GENETICS OF HYBRIDIZATION AND INCOMPATIBILITY BETWEEN ISOLATES OF SORDARIA FIMICOLA

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ABSTRACT

GENETICS OF HYBRIDIZATION AND INCOMPATIBILITY
BETWEEN ISOLATES OF SORDARIA FIMICOLA

By

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Incompatibility and compatibility (hybridization) between isolates of Sordaria fimicola (Rob.) Cesati and DeNotaris were investigated. Eight wild-type isolates of S. fimicola from seven different geographic locations and ascospore color mutants from each were used in this study. Map distances and limited linkage data are presented for each color mutant and germination data for both wild-type and mutant isolates is given.

The eight isolates were crossed in pairs in all possible combinations, employing wild-type as one member of the pair and an ascospore color mutant as the second member, to determine compatibility or incompatibility. Presence of hybrid perithecia, where mycelia from the two isolates intermingled, indicated compatibility; while incompatibility was assumed when examination of 1000 perithecia, showed no hybridization.

Two isolates, 11 and 20, were compatible with all other isolates. Isolates 21 and 23 were compatible with all isolates except 30. Isolate 18 was compatible with all isolates except 19; 19 was compatible with all isolates except 6 and 18. Isolate 6 was compatible with all isolates except 19 and 30.

These initial data were used to determine 11 pairs of isolates which differed in compatibility with a third isolate denoted I_x.

Isolates 18 and 20 (K^{8}) differed in compatibility with isolate 19 and were therefore crossed. F_1 tetrads from this cross were tested for compatibility with I_x19. Similarly, progeny from cross 11 X 19 (K^{12}) were tested for compatibility with I_x18. Other isolate pairs were examined in the same manner.

F_2 tetrads from these $K^{\#}$ X I_x crosses could not be isolated for backcrossing to each $K^{\#}$ parent due to poor germination of spores. Several methods were employed to overcome the reduced germination, but without success. Poor germination was noted at the parental cross and F_1 X I_x cross levels.

On the basis of the data collected, it was obvious that some common factor(s) between two isolates must be present for compatibility to be observed. The nature of the segregations from F_1 X I_x crosses indicates that a multiple-locus system must be operating to confer incompatibility on two paired isolates.

Although a precise incompatibility mechanism cannot be proposed from the data obtained, it appears that isolates of Sordaria fimicola are evolving into species. This hypothesis correlates with Olive's (1954, 1956, 1958) and with Esser's (1965, 1971) definition of heterogenic incompatibility and its consequences.

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INTRODUCTION

Sordaria fimicola (Rob.) Cesati and DeNotaris is a homothallic pyrenomycete belonging to the order Sphaeriales. Members of the Sphaeriales are characterized by the production of dark, pear-shaped, ostiolate perithecia which contain unitunicate asci and paraphyses arising from a hymenial layer (Alexopoulos, 1962). S. fimicola reproduces solely by sexual reproduction resulting in ascospores; the ability to produce conidia having been lost or perhaps never existent.

Eight ascospores are arranged in an orderly, linear manner within each ascus. Normal, wild-type ascospores are dark in color; however, ascospore color mutants can be obtained through ultraviolet radiation treatment. Thus, the ascospore color mutants provide a simple, visual tool for identification of hybrid perithecia (Olive, 1956). These characteristics, in addition to the short generation time of eight days, make Sordaria fimicola a good subject for genetic analysis.

Recently, the subject of heterogenic incompatibility and hybridization in homothallic species of fungi has come under a great deal of review and discussion (Esser, 1971). The best known example of heterogenic incompatibility, described by Karl Esser, is in Podospora anserina. Indeed, the terms heterogenic and homogenic, as applied to incompatibility were introduced by Esser (1959).

Heterogenic incompatibility is defined as the prevent of karyogamy and zygote formation when the two nuclei carry different

incompatibility factors. Heterogenic incompatibility is observed when different geographical races of the same species are crossed. This mechanism may be operating in S. fimicola. Although a homothallic ascomycete, geographical races of S. fimicola, when crossed, frequently produce hybrid perithecia (Fields, 1970). In some crosses, however, no hybrid perithecia are formed. This observation suggests the presence of an incompatibility mechanism.

It is this phenomenon that is the subject of this research. What is the nature of the incompatibility mechanism? What processes are affected by hybridization? How many loci may be involved in incompatibility? Is a heterogenic incompatibility mechanism operating? If so, what are the consequences?

METHODS AND MATERIALS

Cultures

Eight single-spore isolates of Sordaria fimicola were used in this study: 6, 11, 18, 19, 20, 21, 23, and 30. These wild-type isolates and ascospore color mutants for each were obtained from Dr. William G. Fields at Michigan State University. Table I gives the source and geographical location of the wild-type isolates.

Olive (1956) showed that his isolates A-1 and C-7 of S. fimicola were compatible with certain of his isolates but not with others. Dr. William G. Fields (personal communication) indicates that of 40 isolates of S. fimicola in the Michigan State University collection, two distinct groups are found: those compatible with A-1 and those compatible with C-7. The two groups are not intercompatible. All of the isolates used in this study were of the C-7 group and therefore, all were compatible with C-7.

Each ascospore color mutant was backcrossed with its corresponding wild-type for at least three generations to eliminate any structural aberrations that may have been present. Ascospore color mutants are designated as follows: br = brown, g = gray, h = hyaline, t = tan, and y = yellow. Ascospore color mutant 30g was not easily used because of its very slow growth rate as compared with all other isolates. $19y^{1}$(st) is a sterile mutant. It produces no perithecia when grown alone; however, when crossed with a compatible isolate,

TABLE I

Source and geographical location of each wild-type isolate.

Isolate number	Source	Geographical location
6	Cow dung	Pellston, Michigan
11	Rabbit dung	Big Bend National Park, Texas
18	Raccoon dung	Douglas Lake, Michigan
19	Deer dung	Vanderbilt, Michigan
20	Deer dung	Baldwin, Michigan
21	Deer dung	Baldwin, Michigan
23	Horse dung	Mackinac Island, Michigan
30	Cow dung	Paint Rock, Texas

mutant as well as hybrid perithecia are formed. The ascospores in the hybrid perithecia exhibit approximately 70% germination.

Stock cultures were maintained on corn meal + agar slants at 5°C.

Media

Isolates were crossed on B agar: 17g corn meal agar, 2g dextrose, 3g sucrose, 1g yeast extract, and 1000 ml distilled water. Acetate agar (Bretzloff, 1956) was used as germination medium: 17g corn meal agar, 1g yeast extract, 7g sodium acetate, and 1000 ml distilled water. Dissecting agar was used during micromanipulation: 1g corn meal agar, 3g plain agar, 3g sucrose, 2g dextrose, 0.1g yeast extract, 0.7g sodium acetate, and 100 ml distilled water. Stock cultures were maintained on corn meal + agar slants: 17g corn meal agar, 2g glucose, 1g yeast extract, and 1000 ml distilled water.

Isolation of ascospore color mutants

Because of the poor germination of some of the ascospore color mutants, several attempts were made to secure new color mutants. An additional mutant, 11h, was obtained from isolate number 11 using the following method:

Isolates were inoculated onto B agar and allowed to grow for 24 hours. Cultures were then incubated at 5°C for 24 hours before irradiation. Directly after removal from 5°C, the mycelia were irradiated for 5 minutes using a General Electric, 15 watt germicidal lamp at a distance of 5 inches. Plates were left at room temperature for 4 hours at which time mycelia were irradiated a second time for

5 minutes. Petri dishes were kept in the dark between each irradiation period and for 4-6 hours after the second irradiation to prevent photoreactivation and subsequent repair.

At maturity, perithecia were examined singly until one was found which had an approximate 1:1 segregation of mutant to wild-type ascospores. Several mutant spores were dissected out with the aid of a micromanipulator and allowed to germinate and grow. If no sterility was observed, one ascospore isolate was selected and backcrossed to its corresponding wild-type. The new mutant was then checked for adequate germination.

5-bromouracil and hydroxylamine were also used in other attempts to increase the incidence of ascospore color mutants; however, results were negative. A potentially less laborious method of securing mutant ascospores was also attempted. This involved allowing spores from the irradiated mycelia to discharge onto plain agar (no sodium acetate added) to recover mutant spores, which can germinate without the presence of acetate. However, no mutant ascospores could be isolated using this technique.

Crossing of isolates

Since S. fimicola is homothallic, mycelia derived from single ascosporic cultures will produce perithecia with asci homozygous for any genetic factor. However, different isolates may be crossed by placing inocula near one another on agar plates. Mycelia from each inoculum will produce abundant perithecia, but in the area where mycelia from the inocula intermingle, some hybrid perithecia may be produced. That is, if the inocula were different genetically, some

perithecia would be produced which contain asci that are heterozygous (Bistis and Olive, 1954).

In this study, each wild-type isolate was crossed with each ascospore color mutant by placing inocula of each approximately 1 cm apart in the center of a plate of B agar. The plates were incubated at 25°C for 8-9 days. At maturity, 50-1,000 perithecia in the area where the mycelia intermingled were examined for hybrid asci. The number examined depended on how quickly hybrid perithecia were discovered. After 1,000 perithecia were examined (the number chosen arbitrarily) and no hybrid perithecia found, incompatibility between the two isolates of the cross was assumed.

Occasionally, two isolates inhibited each other by restricting growth, halting maturation of perithecia or by forming bands of dark hyphae at the intersection of the isolates in which there were no perithecia. Olive (1956) suggested that this inhibition may be overcome by inoculating the two isolates at the same point on an agar plate. However, this was not successful and subsequent inhibition was interpreted as incompatibility.

RESULTS

The map distance of each ascospore color mutant from the centromere of its linkage group as well as limited linkage data are given in Table II. Each mutant was crossed with its corresponding wild-type and asci from hybrid perithecia were examined. This table shows that the distance of each ascospore color mutant from the centromere of its linkage group, with the exceptions of 1ly, 11br, 19y^{1}(st), and 20t, approaches the limit of resolution for mapping one gene as determined by tetrad analysis. Hence, the distances given in map units are minimum; in reality, the locus for the color mutant may be much further away from the centromere.

From the limited linkage data, it appears that none of the mutants are linked, with the exception of 1ly and 11br. Only parental ditype asci were observed in this cross (no tetratypes) which concurs with the fact that both mutants map at approximately the same distance from the centromere. However, one mutant is apparently modified in hybrid asci, becoming darker in color, but not as dark as wild-type spores. Crosses were also made of 18br X 11h, 18br X 19g, 19g X 11h, 19g X 20t, and 19g X 21t, but no hybrid perithecia were found (300-400 perithecia were examined). Hence the linkage data on these crosses could not be obtained.

Germination percentages for each wild-type isolate and its corresponding color mutant(s) are given in Table III. Germination

TABLE II

Linkage data and map distances of each ascospore color mutant from the centromere of its linkage group.

Ascospore color mutant	1st division segregation	2nd division segregation	Total	Frequency 2nd division segregation	Map distance
6h	58	101	159	63%	31.5
11y	219	141	360	39%	19.5
11br	131	88	219	40%	20.0
11h	109	142	251	62%	31.0
18y	109	152	261	58%	29.0
18br	115	172	281	61%	30.5
$19y^2$	181	126	307	41%	20.5
19g	95	116	211	54%	27.0
$19y^1$(st)	285	0	285	0%	0.0
20t	244	3	247	1.2%	0.6
21t	131	146	277	52%	26.0
23y	115	116	231	50%	25.0
30g	79	99	178	56%	28.0

Linkage data:

Cross	Parental ditype	Nonparental ditype	Tetratype	Conclusion
11y X 11h	66	36	67	unlinked
11br X 11h	58	51	62	unlinked
11br X 11y	76	0	0	linked
18y X 18br	31	31	59	unlinked
19y X 19g	19	20	34	unlinked
18br X 20t	24	20	68	unlinked

TABLE III

Germination of ascospores from wild-type and ascospore color mutant isolates.

Isolate	Germination percentage
6h	43
11+	93
11y	50
11br	18
11h	37
18+	83
18y	*
18br	55
19+	94
19y2	32
19g	82
20+	92
20t	70
21+	83
21t	83
23+	82
23y	48
30+	94
30g	85

*Not tested, ascospores coalesced in homozygous asci.

percentages for all isolates were based on approximately 300 spores. Acetate agar was used as the germination medium.

Crosses of all isolates in pairwise combinations were made to determine whether hybrid perithecia were produced. Table IV shows the results of these crosses. The occurrence of hybrid perithecia indicated that crosses were compatible and the absence of hybrid perithecia indicated incompatibility. These data show that most of the paired isolates were compatible.

Generally, in compatible crosses, only 10% or less of the perithecia along the intersecting isolate lines were found to be hybrid. The only notable exceptions to this were crosses of each mutant with its corresponding wild-type isolate. Here, 20-30% of all perithecia produced were hybrid. On close examination of compatible crosses, it was noted that hybrid perithecia from all crosses (except 18 X 21) were observed only along the intersecting isolate lines. Thus, the extent of nuclear migration was spatially limited in all crosses, except that same-isolate crosses produced more hybrid perithecia. Since S. fimicola produces no antheridia, heterokaryotic mycelium resulting in hybrid perithecia probably originated through hyphal anastomoses (Carr and Olive, 1958). It is not known whether incompatibility between isolates selected for this study is due to failure to anastomose or to unknown factors occuring in some later stage of ascogonial development.

Table IV shows that isolates 11 and 20 have similar compatibility patterns as do isolates 21 and 23. Other pairs of isolates differed from one another in compatibility with one or more isolates. Isolates

TABLE IV

Results of crosses of all isolates in pairwise combinations.

+ denotes hybrid perithecia formed (compatibility)

- denotes no hybrid perithecia formed (incompatibility)

Isolate number	Isolate number							
	6	11	18	19	20	21	23	30
6	+	+	+	-	+	+	+	-
11	+	+	+	+	+	+	+	+
18	+	+	+	-	+	+	+	+
19	-	+	-	+	+	+	+	+
20	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	-
23	+	+	+	+	+	+	+	-
30	-	+	+	+	+	-	-	+

18 and 20 differed from one another in that 20 was compatible with 19, but 18 and 19 were incompatible. The same result was found with 11 and 18. 11 was compatible with 19, but 18 was not. In these examples, 18 and 20 were compatible as were 11 and 18. Thus, in terms of incompatibility, pair 18 and 20 as well as pair 11 and 18, differed from one another in their reactions with only a single isolate, number 19 (denoted as I_x). Likewise, pair 11 and 19 and pair 19 and 20 differed in compatibility with one isolate, number 18 (also denoted as I_x).

Other isolates differed in compatibility with two isolates. Pair 18 and 21 differed in compatibility with both isolates 19 and 30, as did pair 18 and 23. In these cases, 18 was not compatible with isolate 19, but was compatible with 30, while both 21 and 23 were compatible with 19, but not with 30. Isolate 6 was compatible with all isolates except 19 and 30.

For five pairs of isolates which differed in compatibility with a third isolate, I_x, 18 and 20, 18 and 21, 18 and 23, 11 and 19, and 6 and 20, F_1 tetrads were isolated with little difficulty. However, for pairs 11 and 21, 11 and 23, 11 and 18, 19 and 20, 19 and 21, and 19 and 23, complete tetrads could not be recovered. In each case, when the isolates were crossed, hybrid perithecia were produced. When hybrid asci were examined, however, one, two, three, or all four members of the tetrad (spore pairs) failed to germinate. There was no discernible pattern as to which ascospore, mutant or wild-type, would not germinate and segregation of non-germination was not consistent from tetrad to tetrad within each cross. This problem was

especially perplexing in a cross such as 19 X 20, where each isolate alone had at least 70% germination of ascospores.

Olive (1956) reported a similar observation when crossing ascospore color mutants of S. fimicola with wild-type isolates. The mutant spores had a "remarkable decrease" in germination when isolated from heterozygous asci.

Since S. fimicola is a homothallic fungus and is therefore not usually crossed with other isolates, the granular appearance of mutant ascospores and the germination failure of both mutant and wild-type ascospores in heterozygous asci may have been due to some cytoplasmic lethality factor and/or to an unknown genetic mechanism. Structural aberrations did not appear to be the cause of the poor germination since first and second division segregations in hybrid asci were in proportion to those numbers obtained in the original mapping of the isolates in all cases studied escept in the cross of 18 X 21.

In this cross, each wild-type member in each recovered tetrad, when grown out alone on B agar, produced numerous hybrid perithecia containing aberrant 6:2 asci. Most of these asci were 6+:2br, although 6br:2+ appeared as well. Contrary to the pattern of all other crosses where hybrid perithecia were found only along intersecting mycelial lines, these hybrid perithecia containing the aberrant asci were found all over the plate. Original stock cultures of 18^{+}, 21^{+}, 18br, and 21t were then grown out on B agar, but only normal homozygous asci were observed. It appears, therefore, that the cross of 18 X 21, regardless of which isolate was the wild-type parent, results in some chromosomal (probably structual) aberration.

This phenomenon resulted in some difficulties when the tetrads were crossed with the I_x. Asci appearing to be from hybrid perithecia had to be studied closely in order to discern the segregation in the asci. This extreme care was necessary to distinguish aberrant asci from those which may actually have been present in hybrid perithecia from the cross of F_1 tetrads X I_x. The difficulties encountered led to the abandonment of this cross, 18 X 21, as a source of information in this study.

Pairs of isolates which differed in compatibility patterns by one isolate (denoted as I_x in each case) which one of the pair was not compatible with, such as pair 18 and 20, where I_x = 19, were analyzed genetically. Each pair with this characteristic was crossed and 9-10 F_1 tetrads from hybrid perithecia were isolated with the aid of a micromanipulator. The F_1 tetrads were analyzed by crossing each spore pair of the tetrad with the differing isolate, I_x, and noting the segregation of compatibility and incompatibility with that isolate. The results of these crosses are given in Tables V-VII. A tetrad member was classified as incompatible with the I_x isolate only after a minimum of 1000 perithecia were examined.

Table V shows that tetrads from the cross 18 X 20, when crossed with I_x19, demonstrate every conceivable combination of compatibility and incompatibility, i.e. tetrads had from 0 to all 4 members compatible with isolate 19. This was not the case when tetrads from the cross 11 X 19 were crossed with I_x18. Here, as Table VI shows, tetrads had from 0 to 3 members compatible with isolate 18. The

TABLE V

Compatibility and incompatibility of F_1 tetrads from cross 18 X 20 with I_x 19.

Tetrad number	Tetrad spore pair	Compatibility (+) or incompatibility (-) with I_x
1	1	+
	2	+
	3	-
	4	+
2	1	+
	2	+
	3	+
	4	+
3	1	+
	2	+
	3	+
	4	+
4	1	-
	2	-
	3	-
	4	-
5	1	+
	2	-
	3	-
	4	-
6	1	-
	2	+
	3	+
	4	+
7	1	-
	2	+
	3	-
	4	+
8	1	-
	2	-
	3	+
	4	-

Table V (Cont'd)

Tetrad number	Tetrad spore pair	Compatibility (+) or incompatibility (-) with I_x
9	1	+
	2	+
	3	-
	4	-
10	1	+
	2	+
	3	+
	4	-

TABLE VI

Compatibility and incompatibility of F_1 tetrads from cross 11 X 19 with I_x18.

Tetrad number	Tetrad spore pair	Compatibility (+) or incompatibility (-) with I_x
1	1	-
	2	+
	3	-
	4	-
2	1	+
	2	+
	3	-
	4	-
3	1	-
	2	-
	3	+
	4	-
4	1	+
	2	-
	3	+
	4	+
5	1	-
	2	+
	3	-
	4	-
6	1	+
	2	-
	3	-
	4	-
7	1	-
	2	-
	3	-
	4	-
8	1	-
	2	-
	3	-
	4	+
9	1	-
	2	-
	3	-
	4	-

fewer number of different ascal classes recovered from this cross may simple be due to the fact that not enough tetrads were analyzed.

In Table VII, tetrads from cross 18 X 23, when crossed with I_x19, had only 0, 1 or 3 members compatible with isolate 19. The ascospore color mutant for isolate 23, 23y, had relatively poor germination (48%) and since no other mutant could be isolated; this cross was abandoned as a source of further information so that data are incomplete for this table.

Ideally, tetrads were to be isolated from each cross of $K^{\#}$-F_1 tetrads X I_x. However, the phenomenon of reduced germination in heterozygous asci was again encountered (as with some of the original $K^{\#}$ crosses). This second group of tetrads was to be backcrossed to each of the original F_1 parents to secure a more accurate picture of the number of loci involved in the incompatibility mechanism and whether or not these loci are linked or unlinked.

In an attempt to overcome the problem of reduced germination in the $K^{\#}$-F_1 X I_x crosses, several methods were employed. For each method, the cross 18 X 20 (K^8) was used as the test cross. Since dissecting agar, which contains sodium acetate, was used in all cases of tetrad isolation, absence of acetate was ruled out as a cause of the poor germination.

The first method involved the use of a sterile mutant, $19y^1$(st) as the mutant I_x isolate. Although $19y^1$(st) produces no perithecia when grown homozygously, when paired with a compatible isolate, mutant ascospores are produced in heterozygous asci. Wild-type members of the F_1 tetrads of the cross 18 X 20 were paired with $19y^1$(st) and

TABLE VII

Compatibility and incompatibility of F_1 tetrads from cross 18 X 23 with I_x19.

Tetrad number	Tetrad spore pair	Compatibility (+) or incompatibility (-) with I_x
1	1	-
	2	+
	3	+
	4	+
2	1	+
	2	-
	3	-
	4	-
3	1	-
	2	-
	3	-
	4	-
4	1	-
	2	+
	3	-
	4	-
5	1	-
	2	-
	3	-
	4	-
6	1	-
	2	-
	3	-
	4	-
7	1	+
	2	-
	3	-
	4	-
8	1	-
	2	-
	3	-
	4	-
9	1	-
	2	-
	3	-
	4	-

the resultant hybrid asci dissected and spores examined for germinability.

With this method, germination of all spores was increased considerably as is shown in Table VIII. Three to four pairs of spores in each resulting tetrad germinated. However, in many cases, only one or two members of the tetrad would continue to grow while the remaining two or more aborted after growth of only a few millimeters. As the table shows, there seems to be no discernible pattern to predict which spores would germinate and abort and which would continue to grow.

A second attempt to overcome reduced germination involved the use of a new isolate which was employed at the parental, $K^{\#}$ cross level. Several new mutant isolates were tested in order to find one with a compatibility pattern similar to isolate 18. Isolate 6h was selected, although it did not have an identical pattern (see Table IV) and its germination percentage was quite low (43). 6h and 20 were paired and resultant hybrid asci dissected. These F_1 tetrads were then paired with I_x19. Progeny from this cross were isolated, with the same result as all other cross (except those with $19y^1$(st) as the mutant I_x isolate). Only one or two members of each tetrad germinated and grew. There was no germination and subsequent abortion as with K^8 progeny X $19y^1$(st). The cross, 6h X 20, was therefore abandoned as means of securing more data on incompatibility between the isolates chosen for this study. With all the methods attempted failing to eliminate or reduce the germination problem, the F_2 progeny X each parent phase of genetic analysis was omitted.

TABLE VIII

Results of the cross of K^{8} (18 X 20) tetrads X $19y^{1}$(st) to determine germinability and subsequent growth of spores.

M = mutant spore pair
WT = wild-type spore pair
___ indicates germination
* indicates abortion (germination but no subsequent growth)

Cross	Tetrads			
K^{8}-6-3 X $19y^{1}$(st)	M*	M	WT	WT
	WT*	WT	M	M
K^{8}-2-2 X $19y^{1}$(st)	M	M	WT*	WT*
	WT	WT*	M*	M
	M*	M*	WT*	WT
	M*	M*	WT	WT*
K^{8}-2-1 X $19y^{1}$(st)	WT*	WT	M	M*
	M	M	WT	WT*
	WT	WT	M	M*
K^{8}-3-2 X $19y^{1}$(st)	M*	M	WT*	WT*
	WT*	WT*	M	M*
	M*	M	WT*	WT*
	WT*	WT	M*	M
	WT*	WT	M	M
	M*	M	WT	WT
	WT*	WT*	M*	M
K^{8}-1-4 X $19y^{1}$(st)	M*	M*	WT	WT
	M*	M	WT	WT
	M*	M*	WT*	WT
	WT*	WT	M*	M*
K^{8}-10-3 X $19y^{1}$ (st)	M	M	WT	WT*
	WT	WT	M*	M*
	WT	WT*	M	M
	M*	M*	WT	WT

To collect further concrete evidence for an incompatibility scheme, F_1 tetrads of both K^{8} (18 X 20) and K^{12} (11 X 19) were backcrossed to each respective parent. Every member of every tetrad was compatible with both parents. Furthermore, members of each tetrad were intercompatible as well as being compatible with members of other tetrads in the same cross.

DISCUSSION

From the initial compatibility data in Table IV, it appears that among the eight geographical isolates chosen for this study, there are six "incompatibility groups." One such group includes isolates 21 and 23, another 11 and 20, while 6, 18, 19, and 30 each compose one group. These "incompatibility groups" have been referred to as geographical races by Esser (1971) and as subspecies by Caten and Jinks (1966). Although there is some differential compatibility between isolates when paired, incompatibility does not mean that the isolates do not belong to the same species.

When all the data are considered, it appears that for compatibility (hybridization) to be observed, at least one common allele, between two isolates, is required. Considering the nature of S. fimicola as a homothallic fungus and therefore self-fertile, if a genetic system is indeed operating to confer compatibility, it follows that like alleles would be required for compatibility and unlike alleles (at one or more loci) would determine incompatibility. Esser (1965) stated this in the following way: "Since the (homothallic) fungus is self-compatible, the inter-race incompatibility may be due to heterogeneity" (parentheses mine). Data from crosses of F_1 progeny of both K^8 and K^{12} to each respective parent and amongst themselves illustrate the hypothesis that some common factor(s) was inherited by each member of each tetrad resulting from a compatible cross.

Several observations can be made when the data are viewed collectively. First, the "relative heterothallism" noted by Pontecorvo (1953) in Aspergillus nidulans, in which more than 50% of the perithecia from a single cross were hybrid, was not noted in this research. In fact, less than 10% of all perithecia from any one cross were hybrid except those between a wild-type isolate and its ascospore color mutant(s). This suggests that there may be some possible selection against hybridization at some point in the sexual cycle, perhaps in limiting nuclear migration. Cross 18 X 21, however, clearly exhibited a different set of characteristics.

In this cross, hybrid perithecia were found in abundance (although not 50%) all over the plate. There was no limitation to nuclear migration. However, there appeared to be a structural aberration which produced 6:2 asci almost exclusively in hybrid perithecia. These phenomena occurred only in this cross. Thus, isolates 18 and 21 appear to be only semi-compatible, producing hybrid perithecia but with aberrant asci.

An analysis of Tables V-VII shows that at least 5 different ascal classes result from these crosses. Cross 18 X 20 (Table V) produced all 5 classes, i.e. F_1 tetrads were produced with every combination of compatible and incompatible (with I_x19) pairs of spores. These various combinations (of 0-4 compatible spores pairs in each tetrad) and the fact that there is not a 1:1 segregation of the compatibility factor indicate that more than 1 or 2 genes are probably involved.

Also obvious in the data presented are the germination difficulties observed in spores of hybrid asci. These difficulties may be

related or due to a number of factors. Cytoplasmic lethality factors, the phenomenon of hybridization in a homothallic fungus, or one or more nuclear genetic factors including those which may be involved in incompatibility may be a cause of reduced germination. As noted previously, there does not appear to be any pattern as to which type of spore, mutant or wild-type, is subject to reduced germination.

The phenomenon of germ-tube production and subsequent abortion and cessation of growth as observed in F_1 progeny of 18 X 20 (K^8) X $19y^1$(st) (Table VIII) is particularly unique. Although other crosses exhibit this sequence of events occasionally, it is most noticeable in this cross. A possible cause to be considered here is that enough chromosomes are paired to permit development and germination of spores; however, the nucleus may cease to divide after germ-tube production, thereby curtailing any further growth. The block halting the completion of the sexual cycle in this cross seems to affect the growth of mycelium following germination, although germination itself appears to proceed normally. This is unlike other crosses where spore maturation as well as germination are primarily affected at both the parental cross and F_1 X I_x cross levels.

Germination difficulties made complete data collection impossible. Further crossing could have shown how many loci may be involved in incompatibility, whether these loci are linked or unlinked and whether they are indeed separate loci or simply alleles at one locus. However, on the basis of the data that were obtained, several mechanisms for incompatibility can be discussed.

It is obvious from Tables V-VII, that there is not a 1:1 segregation of the incompatibility locus (loci). Also apparent is the fact that some common factor(s) must be present for compatibility to be conferred on two isolates and that this factor(s) is inherited by all progeny of a compatible cross. Any proposed mechanism for incompatibility must take into consideration these facts.

A one-locus, 2-allele (or more) system obviously could not explain the results. The alleles would need to be identical in order for initial compatibility to occur between selected parent isolates: all progeny would then inherit this common allele and incompatibility between one parent with a third isolate could not be explained. A 2-locus-2-alleles at each locus scheme would not explain the data for similar reasons. In keeping with the premise that at least one factor must be in common for compatibility to occur between 2 isolates, one allele at at least 1 locus must be the same in the chosen parent isolates. The result would be that the second locus, if segregating independently, must be different which would give only a 1:1 segregation in the progeny for compatibility at this second locus. This can be better illustrated using the example of cross 18 X 20. Recall that 18 and 20 are compatible with each other; however, 18 is not compatible with 19, while 20 is compatible with 19. Two loci, A and B, with alleles x and y at each locus are chosen with a common allele, x, at the A locus conferring compatibility between 18 and 20. This would fulfill requirements of common alleles for compatibility, however, the cross of 18 X 20 would yield a 1:1 segregation at the B locus (if each parent possessed a different allele at this locus) which

clearly is not represented in the data of the F_1 progeny of 18 X 20 when paired with I_x19.

These arguments can be applied to many schemes involving more than 2 loci. However, as one proposes more and more unlinked loci, one is bound to discover a scheme that will fit the data collected. For instance, a 5 locus system with 2 alleles at each locus and common alleles at either loci A and B or at C and D or at E, where 18 has a genotype of ABCDE, 20 of abcdE, and 19 abcde would fit the data given in Table V. Although this scheme may fit the data collected, it is not feasible to propose it because of the lack of data (due to germination difficulties in backcrossing) to confirm it. It is also unknown as to when in the sexual cycle these incompatibility loci act, whether in pre- or post-plasmogamy.

Although a precise mechanism cannot be proposed, it would appear from the data collected that 1) several loci are involved, 2) like alleles at one or more of these loci confer compatibility, and 3) incompatibility is a function of one or more unlike alleles at major incompatibility loci. This latter condition is, in essence, the basis of a heterogenic incompatibility mechanism. The significance of heterogenic incompatibility in homothallic fungi, such as S. fimicola, has been discussed by Mather (1942), Olive (1958), Esser and Kuenen (1965), Caten and Jinks (1966), and Esser (1966, 1971).

The homothallic fungus, already self-fertile, is prevented from breeding with unlike genotypes in some instances, thereby reducing future variability within the species. With heterogenic incompatibility, an effective inbreeding mechanism, races or "incompatibility groups" become reproductively isolated from other isolates within the same

species which have differing genotypes at the incompatibility loci. According to Esser (1965), it is these races, not the species, which are actually the smallest units of evolution. It can be argued, then, that through geographical separation, races, fitting all the physical and morphological descriptions of the species, may be evolving into new species by the process of reproductive isolation.

Darwin's theory, which explains evolution and survival of the most fit through advantageous selection of small variations in the genetic complement, seems directly opposed to any evolutionary advantages of an inbreeding scheme which would limit variations. What, then, are the advantages and disadvantages of heterogenic incompatibility and homothallism for a fungus like S. fimicola?

A factor to be considered here is that S. fimicola has no asexual reproductive mechanism to produce numerous copies of the genetic complement which seems presently well-adapted. Homothallism accomplishes this through self-fertility, allowing variations only through mutation and perhaps through hybridization with compatible isolates (although the extent of hybridization in nature is not known). Through heterogenic incompatibility, present adaptation to the environment is good, but future adaptations may be limited because of little variation due to a high degree of homozygosity (Mather, 1942). However, as Olive (1954) pointed out, homozygous asci, of either parent, in hybrid perithecia are scarce or absent, indicating that mutant and wild-type nuclei may be attracted to each other oftener than 2 mutant or 2 wild-type nuclei in the same perithecium. This would tend to increase the genetic variation of a given isolate.

Two major theories have been advanced on homothallism and its position in the evolutionary scale in relation to heterothallism. Since compatibility between isolates in this study can be considered "unbalanced heterothallism" (Olive, 1958), a short discussion of these theories in pertinent although concrete evidence for both is lacking.

One theory, advanced by Whitehouse (1949), views homothallism as more highly evolved than heterothallism. He argues that a heterothallic fungus is involved in more outbreeding, therefore more evolutionary change through increased variation. The loss or modification of mating-type loci could develop a homothallic species (homothallic forms having a comparatively fixed genotype). Whitehouse also suggests that homothallism is perhaps more favored by natural selection because it is a scheme for certain sexual reproduction which does not depend on close proximity of spores or hyphae of compatible mating types. He does not, however, consider the reduced variability of homothallism due to inbreeding as a liability.

Opposed to this idea is Olive's theory (1958) which views compound loci, i.e. recombinable groups of loci closely related in function as well as on the chromosome, as the most feasible basis for an explanation of the development of balanced heterothallism from homothallism. Recombination between 2 pseudoallelic mutations, double mutations within a compound locus which separate into homologous chromosomes, or chromosomal deletions may result in self-sterility of normal self-fertile fungi. The cross-fertility then required for sexual reproduction would resemble 1-locus heterothallism. Also

considered in this theory is the fact that only 10% of the Ascomycetes are heterothallic while as high as 65% of Basidiomycetes, generally considered to be more highly evolved, are heterothallic.

Heterogenic incompatibility between two isolates (or geographical races) of a species resulting in unbalanced heterothallism or even the balanced heterothallism observed by Pontecorvo suggests a possible intermediate step between homothallism and heterothallism. Furthermore, the incompatibility between selected isolates, reduced germination of ascospores in hybrid asci, and the character of the segregations of F_1 progeny when crossed with I_x suggest a genetic mechanism for incompatibility that fits the definition of heterogenic incompatibility. If heterogenic incompatibility is indeed operating, the possibility that races may be evolving into separate species cannot be overlooked in S. fimicola.

However, this evolution in S. fimicola may not be strictly separated geographically as is the case with higher plants and animals. Tables I and IV illustrate this point. The source of isolates 11 and 30 is Texas, while the remaining isolates were obtained in Michigan. It is interesting to note that, although from sources 1500 miles apart, 11 is compatible with all Michigan isolates as well as isolate 30, and has a compatibility pattern identical to isolate 20. Thus, a geographical barrier may not produce the profoundly different changes in this fungus that may be noted in a higher organism. Also noted in these tables is the fact that although both isolates 20 and 21 were isolated from deer dung near Baldwin, Michigan, their compatibility patterns differ with one isolate, number 30. Here, no geographical barrier has separated the isolates,

yet they differ in compatibility with other isolates. With further study and a solution to the problem of reduced germination involved in hybrid asci and backcrossing, a more precise picture of the incompatibility mechanism operating between isolates of S. fimicola may be elucidated.

SUMMARY

1. Thirteen ascospore color mutants of Sordaria fimicola were mapped in relation to their centromeres and limited linkage data was collected. Germination data for each wild-type isolate and its ascospore color mutant(s) were also obtained.

2. Each of 8 wild-type isolates was crossed with each ascospore color mutant in pairwise combinations and compatibility (hybridization) and incompatibility were noted.

3. On the basis of the compatibility data, 6 "incompatibility groups" were defined.

4. Pairs of isolates differing in compatibility with a single isolate (I_x) were crossed and 9-10 tetrads for each pair were recovered. These F_1 tetrads were then crossed with the differing isolate (I_x) and compatibility and incompatibility noted.

5. Germination difficulties were encountered in both parental crosses and in F_1 X I_x crosses. No solution was found.

6. One cross, 18 X 21, gave aberrant asci in many hybrid perithecia, apparently due to a structural abnormality.

7. Data indicates that some common factor(s) between two isolates must be present for compatibility to be observed. Also indicated is that a multiple locus system must be operating in the incompatibility mechanism.

8. Although a precise incompatibility mechanism cannot be proposed from the data given, it appears that isolates of S. fimicola are evolving into species.

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