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Sexuality in *Neurospora crassa*

I. Mutations to male sterility

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SUMMARY

Mutations giving rise to sexual sterility were induced in *Neurospora crassa* macroconidia by ultraviolet-light irradiation. Thirty mutants were isolated on the basis of their male sterility in crosses with a wild-type strain. When used as the male parent these mutants exhibited a wide spectrum of sexual behaviour patterns ranging from the production of only small brown protoperithecia (complete male sterility) to the production of large and normally pigmented perithecia but with an undeveloped ostiole and very few if any spores. For many of the mutants the behaviour pattern is different when the strain is used as the female parent. Segregation data reveal that none of these mutants represent mutations of the mating-type locus. These findings suggest that the sexual development cycle is blocked at various stages in the different mutant strains. All attempts to restore fertility by supplying various additives to the medium or by varying the incubation time and temperature were unsuccessful. Conidial viability tests carried out on many of the strains revealed no abnormality in this respect. The aberrant segregation patterns exhibited by many of the mutants are discussed.

1. INTRODUCTION

Raper (1960) refers to two genetic control systems over reproduction in the fungi; the primary control is responsible for determining compatibility, whereas the secondary control is imposed at the level of the sexual development cycle, namely the sequence of events from plasmogamy through to ascospore discharge. The sequential nature of the cycle was first demonstrated by Dodge (1935) in *Neurospora sitophila* and *N. tetrasperma*. A more detailed account of sexual development, including an account of the hormonal control system involved, was reported in the genus *Achlya* by Raper (1940, 1951, 1957). Extensive studies have since been conducted on three homothallic Ascomycetes, through the analysis of mutant strains which arrest sexual development at various stages. Nine genes have been described in *Glomerella cingulata*, the effect of mutations of these genes ranging from inability to produce perithecia to the production of normal perithecia with aborted ascospores (Wheeler & McGahen, 1952; Wheeler, 1954). In addition to demonstrating complementation of function between two self-sterile

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mutants (McGahen & Wheeler, 1951), Wheeler (1954) has shown how these mutants could be arranged in a series of steps leading to completion of the sexual developmental cycle. In *Sordaria macrospora*, Esser & Straub (1956, 1958) have described 15 genes controlling the sexual cycle in the organism, these genes exerting their effect at one of six definitive stages of the cycle. In *Sordaria fimicola*, seven reproductive anomalies have been described (Olive, 1956; Carr & Olive, 1959).

Similar studies in heterothallic Ascomycetes have been somewhat less extensive. Through the study of normal sexual development in *Ascobolus stercorarius*, several stages of the cycle have been defined (Bistis, 1956, 1957; Bistis & Raper, 1963). In *Cochliobolus heterostrophus*, Nelson (1959*a*,*b*,*c*) has isolated mutant strains which produce sterile asci, sterile perithecia, or absence of perithecia, respectively. In *Neurospora crassa* various isolated reports of sterility appear in the literature, primarily concerning female sterility, namely failure to produce normal protoperithecia. Westergaard & Hirsch (1954) reported two such anomalies – lack of protoperithecia and abnormal protoperithecia – to be due to mutations of different genes. Fitzgerald (1963) has also shown that protoperithecial production is controlled by two genes. Tan & Ho (1970) have reported a mutation which destroys the ability to produce protoperithecia. Finally, evidence that environmental factors play a significant role in female fertility has been presented by Hirsch (1954) and Fitzgerald (1963).

It is evident from the above that a thorough study of the sexual development cycle in a heterothallic Ascomycete is still lacking. The present report treats the first phase of a such a study, namely, the description of 30 male sterile mutant strains in *Neurospora crassa*, all of which appear to affect various stages of sexual development.

2. MATERIALS AND METHODS

(i) *Strains*. The strains *pan*-1 (allele no. 5531)* and *leu*-3 (allele no. R 156)* of *Neurospora crassa*, chosen for their high degree of fertility and non-leakiness, were used as parental strains for mutation induction. The *fl* strains (locus no. L)* were employed as tester stocks for mating-type and sterility tests. St Lawrence stocks were used in crosses with mutant strains to demonstrate segregation of the mutant sterility gene. All strains were obtained from the Fungal Genetics Stock Center.

(ii) *Media*. All cultures were maintained on standard complete medium consisting of Vogel's Medium N (Vogel, 1956) supplemented with 0·5 % yeast extract, 0·5% casein hydrolysate, 0·1% standard vitamin solution and 0·005% tryptophan. All crosses were carried out on standard crossing medium (Westergaard & Mitchell, 1947) with L-leucine or calcium pantothenate added as required at the rate of 50 mg/l. Sorbose medium was used to obtain restricted colonial growth to facilitate single colony isolation (Tatum, Barratt & Cutter, 1949).

(iii) *Mutagenesis*. A 15 W General Electric G15T8 Germicidal Lamp was used to induce mutation. Conidial suspensions of 1×10^7 conidia/ml were irradiated in

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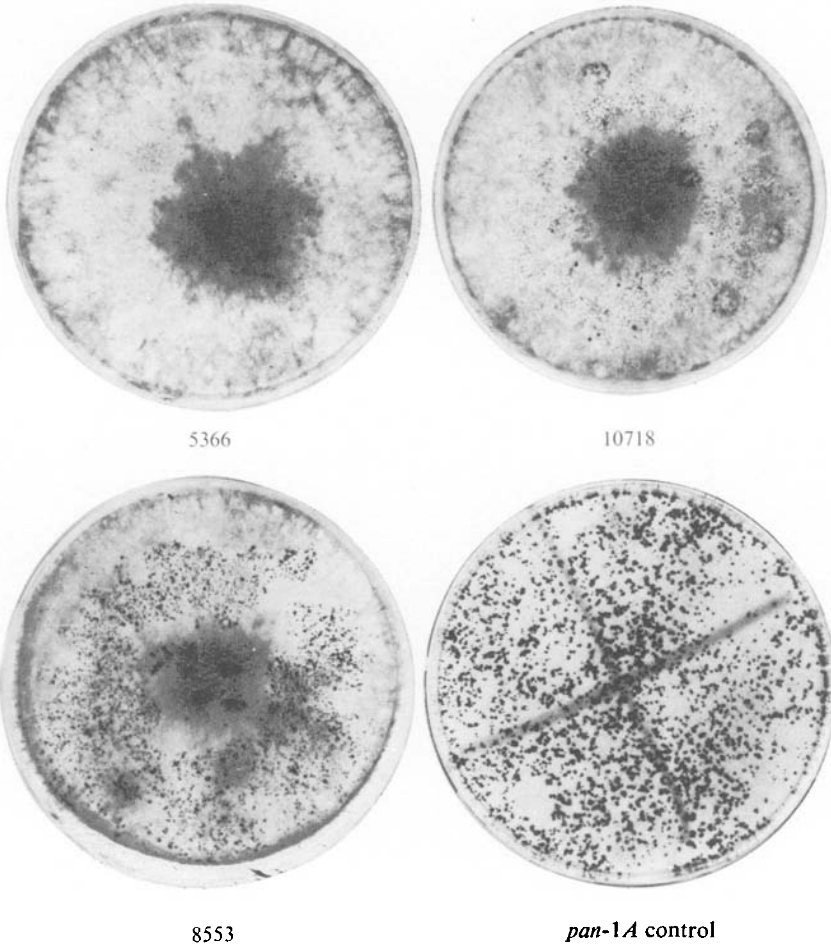

Photographs of crossing plates showing the sexual behaviour patterns of three group 1 and 2 male sterile mutants when crossed with a wild-type strain as compared to a control plate of a fertile (*pan*-1*A*) strain.

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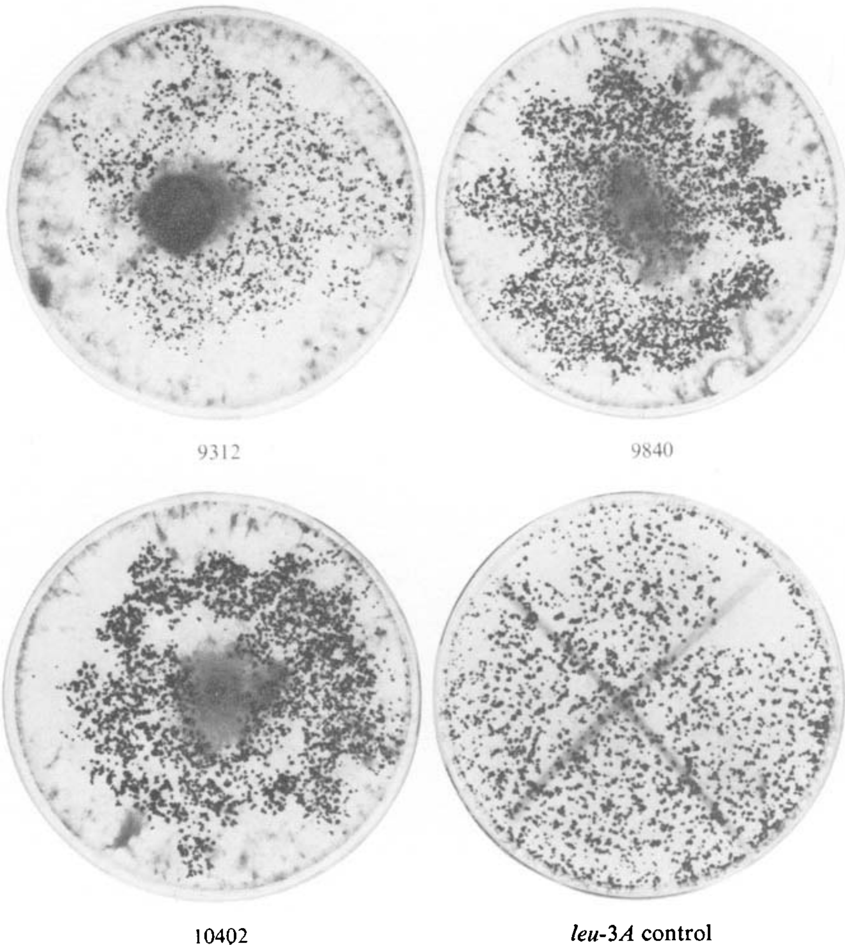

Photographs of crossing plates showing the sexual behaviour patterns of three group 3 and 4 male sterile mutants when crossed with a wild-type strain as compared to a control plate of fertile (*leu*-3 *A*) strain.

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a 100 × 20 mm Petri dish for 4–5 min at a distance of 25 cm from the source. Appropriate dilutions were then made and plated on sorbose medium under red light followed by incubation in the dark to prevent photoreactivation.

(iv) *Isolation and selection of mutants.* Initial testing of irradiated cultures was carried out by a replica plating method (Vigfusson & Weijer, 1969). All mutants were selected on the basis of male sterility, selection and isolation being limited to those showing drastic reduction in male fertility. Following original isolation of the mutant from the master plate, the strain was tested more extensively for fertility by inoculating 1 ml of a conidial suspension of 5×10^3 conidia/ml on to a crossing plate containing 10 ml of medium and a 2- to 3-day-old culture of the tester stock of opposite mating type. After a 14-day incubation period at 25 °C plates were inspected for total number of perithecia and perithecial morphology. Strains were only chosen if they showed a drastic reduction in fertility (0–30 normal mature perithecia per plate as compared to 10^3–10^4 for wild-type crosses) and similar morphology of all immature perithecia (depending on the stage of perithecial development which was arrested in each case). To ensure that all strains were homokaryotic, four serial subcultures of each strain were prepared, each of which was tested as outlined above. The strain was discarded if there were any discrepancies between these results and that of the original strain.

(v) *Test crosses.* All other test crosses for mating type and sterility were carried out by inoculating a small amount of dry conidia on to marked sectors of paired *fl A* and *fl a* protoperithecial plates similar to the method outlined by Smith (1962). As before, crosses were carried out in plates containing 10 ml of medium and incubated 14 days at 25 °C.

Tests for female fertility were conducted by inoculating conidial suspensions of St Lawrence strain on to crossing plates containing a 2–3-day culture of the mutant strain to be tested. Plates were inspected after a 14-day incubation period at 25 °C.

(vi) *Ascospore isolation.* Random spores and tetrads were isolated on 5% agar blocks (Ryan, 1950).

(vii) *Recombination tests.* Crosses for recombination tests were carried out by inoculating on to a crossing plate containing a culture of St Lawrence wild-type, a conidial suspension of the strain to be tested.

3. RESULTS

Thirty mutants were isolated, all of which exhibited varying degrees of male sterility. The phenotypic behaviour of these mutants in crosses with a wild-type strain (Table 1) indicates a wide range of patterns ranging from the production of only very small brown protoperithecia to the production of almost mature perithecia with few spores. Mutants have been grouped according to the stage of perithecial development that is arrested based on such morphological perithecial characteristics as pigmentation, ostiole development, and presence or absence of spores. Photographs showing the appearance of the crossing plate with respect to density of perithecia of representatives from most of the groups are shown in Plates 1 and 2.

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Table 1. *Phenotypic behaviour exhibited in crosses of male sterile mutants with wild-type*

Group	Isolate no.	Parental strain	Mating type	Description
1	5366	*pan*-1	*A*	Completely sterile, producing only very small, brown protoperithecia.
2	7232	*leu*-3	*A*	Abundant small, brown protoperithecia with occasional mature perithecia (0–10 per plate) which contain abundant normal spores. Strains 7334, 8553 and 5926 produce a slightly greater number of mature perithecia (20–30) per plate
	7384	*leu*-3	*A*	
	8455	*leu*-3	*A*	
	8553	*leu*-3	*A*	
	10299	*leu*-3	*A*	
	10710	*leu*-3	*A*	
	10718	*leu*-3	*A*	
	5538	*pan*-1	*A*	
	5926	*pan*-1	*A*	
	16009	*pan*-1	*A*	
3	7065	*leu*-3	*A*	Abundant immature brown perithecia, slightly larger than in group 2 mutants, no ostiole, no spores
	9312	*leu*-3	*A*	
	10233	*leu*-3	*A*	
	10734	*leu*-3	*A*	
4	7341	*leu*-3	*A*	Abundant normally pigmented but immature perithecia. Ostiole development starting. Perithecia empty or containing very few spores
	9840	*leu*-3	*A*	
	10402	*leu*-3	*A*	
	10528	*leu*-3	*A*	
	10589	*leu*-3	*A*	
	10979	*leu*-3	*A*	
	11042	*leu*-3	*A*	
	P-D-11-1	*pan*-1	*A*	
5	10982	*leu*-3	*A*	Abundant normally pigmented but slightly immature perithecia. Ostiole development almost complete. Perithecia empty or containing very few spores
	16044	*pan*-1	*A*	
	P-B-13-1	*pan*-1	*A*	
	P-D-12-1	*pan*-1	*A*	
6	12042	*leu*-3	*a*	Same as group 5 above
	12365	*leu*-3	*a*	
	15218	*leu*-3	*a*	
	leu-3 and *pan*-1 parentals	—	*A*	10^3–10^4 normal mature perithecia per plate
	leu-3 and *pan*-1 parentals	—	*a*	

In order to demonstrate genetic segregation of male sterility (*mst*) mutant strains were crossed with St Lawrence wild-type and progeny analysed for mating type and male sterility. Results are shown in Table 2 along with ascospore germination percentages and recombination frequencies between male sterility and mating type. Reciprocal crosses were also carried out with the St Lawrence wild-type strain to detect any differences in segregation of male sterility. Although all the strains were tested, in many cases the crosses were infertile and could not be analysed. Data for the successful crosses are presented in Table 3.

It is evident from Table 2 that segregation patterns for male sterility and mating

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Table 2. *Segregation of male sterility* (mst) *and mating type in random spores from* mst × mst$^+$ *crosses*

Mutant number	Mating type	Ascospore germination (%)	No. of spores analysed	Segregation of male sterility and mating type				Recombination between male sterility and mating type (%)
				a mst$^+$	*a mst*	*A mst*$^+$	*A mst*	
5366	*A*	—	21 tetrads, 15 PD : 6 TT : ONPD					—
7232	*A*	50	199	61	27	40	71	33·6
7384	*A*	NT						
8455	*A*	54·6	365	114	72	90	89	45·0
8553	*A*	74·5	511	206	63	57	185	23·5
10299	*A*	55·0	220	84	36	35	65	32·2
10710	*A*	51·3	309	91	66	67	85	43·0
10718	*A*	86·0	315	103	55	54	103	31·6
5538	*A*	80·0	171	51	32	28	60	35·0
5926	*A*	NT						
16009	*A*	75·5	169	65	28	20	56	27·8
7065	*A*	62·0	124	58	0	66	0	—
9312	*A*	73·0	146	77	0	70	0	—
10233	*A*	19·0	56	33	0	23	0	—
10734	*A*	NT						
7341	*A*	NT						
9840	*A*	48·0	158	85	0	73	0	—
10402	*A*	49·0	162	82	0	80	0	—
10528	*A*	54·2	163	88	0	75	0	—
10589	*A*	69·0	69	30	0	39	0	—
10979	*A*	89·0	178	86	0	92	0	—
11042	*A*	58·0	174	87	0	87	0	—
P-D-11-1	*A*	67·0	67	35	0	32	0	—
10982	*A*	31·7	255	140	57	27	31	33·0
16044	*A*	67·0	125	33	27	32	33	47·3
P-B-13-1	*A*	34·0	102	61	4	15	22	18·6
P-D-12-1	*A*	52·2	62	28	5	24	5	47·0
12042	*a*	17·5	94	18	0	76	—	—
12365	*a*	67·8	252	128	0	124	—	—
15218	*a*	51·5	181	47	0	134	—	—

NT = not tested.

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Table 3. *Analysis of random spores from reciprocal crosses of some male sterile mutants with wild-type St Lawrence*

Mutant crossed and direction of cross*	No. of spores analysed	Segregation of mating type and sterility			
		a mst⁺	*a mst*	*A mst*⁺	*A mst*
wt. × 9312	89	49	0	40	0
9312 × wt.	57	28	0	29	0
wt. × 9840	26	15	0	11	0
9840 × wt.	132	70	0	62	0
wt. × 10402	82	40	0	42	0
10402 × wt.	80	42	0	38	0
wt. × 10982	55	28	19	3	5
10982 × wt.	42	29	5	5	3
wt. × P-B-13-1	55	30	2	10	13
P-B-13-1 × wt.	30	22	2	1	5
wt. × 8455	168	46	39	37	36
8455 × wt.	89	19	29	14	27
wt. × 8553	100	36	10	13	41
8533 × wt.	128	56	14	15	43
wt. × 10299	99	38	18	19	24
10299 × wt.	121	46	18	16	41
wt. × 10718	88	26	9	18	35
10718 × wt.	92	35	18	9	30
wt. × 5538	71	21	17	11	22
5538 × wt.	100	30	15	17	38
wt. × 16009	69	30	12	6	21
16009 × wt.	100	35	16	14	35

* The first parent listed is the protoperithecial parent.

Table 4. *Analysis of germinated spores from 30 unordered tetrads isolated from a cross of St Lawrence wild-type × male sterile mutant 10982*

Ascus	No. of spores germinated	Ascospore genotypes	Ascus	No. of spores germinated	Ascospore genotypes
1	8	2 *a mst*⁺ : 2 *a mst* : 2 *A mst*⁺ : 2 *A mst*	13	2	2 *A mst*
2	4	2 *a mst*⁺ : 2 *A mst*⁺	14	2	2 *a mst*⁺
3	4	4 *a mst*⁺	15	2	2 *a mst*⁺
4	4	2 *a mst*⁺ : 2*A mst*	16	2	2 *a mst*
5	4	2 *a mst*⁺ : 2 *A mst*	17	2	2 *a mst*⁺
6	4	4 *a mst*⁺	18	2	*a a mst*⁺
7	4	2 *a mst*⁺ : 2 *A mst*⁺	19	2	2 *a mst*⁺
8	4	4 *a mst*⁺	20	2	2 *a mst*⁺
9	3	3 *a mst*⁺	21	2	2 *a mst*⁺
10	3	3 *a mst*⁺	22	1	1 *A mst*⁺
11	3	2 *a mst*⁺ : 1 *A mst*	23	1	1 *a mst*⁺
12	3	1 *a mst* : 2 *A mst*⁺	23	1	1 *a mst*⁺

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Table 5. *Unordered tetrad analysis of some group 3, 4 and 6 mutants crossed with wild-type St Lawrence*

Mutant	Ascus	No. of spores germinated	Ascospore genotypes
9312	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	7	3 $a\ mst^+$: 4 $A\ mst^+$
	3	7	4 $a\ mst^+$: 3 $A\ mst^+$
9840	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	8	4 $a\ mst^+$: 4 $A\ mst^+$
	3	8	4 $a\ mst^+$: 4 $A\ mst^+$
	4	8	4 $a\ mst^+$: 4 $A\ mst^+$
	5	8	4 $a\ mst^+$: 4 $A\ mst^+$
	6	8	4 $a\ mst^+$: 4 $A\ mst^+$
	7	7	3 $a\ mst^+$: 4 $A\ mst^+$•
	8	7	4 $a\ mst^+$: 3 $A\ mst^+$
	9	7	3 $a\ mst^+$: 4 $A\ mst^+$
10233	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	7	3 $a\ mst^+$: 4 $A\ mst^+$
	3	4	2 $a\ mst^+$: 2 $A\ mst^+$
	4	4	0 $a\ mst^+$: 4 $A\ mst^+$
10402	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	7	3 $a\ mst^+$: 4 $A\ mst^+$
	3	5	4 $a\ mst^+$: 1 $A\ mst^+$
	4	5	3 $a\ mst^+$: 2 $A\ mst^+$
	5	8	4 $a\ mst^+$: 4 $A\ mst^+$
	6	7	4 $a\ mst^+$: 3 $A\ mst^+$
	7	7	4 $a\ mst^+$: 3 $A\ mst^+$
	8	7	4 $a\ mst^+$: 3 $A\ mst^+$
	9	7	3 $a\ mst^+$: 4 $A\ mst^+$
10528	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	8	4 $a\ mst^+$: 4 $A\ mst^+$
	3	8	4 $a\ mst^+$: 4 $A\ mst^+$
	4	8	4 $a\ mst^+$: 4 $A\ mst^+$
	5	7	4 $a\ mst^+$: 3 $A\ mst^+$

Mutant	Ascus	No. of spores germinated	Ascospore genotypes
10589	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	8	4 $a\ mst^+$: 4 $A\ mst^+$
	3	8	4 $a\ mst^+$: 4 $A\ mst^+$
10979	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	8	4 $a\ mst^+$: 4 $A\ mst^+$
	3	8	4 $a\ mst^+$: 4 $A\ mst^+$
	4	8	4 $a\ mst^+$: 4 $A\ mst^+$
	5	8	4 $a\ mst^+$: 4 $A\ mst^+$
	6	8	4 $a\ mst^+$: 4 $A\ mst^+$
	7	8	4 $a\ mst^+$: 4 $A\ mst^+$
	8	7	3 $a\ mst^+$: 4 $A\ mst^+$
	9	7	4 $a\ mst^+$: 3 $A\ mst^+$
	10	6	3 $a\ mst^+$: 3 $A\ mst^+$
12042	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	8	4 $a\ mst^+$: 4 $A\ mst^+$
	3	8	4 $a\ mst^+$: 4 $A\ mst^+$
	4	8	4 $a\ mst^+$: 4 $A\ mst^+$
	5	8	4 $a\ mst^+$: 4 $A\ mst^+$
	6	8	4 $a\ mst^+$: 4 $A\ mst^+$
	7	9	4 $a\ mst^+$: 4 $A\ mst^+$
	8	7	3 $a\ mst^+$: 4 $A\ mst^+$
	9	7	4 $a\ mst^+$: 4 $A\ mst^+$
12365	1	8	4 $a\ mst^+$: 4 $A\ mst^+$
	2	8	4 $a\ mst^+$: 4 $A\ mst^+$
	3	8	4 $a\ mst^+$: 4 $A\ mst^+$
	4	7	3 $a\ mst^+$: 4 $A\ mst^+$
	5	5	0 $a\ mst^+$: 4 $A\ mst^+$
	6	4	0 $a\ mst^+$: 4 $A\ mst^+$
	7	4	0 $a\ mst^+$: 4 $A\ mst^+$
	8	4	0 $a\ mst^+$: 4 $A\ mst^+$
	9	6	3 $a\ mst^+$: 3 $A\ mst^+$

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Table 6. *Conidial viability with age in male sterile strains of* Neurospora crassa *stored at* 4 °C

Strain	% viability at the age of: 4 days	8 days	38 days	60 days
5366	88·0	89·5	82·5	73·5
7232	94·0	92·0	81·0	65·0
8455	70·5	92·0	71·0	72·5
8553	78·0	88·0	72·0	70·0
10299	61·5	53·5	72·5	50·5
10710	90·0	100·0	86·5	71·0
10718	100·0	82·5	79·5	68·5
5538	100·0	73·0	58·5	72·5
16009	68·5	79·5	61·5	45·0
Control (*leu*-1 *A*)	79·0	84·5	62·0	51·0
Control (*pan*-3 *A*)	99·5	74·0	54·5	40·0

type are aberrant for all but the groups 1 and 2 mutants. Group 5 mutants (with the exception of mutant 16044) display a skewed segregation pattern while groups 3, 4 and 6 produce only male fertile progeny. Mutant 10982 was arbitrarily chosen as a typical representative of Group 5 mutants and subjected to unordered tetrad analysis to determine whether the skewed segregation was due to reduced viability of *mst* ascospores. Results, shown in Table 4, strongly support this hypothesis.

Nine mutants from groups 3, 4 and 6 were similarly analysed and results are shown in Table 5. Several tetrads from four of these crosses were arbitrarily chosen and backcrossed to the wild-type St Lawrence strain. Tetrad analysis from these crosses indicate only wild-type (*mst*$^+$) progeny with normal segregation of mating type.

An attempt was then made to restore fertility by varying the standard crossing medium (SCM) to determine whether the behaviour of some of these mutants was a result of their inability to synthesize some common nutrient requirement. The following variations in media were used:

(1) SCM plus 0·15 % standard vitamin solution.

(2) SCM plus 0·005 % tryptophan.

(3) SCM plus 0·25 % yeast extract, 0·50 % malt extract, 0·5 % casein hydrolysate, and 0·10 % vitamin solution.

(4) SCM plus fivefold increase in the amount of leucine or calcium panthothenate added.

(5) SCM plus tenfold increase in the amount of leucine or calcium panthothenate added.

(6) SCM with glucose substituted for sucrose.

(7) SCM with glycerol substituted for sucrose.

(8) SCM with sodium acetate substituted for the sucrose.

(9) 2·0 % cornmeal plus 2·0 % glucose.

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Table 7. *Effect of male fertility of increasing the concentration of spermatia in crosses of wild-type ♀ × mutant ♂*

Strain	No. of mature perithecia produced by spermatial concentrations of: 5×10^3 per plate	5×10^6 per plate
5366	0	0
7232	0	3
8455	0	0
8553	12	34
10299	1	6
10710	1	4
10718	1	5
5538	2	10
16009	2	12
Average (all mutants)	2·1	8·2
Control (*leu*-1 *A*)	2800	33000
Control (*pan*-3 *A*)	3200	29000

Using these media, each of the mutants was crossed using St Lawrence as the protoperithecial strain. In no case was there any significant increase in fertility of the mutants or the controls (*pan A* and *leu A*) over that obtained with SCM. Variations in amount of medium and incubation temperature were then employed. Crosses were made on 10, 15, 20, 30 ml of SCM and incubated at 20, 25 and 30 °C for 15 days. In no case was there any significant increase in fertility as a result of these variations.

The relatively high proportion of mutants blocking 'early' stages of the sexual development cycle (probably prior to karyogamy) suggests the possibility of several genes controlling these early stages. Investigations were conducted to determine (i) whether reduced conidial viability in the mutant strains was a cause of sterility* and (ii) whether increasing the density of the spermatia used in crosses would restore fertility significantly. Tests were carried out on conidia of the same culture (stored at 4 °C) at 4, 8, 38 and 60 days of age. Groups 1 and 2 mutants as well as the parental *leu A* and *pan A* strains were tested. Results are shown in Table 6. Crosses were then carried out by inoculating, respectively, 1 ml of 5×10^3 and 5×10^6 concentrations of conidia of each of these strains and parental controls on the paired *fl a* protoperithecial plates. Results are shown in Table 7.

Finally, all strains were analysed for their behaviour when used as the female, protoperithecial strain. Results are shown in Table 8. Two strains, 7232 and 10710, which are both male and female sterile, were further tested to assess any differences in segregation of male and female sterility. Results are shown in Table 9.

* Conidia were used as the spermatizing element in all tests for fertility.

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Table 8. *Phenotypic behaviour of male sterile mutants with respect to female fertility in crosses with wild-type St Lawrence strain*

Mutant	Mating type	Male sterile group	Description
5366	*A*	1	Female sterile, producing only small brown protoperithecia
7232	*A*	2	
10710	*A*	2	
10233	*A*	3	
10528	*A*	4	
8455	*A*	2	Completely fertile, similar to wild-type
8553	*A*	2	
10299	*A*	2	
10718	*A*	2	
5538	*A*	2	
16009	*A*	2	
9312	*A*	3	Produce immature, pigmented perithecia with some ostiole development. Perithecia empty or containing very few spores
9840	*A*	4	
10402	*A*	4	
10589	*A*	4	
10979	*A*	4	
10982	*A*	5	
16044	*A*	5	
P-D-11-1	*A*	4	
P-D-12-1	*A*	5	
P-B-13-1	*A*	5	
12042	*a*	6	
12365	*a*	6	
15218	*a*	6	

4. DISCUSSION

It seems apparent from the data in Table 1, 2 and 3 that the mutant strains herein described represent mutations of various genes controlling different stages of the sexual development cycle. Mutant strains of groups 1 and 2 seem to block some stage (stages) early in the cycle, e.g. plasmogamy. Mutants in groups 3–6, however, manifest their specific phenotypes at later stages, possibly controlling such steps as karyogamy, meiosis, spore delimitation or spore maturation. Tests conducted to determine whether some of the strains are sterile by virtue of some other deficiency, namely variation in type and amount of medium, and variation in incubation temperature, all produced negative results. This provided additional evidence that these are mutations of genes each of which affect a specific function related to sexual development.

Results of the conidial viability tests (Table 6) reveal that the sterility is not likely an indirect result of drastic reduction in conidial viability of the mutant strains. Similarly increasing the conidial concentration in crosses 5×10^3/plate to 5×10^6/plate (relative conidial densities on the plate of 0·8 conidia/mm^2 and $8{\cdot}0 \times 10^2$ conidia/mm^2, respectively) resulted only in an average of a fourfold increase in the number of perithecia compared to a tenfold increase for the fertile parental strain (Table 7). This would indicate that the impairment in the mutant strains is not

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Table 9. *Segregation of mating type (m.t.), male sterility* (mst), *and female sterility* (fst) *in random spores from crosses of wild type St Lawrence* $a \times A$ mst⁻ fst⁻

Cross	No. of spores analysed	Segregation of mating type, *mst* and *fst*								Recombination between		
		a				*A*						
		mst+		*mst*−		*mst*+		*mst*−				
		fst+	*fst*−	*fst*+	*fst*−	*fst*+	*fst*−	*fst*+	*fst*−	m.t. and *mst*	m.t. and *fst*	*mst* and *fst*
w.t. × 7232	37	10	3	4	4	3	4	4	5	40.5 %	36·5 %	40·5 %
w.t. × 10710	80	15	8	6	10	9	4	12	16	36·3 %	48·8 %	37·5 %

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related to a function of attraction of the trichogyne since under those circumstances one would expect increasing densities of conidia to have a greater effect in improvement of fertility. Preliminary studies in this laboratory, however, have indicated that attraction of the trichogyne for the conidium may not exist in *N. crassa*. Cytological studies are presently underway to determine the exact stage blocked in each strain. These latter studies will ultimately enable a thorough description of the stages of the sexual development cycle in *n. crassa*, complementing previous work carried out in this field by McClintock (1945), Singleton (1953) and Pinchiera & Srb (1969) in *N. crassa* and in related species (Colson, 1934; Dodge, 1935).

As Table 2 indicates, all of the groups, 3–6 mutants display aberrant segregation patterns of the sterility genes (ratio of *mst* to mst^+). In the group 5 mutants with the exception of mutant 16044, the mst^+ ascospores are found far more frequently than the *mst* ascospores (a combined total of 360 mst^+ : 184 *mst* for the four strains in this group). This suggests that *mst* ascospores are less viable. The data in Table 4 for strain 10982 support this explanation. The mutant gene appears to effect partial ascospore lethality similar to the self-sterile l–1 and l–2 mutants of Carr & Olive (1959). The ascospore germination percentages for these four mutants (31·7 %, 67·0 %, 34·0 % and 52·5 %) further support this hypothesis.

All of the groups 3, 4 and 6 mutants, on the other hand, give rise to only male fertile progeny when crossed with a wild-type strain; this in spite of the fact that all of the mutants had been previously subcultured vegetatively four times and were found to be stable with respect to the mutant phenotype. Tetrad analysis of some of these mutants (Table 5) reveals normal viability of the ascospores. No further tests have been carried out on these strains and any explanatory attempts at this point would be conjecture.

A comparison of Tables 1 and 8 reveals different behaviour patterns for some of these strains, depending upon whether they are used as the male or the female parent in the cross. This applies particularly to the early (groups 1 and 2) mutants and suggests that some of the mutations affect specific male functions. The data in Table 9 provide additional evidence for this. It will be noted from this Table that analysis of progeny from two strains which are both male and female sterile demonstrates almost independent segregation for male and female sterility markers. It appears therefore that for the early mutants the male sterile block affects a specifically male function since these mutants are female-fertile. The exceptions to this, strains 5366, 7232 and 10710, represent double mutations male-sterile (*mst*) and female-sterile (*fst*) (Table 9).

The strains which block later stages of development (groups 3–6) demonstrate somewhat similar behaviour patterns whether used as the male or the female parent, with the exception of two strains (Tables 1, 8). This similarity might suggest that the block in these strains occurs after the association of the male and female nucleus or possibly even after karyogamy, in which case behaviour patterns would be expected to be the same. The two exceptions, 10233 and 10528, might then represent double mutations, like 5366, 7232 and 10710.

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On the basis of the published literature, this study is believed to represent a relatively extensive search for sterility mutants in *N. crassa*, and with the selection procedure employed, detection of mutations of the mating-type locus proper would be anticipated. No such mutants, however, have been isolated. This adds support to the long-held theory that the one-locus, two-allele incompatibility system as found in filamentous Ascomycetes is very stable (Burnett, 1956; Raper & Esser, 1964).

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