetic studies often require different strains to be crossed. When a fungus is homothallic and self-fertile, one needs to obtain crossed fruiting bodies such as perithecia, and to distinguish them from selfed fruiting bodies. The classic methods in *Sordaria fimicola* are: (i) to use crosses of wild-type (black ascospores) x an autonomous ascospore colour mutant (e.g., gray, brown or hyaline), looking for perithecia showing asci with mainly 4 wild-type: 4 mutant colour segregations; (ii) to use two complementary self-sterile mutants, when only crossed perithecia should be produced (Olive 1956 *Am. J. Bot.* 43: 97-107). The aim here was to test whether Israeli strains of *S. fimicola* were cross-fertile with American strains (from A1) or Canadian strains (from C7) used by Olive (1956, *ibid.*), and to study the cross-fertility of different Israeli strains from the same general area. Method (i) was preferred to method (ii) because it did not require the isolation of self-sterile mutants in all strains to be tested, and because self-sterile mutants might reduce cross-fertility.

In 'Evolution Canyon', Lower Nahal Oren, Mount Carmel, Israel, the south-facing slope (SFS) has African and Asian xeric tropical species, has much more solar radiation, and is warmer, drier and more heterogeneous than the lush temperate north-facing slope (NFS), which has a more European flora. The two slopes are 200 to 500 metres apart (Nevo 1995 *Proc. R. Soc. Lond. Ser. B.* 262: 149-155). We used the following *S. fimicola* strains, where S stands for the SFS and N stands for the NFS: S1, N7(i) and N7(ii) from 120 m above sea level; S2 and N6 from 90 m; S3, N5(i) and N5(ii) from 60 m. They were all isolated vegetatively, not from ascospores, and spontaneous ascospore colour mutants were easy to find and isolate (Lamb *et al.* 1998 *Genetics* 149: 87-99, which has maps of the colour mutations).

Unless otherwise stated, crosses were made by inoculation of strains on opposite sides of 9 cm diameter petri dishes of minimal medium (Olive 1956 *Am. J. Bot.* 43: 97-107); if the two strains grew at very different speeds, the slower strain was inoculated earlier than the faster one, so that about equal amounts of growth met at the centre of the dish. In another method, inocula of the two strains were grown together in 100 ml of liquid minimal medium in 250 ml flasks on a shaker for 24 h, then the hyphae were blended (Funkenstori T1500 blender, speed 5, 90 s). 1 ml of the blend was pipetted onto the centre of a plate of cornmeal agar (Kitani and Olive 1967 *Genetics* 57: 767-782). Most incubations in Israeli x American crosses and Israeli x Canadian strains were at 25°, with two or more replicate plates, but some further replicates were crossed at 17.5°, producing no differences in cross-fertility results but a longer maturation time. Mature perithecia were dissected in 2M sucrose solution and the asci were viewed at x 80 to see the ascospore colour segregation ratios.

Strains often become less self-fertile with time, especially with repeated subculturing. The original Israeli strains were isolated in 1994 and 1995, but for this work (mainly done in 1999), very self-fertile reisolates were obtained by germinating black ascospores from self-crosses of the original Israeli wild isolates. All the American isolates used were strongly self-fertile, except for *sp g⁺ i⁻ cor*, for which three different subcultures of ours had different self-fertilities ranging from slight to strong. El-Ani *et al.* (1961 *Am. J. Bot.* 48: 716-723) stated that *corona* gave reduced self-fertility and the FGSC Catalogue of Strains 1998 states (p176) that *corona* is self-sterile, which is not our experience. Of the Canadian strains, C7 translocation was very self-fertile but C7 *hyaline* was self-sterile. Most American and Canadian strains were reisolates of Olive's original ones, from ascospores from selfed crosses. Our strains were obtained from Olive, Kitani and Cox, not from FGSC, but strain *sp g⁺ i⁻ cor* corresponds to FGSC 6307, *g⁺* to FGSC 2770, and *Kg⁺* to FGSC 2918; those three are derived from Olive's A1 wild strain from dung in New York City, while strains C7 translocation and C7 *hyaline* are derived from wild strain C7, from dung in Ontario (Olive 1956 *ibid.*). The American and Canadian strains do not cross with each other (Olive 1956 *ibid.* and B. C. Lamb, unpublished results), so the allelism of C7's *hyaline* with A1's mutations is untested. The C7 translocation strain (Cox and Gill 1967 *New Phytol.* 66: 653-664) was used because it was self-fertile and Canadian, with black ascospores (*g⁺*); our culture of the original C7 wild-type did not revive from storage.

The strains crossed here, their genotypes and their cross-fertility results are given in Tables 1 to 3. At least 20 perithecia per dish were examined from each cross, mainly from near the line where the two strains met, or from either side of the visible fertility barrier where one was formed. In the latter case, there were usually many selfed perithecia on either side of the barrier.

Table 1. Israeli x American and Israeli x Canadian crosses and their cross-fertility results

Israel strains	American or Canadian strains they were crossed with
(i) Crosses with no hybrid perithecia and showing a visible fertility barrier, as a gap between hyphae of the two strains. Inocu. 8 cm apart.	
S1 reisolates, wt, R2.3; R4.1	<i>g</i> ⁻ ; two isolates of <i>sp g⁺ i cor</i>
S3 reisolate, wt, R1.2	<i>g</i> ⁻ ; two isolates of <i>sp g⁺ i cor</i>
N5 reisolate, wt, R2.3	<i>g</i> ⁻ ; two isolates of <i>sp g⁺ i cor</i>
N7 reisolates, wt, R1.4; R4.2	<i>g</i> ⁻ ; two isolates of <i>sp g⁺ i cor</i>
(ii) Crosses with no hybrid perithecia and no visible fertility barrier. Inoculated 1 cm apart, at one side of the dish.	
S1 reisolates, wt, R2.3; R4.1	two isolates of <i>g</i> ⁻ ; C7 <i>hyaline</i>
S3 reisolate, wt, R1.2	two isolates of <i>g</i> ⁻ ; C7 <i>hyaline</i>
N5 reisolate, wt, R2.3	two isolates of <i>g</i> ⁻ ; C7 <i>hyaline</i>
N7 reisolates, wt, R1.4; R4.2	two isolates of <i>g</i> ⁻ ; C7 <i>hyaline</i>
(iii) Crosses made by inoculation together in liquid medium and then blending; no hybrid perithecia	
<i>grey 4.3</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation
<i>grey 16.2</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation
<i>grey 21.3</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation
<i>light grey 60.3</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation
<i>white 17.2</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation
<i>white 18.2</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation
<i>white 41.3</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation
<i>white 67.1</i>	<i>sp g⁺ i cor</i> ; two isolates of <i>Kg⁺</i> ; C7 translocation

Key to strains. Israeli: (i), (ii), wt., black ascospores; (iii), ascospore colour mutations with the stated phenotype, e.g., *white*. American and Canadian: black ascospores in *g⁺* and C7 translocation; *g*⁻ (gray) and *hyaline* (clear ascospores) are ascospore colour mutations; *i* is at a separate locus, giving indigo coloured spore walls. Mutations *sp* (spotty distribution of perithecia) and *cor* (corona) do not affect ascospore pigmentation. K stands for a strain from Y. Kitani.

In the Israeli x Israeli crosses, made at 17.5° with four replicates of each cross, six different ascospore colour mutants derived from original SFS wild-types S2 and S3 were crossed to two different south-facing slope wild-types and to two different north-facing slope wild-types. All crosses showed cross-fertility, with hybrid perithecia (Table 2, i and ii). Five different ascospore colour mutants derived from original NFS wild-type N5 were crossed to two SFS and to two NFS wild-types (Table 2, iii and iv). All crosses to NFS wild-types were cross-fertile (Table 2, iv), but crosses of NFS strain *grey 21.3* to two SFS wild-types were not cross-fertile (Table 2, iii), so the overall frequency of crosses with no hybrid perithecia was 2 out of 42, or 5%, in crosses of wild-type x mutant for ascospore colour.

In crosses of ascospore colour mutant x different ascospore colour mutant, 11 such crosses between pairs of mutant SFS strains were cross-fertile, as were all seven such crosses between pairs of mutant NFS strains (Table 3, i and ii). In 31 such crosses between mutant SFS strains and mutant NFS strains, 25 were cross-fertile, and 6 (19%) produced no hybrid perithecia (Table 3, iii). The perithecia in many such mutant x mutant crosses had pale walls, so it was easy to look through the walls for black recombinant ascospores, as well as dissecting perithecia to look at intact asci.

In Olive's original work (Olive 1956 *ibid.*), strain A1 from New York City dung was infertile with another New York dung strain and with all strains from Ontario and Ithaca, NY, but was cross-fertile with one from Manitoba and one from Michigan. Which ascospore colour mutant was carried in strain C7 also affected which strains would cross. In the present work, there was no cross-fertility between the American and Israeli strains, or Israeli and Canadian strains. There was full cross-fertility between strains within each slope of "Evolution Canyon", even when isolated from different altitudes; crosses between strains from different slopes were generally but not always cross-fertile, so there was less cross-fertility between geographically more distant strains.

The present results show that cross-fertility was very common between homothallic strains of *S. fimicola* from one locality, such as one slope of 'Evolution Canyon', but diminished slightly for strains somewhat further apart, such as the two slopes of 'Evolution Canyon'. There was no cross-fertility between strains from widely separated areas Israel, America (New York) and Canada (Ontario). This suggests that the increasing genetic divergence between strains occurring further apart usually results in a complete loss of cross-fertility in this homothallic fungus.

Acknowledgments

We are grateful to Lindsay Olive, Yoshiaki Kitani, Brian Cox and Eviatar Nevo for supplying the various strains used.

Table 2. Israeli x Israeli strain wild-type x spore colour mutant crosses and their cross-fertility results

(i) South slope wild-type x south slope spore colour mutants crosses; all fertile

S2 reisolate, wt, R4.2 fertile with w18.2, w24.3, w92.1, l60.3, w67.1, g4.3.

S3 reisolate, wt, R2.3 fertile with w18.2, w24.3, w92.1, l60.3, w67.1, g4.3.

(ii) North slope wild-type x south slope spore colour mutant crosses; all fertile

N5 reisolate, wt, R1.4 fertile with w18.2, w24.3, w92.1, l60.3, w67.1, g4.3.

N7 reisolate, wt, R5.3 fertile with w18.2, w24.3, w92.1, l60.3, w67.1, g4.3.

(iii) South slope wild-type x north slope spore colour mutant; most but not all were fertile

S2 reisolate, wt, R1.3 fertile with w17.2, infertile with g21.3

S2 reisolate, wt, R2.2 fertile with lg14.3, w41.3, infertile with g21.3

S2 reisolate, wt, R4.3 fertile with g16.2, infertile with g21.3

S3 reisolate, wt, R10.2 fertile with w17.2, infertile with g21.3

S3 reisolate, wt, R8.4 fertile with lg14.3, infertile with g21.3

S3 reisolate, wt, R4.2 fertile with w41.3, infertile with g21.3

S3 reisolate, wt, R6.3 fertile with g16.2, infertile with g21.3

(iv) North slope wild-type x north slope spore colour mutant crosses; all fertile

N5 reisolate, wt, R1.4 fertile with w17.2

N5 reisolate, wt, R3.3 fertile with lg14.3, g21.3

N5 reisolate, wt, R4.5 fertile with w41.3

N5 reisolate, wt, R1.1 fertile with g16.2

N7 reisolate, wt, R2.3 fertile with w17.2

N7 reisolate, wt, R4.1 fertile with lg14.3, g21.3

N7 reisolate, wt, R2.1 fertile with w41.3, g16.2

Key to strains. wt, black ascospores; w, white; lg, light gray; g, gray.

Table 3. Israeli x Israeli strain spore colour mutant x different spore colour mutant crosses and their cross-fertility results

(i) South slope x south slope crosses; all fertile

w18.2 fertile with w24.3, w92.1, lg60.3, w67.1, g4.3

w24.3 fertile with w92.1, lg60.3, w67.1

w92.1 fertile with lg60.3, w67.1

lg60.3 fertile with w67.1

(ii) North slope x north slope crosses; all fertile

w17.2 fertile with lg14.3, w41.3, g16.2

lg14.3 fertile with w41.3, g16.2

w41.3 fertile with g16.2

g16.2 fertile with g21.3

(iii) South slope (listed first) x north slope crosses; mostly fertile, but some sterile

w18.2 fertile with w17.2, lg14.3, g16.2 but sterile with g21.3

w24.3 fertile with w17.2, lg14.3, w41.3, g16.2, but sterile with g21.3

w92.1 fertile with w17.2, w41.3, g16.2, but sterile with lg14.3, g21.3

lg60.3 fertile with w17.2, lg14.3, w41.3, g16.2, g21.3

w67.1 fertile with w17.2, w41.3, g16.2, g21.3, but sterile with lg14.3

g4.3 fertile with w17.2, lg14.3, g16.2, but sterile with w41.3, g21.3

Key: as in Table 2.