Genetic Evidence for Hybridization in an Interspecific Cross in the Genus *Sordaria*

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(Accepted for publication 15 September 1969)

SUMMARY

When the two heterothallic species *Sordaria heterothallis* and *S. thermophila* are crossed, two types of viable ascospores are recovered. The first, the mature black ascospore, is produced with a mature eight-spored ascus formed from an intraspecific fusion of nuclei of the same mating type. The second, the non-pigmented germinable ascospore, is produced by the interspecific fusion of nuclei of opposite mating types within an ascus in which all other ascospores are aborted. The non-pigmented ascospore is the product of the mixed genomes of the two species. It is a recombinant spore from which either species may be obtained by the partial substitution of its genome through successive backcrosses. Its inability to develop pigmentation, the irregular growth patterns of some of the strains which it produces and their inability to react in complementation tests, appear to be the result of a lack of affinity between the two species.

INTRODUCTION

Two new heterothallic species, *Sordaria heterothallis* (Fields & Maniotis, 1963) and *S. thermophila* (Fields, 1968), have recently been described. Previously reported studies on the hybridization experiments between these species (Fields, 1963; Lewis, 1968*a*, 1969) have indicated that the primary result was the formation of perithecia in which most of the asci were aborted at varying stages of maturation, and in which an occasional mature eight-spored ascus was recovered. These asci (recovered at a frequency of 1 per 10,000 asci examined) were found to have been homozygous for the mating type factor. Lewis (1968*b* and 1969) described evidence which suggests that they were formed by the intraspecific fusion of nuclei of the same mating type. Although most of the spores formed in these asci were normal in colour and shape and showed reasonable viability, these studies also indicated that aneuploid ascospores were formed during meiosis and that the resultant loss of chromosomes contributed to the inviability observed in some ascospores.

Another type of viable ascospore produced from the hybridization attempts was the non-pigmented germinable ascospore. The aim of this study was to determine whether this ascospore was the product of intra- or interspecific nuclear fusions. Proof of the latter would determine whether hybridization had occurred between the two species.

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MATERIALS

Wild-type strains of *Sordaria heterothallis* and *S. thermophila* were supplied by Dr W. G. Fields. The wild-type strains of *S. heterothallis* were designated as WT 5-*A* and WT 16-*a*, and those of *S. thermophila* as WT 7-*A* and WT 17-*a*. In each case *A* and *a* refer to the two mating types. Differences between the two species, and descriptions of the media, the cultural conditions employed and the techniques used in the isolation, characterization and mapping of mutants have been previously reported (Lewis, 1969). The *S. heterothallis* mutants used in this study were *stu*₁, *stu*₄, *stu*₅, *stu*₆ (strains with stunted growth) and *ol*₃ (olive-green ascospores). Those of *S. thermophila* were *col*₁, *col*₂, *col*₃ (strains with a colonial type of growth) and *t* (tan coloured ascospores). In *S. heterothallis*, *stu*₁ and *stu*₆ were 0.1 units apart and 1.0 unit from the mating type locus. *Stu*₄ and *stu*₅ were 2.0 units apart on another chromosome.

Several types of interspecific crosses were made: (1) Between the two wild-type strains. (2) *Stu*₆ × *t* (cross no. 4, Table 1). (3) Heterocaryons forced between *stu* strains of *Sordaria heterothallis* were crossed with wild-type strains of *S. thermophila* (crosses 1 to 3, Table 1). From these crosses it was hoped to determine whether the *f*₁ progeny (1) segregated only as parentals and whether strains recombinant for the markers in the constituent strains of the heterocaryon could be recovered; (2) were the products of interspecific fusions. Progeny were analysed for recombination between the *stu* markers and the mating type factors in order to determine whether the non-pigmented germinable ascospores were derived from interspecific nuclear fusions. *f*₁ strains produced by non-pigmented germinable ascospores were classified to species by back-crossing to wild-type strains of both species. The genotypes of stunted progeny from interspecific crosses were determined by complementation tests with *stu*_{1, 4, 5} and *stu*₆ of *S. heterothallis* and with the restricted colonial mutants of *S. thermophila*.

RESULTS

Fields (1963) carried out the initial interspecific matings between *Sordaria heterothallis* and *S. thermophila*. He found that there was an abundant production of perithecia in which almost all the asci contained aborted protoplasts or delimited ascospores which did not mature. He also observed (personal communication) that these 'immature' ascospores occasionally germinated.

A closer study of these non-pigmented germinable ascospores showed that they were produced in asci which contain no black spores, and in which only one spore germinated. The other seven ascospores in the asci showed various degrees of abortion and no germination (Pl. 1, fig. 1). These asci were produced at the rate of 20 to 30 per perithecium, and the delimited ascospores ranged in colour from hyaline to beige. Most asci contained eight aborted ascospores and made up the bulk of the cluster in interspecific matings (Pl. 1, fig. 2). The germinable ascospores germinated without any of the pre-germination activation that was mandatory for *Sordaria heterothallis* and *S. thermophila*. The ascospores of *S. heterothallis* are only known to germinate when incubated on a medium containing 7 % sodium acetate (Fields & Maniotis, 1963), and those of *S. thermophila* require a 2-hr heat shock treatment and incubation on a medium containing 0.55 % ammonium acetate (Lewis, 1969).

Non-pigmented germinable spores isolated from wild-type interspecific matings

Table 1. Analysis of non-pigmented germinable ascospores isolated from *Sordaria heterothallis* × *S. thermophila* crosses

Cross no.	<i>S. heterothallis</i> × <i>S. thermophila</i>	Spore no.	Phenotypes		Complementation results*	MT	f ₁ backcross reactions with		Recombinant spores
			Colour	Growth			<i>S. thermo-</i> <i>phila</i>	<i>S. hetero-</i> <i>thallis</i>	
1	$(stu_6 + stu_6)a \times WT\ 7, A$	1, 4, 7, 10, 11	Hyaline	WT	—	<i>A</i>	Inter†	Inter	—
		2	Hyaline	WT	—	<i>a</i>	Inter†	Inter	—
		3, 12	Hyaline	<i>stu</i>	Not tested	<i>a</i>	Inter	Inter	+
		5, 6, 8	Hyaline	Sub WT	Not tested	<i>A</i>	Inter	Inter	—
		9	Light brown	WT	—	<i>A</i>	Inter	Inter	—
2	$(stu_1stu_4ol_3 + stu_5)a \times WT\ A$	1	Hyaline	Sub WT	Negative	Not tested	—	—	—
		2, 5	Hyaline	<i>stu</i>	Negative	Not tested	—	—	—
		7-12, 14, 15, 17-20	Hyaline	<i>stu</i>	Negative	Not tested	—	—	—
		6, 13, 16	Beige	WT	—	Not tested	—	—	—
		21-22	Hyaline	WT	—	Not tested	—	—	—
3	$(stu_1stu_4ol_3 + stu_5stu_6)a \times WT\ 7, A$	24-25	Hyaline	WT	—	<i>a</i>	Inter	Inter	+
		23	Hyaline	<i>stu</i>	Negative	<i>A</i>	Inter	Inter	+
		1, 8, 3, 4, 16, 17	Hyaline	<i>stu</i>	Negative	<i>A</i>	Inter	Inter	+
		2, 10, 12, 15	Hyaline	<i>stu</i>	Negative	<i>a</i>	Inter	Inter	—
		5, 9, 14	Hyaline	<i>stu</i>	Negative	<i>a</i>	Inter	Inter	—
		8, 13	Hyaline	Sub WT	—	Not tested	Inter	Inter	—
		11	Hyaline	WT	—	<i>A</i>	Inter	Inter	+
		6	Beige	<i>stu</i>	Negative	Not tested	Inter	Inter	—
		1-3	Hyaline	WT	—	<i>A</i>	Inter	Inter	—
		5-7	Hyaline	<i>stu</i>	Not tested	<i>a</i>	Inter	Inter	—
4	$stu_6\ a \times t_1\ A$	4	Hyaline	<i>stu</i>	Not tested	<i>a</i>	Inter	Inter	—

* Complementation tests were carried out with *stu₁*, *stu₂*, *stu₃*, *stu₄* and *stu₆* of *S. heterothallis* and *col₁* of *S. thermophila*. A positive complementation test is scored when pairs of *stu* cultures of the same mating type produce fans of wild type growth, when the control, *stu* cultures of opposite mating types remain stunted. In a negative test only stunted growth is observed.

† Interspecific.

‡ This strain produced an intraspecific reaction with *S. thermophila* after three subculturings.

§ These strains produced a few perithecia in which some asci had 4 to 8 black spores.

** See text: formation of strains phenotypically resembling *S. heterothallis* from a non-pigmented ascospore.

showed two basic types of growth, normal wild type growth and a sub-wild type growth. The sub-wild type growth microscopically had an abnormal mycelial development, with much spiralling and more branching than the wild type. The growth rate was slower than the wild type, but the colony eventually grew to the edge of the Petri dish. In some sub-wild type strains, the colony gave rise to numerous sectors. These observations and the inability of the spores to develop normal pigmentation suggest that factors inhibiting the phenotypic expression of wild type were present in these non-pigmented ascospores, although some had become sufficiently mature physiologically to germinate and grow. When strains from the non-pigmented germinable ascospores were crossed among themselves, the asci produced showed an even greater degree of ascospore abortion than that produced in interspecific crosses. The non-pigmented germinable ascospores produced showed no phenotypic differences from their f_1 counterparts, and their mycelial strains had similar characteristics.

Progeny from the four matings shown in Table 1 are described phenotypically. f_1 cultures showed the phenotypes of either the mutant parental strains used in these matings, the sub-wild type mycelium, with its characteristic irregular growth and sectoring, or wild type growth with the normal phenotype. Thirty-five slow growing isolates were tested for complementation with stunted and colonial strains of both species. The results in Table 1 show that no complementation took place. It was assumed that failure of complementation might be due to the recombinant nature of the non-pigmented ascospores.

Twenty-nine of the isolated non-pigmented germinable ascospores were backcrossed to the wild type strains of both species. All strains showed an interspecific reaction with both species. This result led to the conclusion that the genomes of these strains were interspecifically recombinant, and that both parents contributed to their genotypes. Genetic proof of this is described in the following sections.

Twelve of the f_1 non-pigmented ascospores showed apparent recombination between the *stu* markers and the mating type factors (Table 1). The apparent recombinant spores with stunted growths, no. 23 of cross no. 2, and nos. 1, 3, 4, 16 and 17 of cross no. 3, could not be classified as true recombinants without further study, since the *stu* markers could not be identified through complementation, and a 1:1 segregation could not be determined from the aborted f_2 asci and ascospores. Proof of their recombinant nature is provided in the crosses discussed below.

(1) Cross no. 3, ascospore no. 3 (Table 1). This spore, an apparent recombinant, produced a culture which had a stunted growth and carried the *A* mating type allele. The backcross reactions with both *Sordaria heterothallis* and *S. thermophila* were interspecific. The *S. thermophila* backcross produced aborted asci with no germinable ascospores. In the *S. heterothallis* backcross, a few aborted asci with 1 to 2 mature spores were produced, and one mature eight-spored ascus was recovered. The eight black spores which germinated were wild type in growth and *a* in mating type. Three non-pigmented germinable spores—two of stunted and one of wild type growth—were recovered and analysed. The stunted strains were determined to be *stu₅a* through complementation tests. The recovery of the *stu₅* marker in the backcross progeny proves that the original non-pigmented ascospore must have been recombinant, with the *stu₅* marker derived from *S. heterothallis* and the *A* mating type locus from *S. thermophila*. This ascospore could only have resulted from an interspecific nuclear fusion.

(2) Cross no. 3, ascospore no. 14 (Table 1). This spore produced a mycelium with stunted growth, which was *a* in mating type and gave a negative complementation test. The backcross with *Sordaria heterothallis* produced aborted asci with 1 to 2 mature ascospores; several non-pigmented germinable ascospores were isolated. Of these, two were wild type, one sub-wild type and four stunted in phenotype. One of these stunted strains was determined to be *stu₁stu₄ a*, through complementation tests. Thus the *f₁* non-pigmented ascospore which was stunted in phenotype must have carried the *stu* markers. That these *f₂* non-pigmented ascospores could take part in complementation reactions with *stu* strains of *S. heterothallis* reflects the fact that they contained a larger part of the *S. heterothallis* genome than the *f₁* ascospores. That they were non-pigmented reflects the fact that they were the products of mixed genomes. From this result it may be concluded that ascospores no. 1, 4, 16 and 17 from cross no. 3, which were phenotypically stunted, carried the *stu* markers and were recombinant. Ascospore no. 11 was also shown to be recombinant for the *stu₅* marker and mating type factor, as is described in the section in which strains phenotypically resembling *S. heterothallis* are obtained through backcrossing.

Ascospore no. 2, cross no. 1 (Table 1), and ascospores 21, 22, 24 and 25 of cross no. 2 were also interspecific recombinants. The initial backcross reactions of spore no. 2, cross no. 1 with *Sordaria heterothallis* and *S. thermophila* were interspecific. This strain was however repeatedly subcultured over 3 months while other experiments were in progress. On further testing, the backcross was found to produce an interspecific reaction with *S. heterothallis* but an intraspecific reaction with *S. thermophila*. This *f₁* segregant, *a* MT, was clearly an interspecific recombinant.

These results demonstrated that the non-pigmented germinable ascospores contained a mixture of the genomes of *Sordaria heterothallis* and *S. thermophila*. Such mixtures appeared to impair some of the physiological functions of the cells; spore pigmentation is prevented, and *stu* strains failed to show complementation either among themselves or when mixed with *stu* strains of *S. heterothallis*. Proof that these strains were the products of mixed genomes was also shown by the recovery of both of the original species from them as is demonstrated below.

*Formation of strains phenotypically resembling S. thermophila
from a non-pigmented ascospore*

Strains phenotypically resembling *Sordaria thermophila* were obtained from *f₁* non-pigmented ascospore cultures by successive backcrossing to the *S. thermophila* wild type strain. One such series of backcrosses (Fig. 1) was started with the culture from ascospore no. 1 of cross no. 3 (Table 1). The mycelium from this ascospore had a stunted growth and was *A* in mating type. Only non-pigmented ascospores were recovered from the interspecific backcross with *S. heterothallis* but none could be induced to germinate. The backcross with *S. thermophila* produced two types of perithecia. (1) Several perithecia contained asci with 1 to 8 black spores (Pl. 1, fig. 3). Six asci with eight black spores were isolated; the ascospores germinated on ammonium only after the 2-hr heat treatment at 50°. All germinating ascospores were wild type in growth, although one parent appeared to carry the *stu* marker. This and the fact that this culture gave rise to two types of perithecia in this cross suggests that strain 1 of cross no. 3 was a heterocaryotic culture. Figure 1 shows the results of the analysis of ascus no. 4 of the *f₂* backcross. Both mating type factors were recovered.

Mycelia from each of three pairs of ascospores of this ascus were backcrossed to the wild type strains of *S. heterothallis* and *S. thermophila* respectively. Normal segregation of the mating type alleles was observed in all crosses with *S. thermophila*, with asci and ascospores showing the typical shape and size of this species (Pl. 1, fig. 5). Strains with the *S. thermophila* phenotype were thus isolated among the f_3 progeny. (2) The majority of f_1 perithecia in this backcross contained asci which showed an interspecific reaction, with some asci containing 1 to 2 black ascospores (Pl. 1, fig. 4). One mature eight-spored ascus which was recovered from these perithecia was homozygous for the wild type growth and *a* mating type of *S. thermophila*. This confirms the results described previously (Lewis, 1969) that the formation of the mature eight-spored ascus was exclusively an intraspecific event.

*Formation of strains phenotypically resembling S. heterothallis
from a non-pigmented ascospore*

Strains phenotypically resembling *Sordaria heterothallis* were obtained from recombinant f_1 non-pigmented germinable ascospores through successive backcrosses to the *S. heterothallis* wild type. Figure 1 shows a series of backcrosses which were started with ascospore culture no. 11 of cross no. 3 (Table 1). This spore produced a wild type mycelium of *A* mating type. Backcrosses to *S. heterothallis* and *S. thermophila* were both interspecific, but perithecia from the former produced numerous aborted asci with 1 to 4 black ascospores. Cultures from eight of the 16 isolated black spores were *stu₅a* in genotype. Thus the original f_1 ascospore was interspecifically recombinant. One of the wild type f_2 ascospores of *a* mating type was backcrossed to *S. heterothallis A*. The f_3 asci contained mostly 4 to 8 black spores (Pl. 1, fig. 6). Six asci with eight black spores were dissected, and all ascospores showed wild type growth. Figure 1 shows the analysis of ascus no. 1. A mycelium from each of three spore pairs was backcrossed to both *S. heterothallis* and *S. thermophila*; all produced intraspecific reactions with *S. heterothallis* (Pl. 1, fig. 7) and interspecific reactions with *S. thermophila*. Strains with the *S. heterothallis* phenotype were thus isolated among the f_4 progeny. The replacement of parts of the genomes of the non-pigmented germinable ascospores with parts of the genomes of *S. heterothallis* or *S. thermophila*, the demonstration of interspecific recombination and the fact that these spores react inter-specifically with both species, is evidence that these non-pigmented spores are inter-specific recombinants.

DISCUSSION

The non-pigmented germinable ascospore is a cell containing parts of the genomes of the two species *Sordaria heterothallis* and *S. thermophila*. The mixture of chromosomes appears to impair the growth rate in some strains and inhibits spore pigmentation in all cases. It is probable that the synthesis of the spore pigment is controlled by several loci on a number of chromosomes, and that the necessary complementation between the products of these loci which is needed for pigment synthesis cannot or can only partially take place, as in the production of beige coloured germinable ascospores, when the chromosomes are from different species. This may reflect the evolutionary differences between the chromosomes of these two species. A hypothesis of this nature can be formulated on the basis of the results obtained from attempts to force heterocaryons between strains carrying the *stu* markers. If one or both of these strains is

derived from a non-pigmented germinable ascospore no complementation takes place, although the *stu* markers were originally derived from the same species. It cannot be assumed that the differences in cytoplasm may be responsible for this result; for example, the inability of *S. heterothallis* proteins to be formed on *S. thermophila* polysomes. Lewis (1969) has shown that the genome of *S. heterothallis* can express itself normally in the cytoplasm of *S. thermophila* and vice versa. Another explanation for these irregular phenotypic expressions may be that the nuclei in these ascospores are aneuploid. This appears to be the explanation for the ascospore culture no. 2 of cross no. 1, which may have lost its extra chromosomes of *S. heterothallis* during successive subculturing. In this case, however, the *a* mating type allele from *S. heterothallis* appears to have been incorporated into the mating type chromosome of *S. thermophila* resulting in a recombinant genome. In the genus *Neurospora* Dodge (1931) was able to introduce certain factors from *N. sitophila* into *N. crassa* and show their normal segregation in eight-spored asci from the $f_1 \times N. crassa$ backcross. More detailed work along these lines in *N. crassa* has been done by Fincham (1951), and Scott-Emuakpor (1965), who have compared the recombination frequencies of markers first in one species and then after transfer into the genome of the other. One result of the presence of aneuploid nuclei would be the eventual formation of heterocaryotic strains (Pittenger, 1954) as in ascospore culture no. 11 of cross no. 3. This phenotypically wild type culture was shown to contain nuclei containing the *stu₅* marker. On the other hand the phenotypically stunted culture of ascospore no. 1 cross no. 3 when crossed with the wild type strain of *S. thermophila* gave rise to asci with eight spores which formed cultures with wild type growth. This suggested that only nuclei without the *S. heterothallis stu* marker took part in the formation of these asci.

The recovery of *Sordaria heterothallis* and *S. thermophila* from recombinant ascospores by backcrossing is similar in some respects to the work done with *Neurospora* by Dodge (1928, 1931, 1936).

The hybrid asci obtained from crosses between *Neurospora sitophila* and *N. tetrasperma* (Dodge, 1928) showed less abortion than did those in the hybridization of our *Sordaria* species. There was better delimitation of ascospores, and a large number of mature ascospores were obtained, although the number of perithecia produced seems to have been very small. Dodge was able to recover *N. tetrasperma* after two backcrosses, as has been the case with *Sordaria thermophila* in the present study. Dodge recovered the *N. sitophila* genome by intercrossing certain f_1 isolates. When f_1 from interspecific crosses in *Sordaria* were crossed among themselves the asci contained more rather than fewer aborted ascospores.

Hybridization studies in *Sordaria* suggest that these two species are less closely related than the two species, *Neurospora sitophila* and *N. crassa*. A large number of f_1 ascospores with mixed genomes which were recovered from the *Neurospora* hybridization appeared morphologically and physiologically normal, and markers introduced from both species were expressed normally (Dodge, 1931). Very similar results were obtained in crosses involving *N. sitophila* and *N. tetrasperma* (Dodge, 1931, 1936). The fact that strains from the f_1 non-pigmented ascospores reacted interspecifically with both species of *Sordaria*, although they were composed of parts of the genomes of both species, as has been shown through the results of genetic analysis, suggests that there must be very little affinity between the two species.

The author wishes to express his appreciation to Professor Lindsay S. Olive for his guidance and support, and for his many helpful suggestions during the course of these studies, and to Dr W. G. Fields for his advice and suggestions. The willingness of Dr Fields to supply the cultures used in this study is gratefully acknowledged.

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EXPLANATION OF PLATE

PLATE I

Fig. 1. Portion of Fig. 2 enlarged to show non-pigmented ascospore germinating in an ascus plated in corn meal agar. $\times 2500$.

Fig. 2. Interspecific cluster from *Sordaria heterothallis* $\times$ *S. thermophila* cross, showing asci with aborted ascospores in all stages of development. Note ascus with germinating ascospore. $\times 550$.

Fig. 3 to 4. Magnifications, $\times 350$.

Fig. 3. Clusters of asci with 1 to 8 mature ascospores from the following cross: Mycelium from non-pigmented f_1 ascospore no. 1 of cross no. 3 (Table 1) *A* $\times$ *S. thermophila a*.

Fig. 4. Cross similar to that in Fig. 3. Asci from perithecia which produced interspecific clusters.

Fig. 5 to 7. Magnifications, $\times 350$.

Fig. 5. f_3 *S. thermophila* ascospores from the f_2 back-cross of mycelium derived from spore of cluster shown in Fig. 3, to *S. thermophila* wild type.

Fig. 6. f_3 asci mostly with 6 to 8 mature ascospores from the following cross: f_2 ascospore progeny *a* (Fig. 3 ascospore 26₁) $\times$ *S. heterothallis A*. Most of these f_3 ascospores show a resemblance to *S. heterothallis* wild type ascospores.

Fig. 7. f_4 *S. heterothallis* ascospores obtained by crossing progeny from clusters like those in Fig. 6 with *S. heterothallis* wild type.