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Sexuality in *Neurospora crassa*

II. Genes affecting the sexual development cycle

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SUMMARY

Eighteen male sterile mutants of *N. crassa* which block various stages of the sexual development cycle have been functionally categorized on the basis of complementation tests. Nine of these (those which block early stages of the sexual cycle) have also been analysed through recombination studies. On the basis of these data, a minimum of four genes appear to be involved in early stages of sexual development (i.e. probably prior to karyogamy) and several genes are indicated which control later stages of the cycle. Complementation phases with respect to sexual development in heterothallic Ascomycetes are discussed as well as a comparison of results obtained with expectations and with those obtained in related Ascomycetes.

1. INTRODUCTION

Considerable information regarding the physiology and genetic control of the sexual development cycle in the fungi has been obtained through the study of mutants which block various stages of this cycle. The most extensive of these studies has been carried out on three homothallic Ascomycetes: *Glomerella cingulata* (McGahen & Wheeler, 1951; Wheeler & McGahen, 1952; Wheeler, 1954), *Sordaria macrospora* (Esser & Straub, 1956, 1958) and *S. fimicola* (Olive, 1956; Carr & Olive, 1959). These studies have not only served to substantiate previous reports regarding the sequential nature of the sexual cycle (e.g. Dodge, 1935; Raper, 1951, 1952) but have also revealed that the various stages of the cycle are under direct genetic control such that a mutation of a gene controlling a specific stage of the cycle prevents all development beyond that stage (Wheeler, 1954; Esser & Straub, 1958). A delineation and detailed description of the various stages in normal sexual development has thus been possible (Esser & Straub, 1958).

Similar studies in heterothallic Ascomycetes have been somewhat less extensive, consisting largely of isolated reports of single blocks in the sexual cycle (Nelson, 1959*a*, *b*, *c*; Westergaard & Hirsch, 1954; Fitzgerald, 1963; Tan & Ho, 1970). Weijer & Vigfusson (1972) have isolated and described 30 mutant strains of the heterothallic Ascomycete *Neurospora crassa*, each of which exhibits an impairment in fertility when used as the male parent. The various strains reported

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block different stages of the sexual development cycle. The present study treats the categorization of these mutants (i) in terms of function by assessing the degree of improvement in male fertility in a heterokaryon composed of two mutant strains and (ii) in terms of structure by means of recombination analyses.

2. MATERIALS AND METHODS

Wild-type St Lawrence strains (FGSC nos. 262 and 533) were obtained from the Fungal Genetics Stock Center at Dartmouth College. Male sterile mutants were induced by UV irradiation and have previously been described (Weijer & Vigfusson, 1972).

Complementation studies were carried out by testing the male fertility of heterokaryotic combinations of mutant strains. In any given combination the mutant strains involved carried a *pan*-1 and *leu*-3 marker respectively, facilitating forced heterokaryon production. Although all parent strains used for mutant induction were originally *het*-compatible, some combinations could not be successfully produced. Other grew to a limited extent but produced only homokaryotic (*pan*-1 or *leu*-3) conidia. Only heterokaryons with at least 10 % heterokaryotic conidia were used. All crosses were carried out on 15 ml of standard crossing medium Westergaard & Mitchell, 1947) in 100 × 15 mm Petri plates. Known concentrations of conidia (2×10^4) of the male parent (heterokaryon) were added to a 2 to 3-day-old culture of the protoperithecial parent (St Lawrence). After 14 days of incubation at 25 °C presence or absence of complementation was judged by comparing the results, in terms of total number of mature perithecia, with control plates of the individual heterokaryon component strains and with the fertile *pan*-1 + *leu*-3 heterokaryon. In almost all cases results were well defined, namely either definitely negative or showing marked improvement in fertility often equalling that of the fertile *pan*-1 + *leu*-3 heterokaryon. Photographs of crossing plates of some of the mutant strains and some heterokaryons are shown in Plates 1–3.

Crosses for recombination tests were carried out by preparing protoperithecial plates of one of the strains and subsequently adding a conidial suspension of the other strain to be tested. When fertile perithecia were obtained tetrad analysis was carried out if possible, otherwise random spore analysis was used.

3. RESULTS

Complementation data for all successful combinations of the mutants tested are shown in Table 1. Recombination tests were limited to those mutants which appear to block sexual development at an early stage of the sexual cycle. These crosses are by and large infertile and mature perithecia are usually only obtained after several attempts. In 12 of the 36 combinations fertile perithecia were never obtained. For this reason the recombination data are limited, representing the analysis of approximately 15 tetrads, or, if these were not obtainable, approximately 100 random spores. These data are presented in Table 2.

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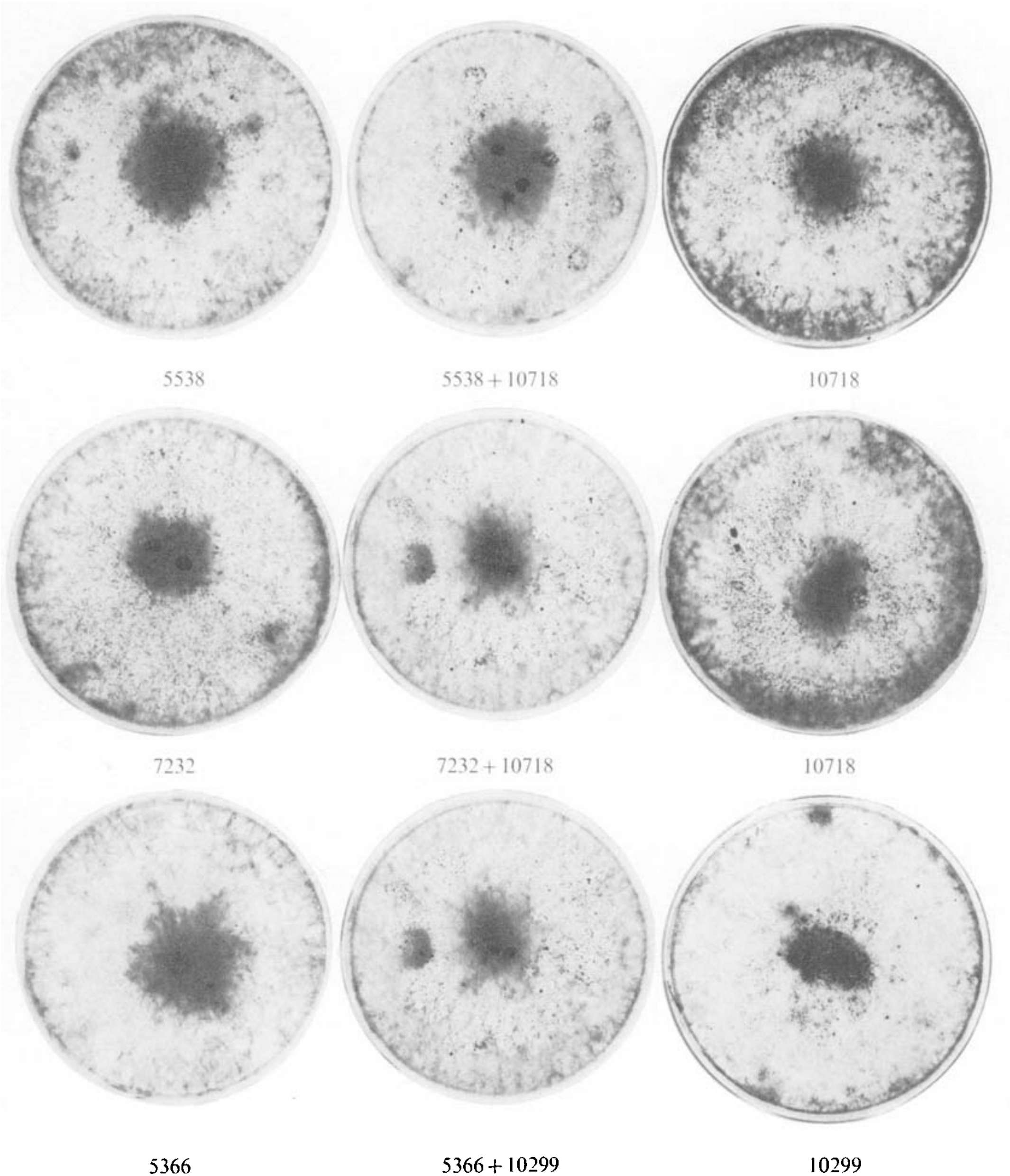

Photographs of crossing plates of three heterokaryons of male sterile mutants which show lack of complementation, along with control plates showing the behaviour of each of the components of the heterokaryon in crosses with a wild-type strain.

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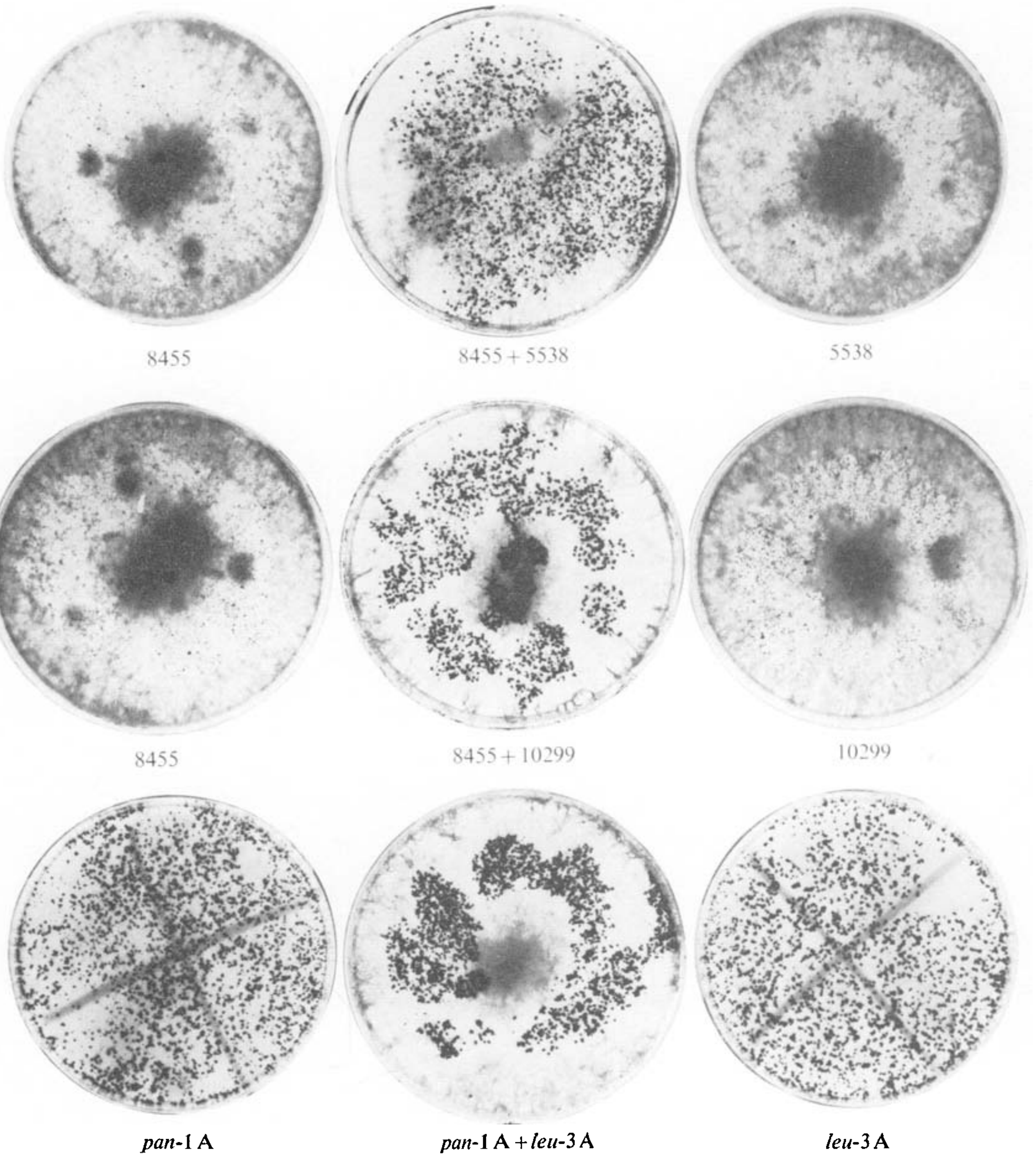

Photographs of crossing plates of two heterokaryons of male sterile mutants which show a high degree of complementation along with control plates of *pan*-1 A and *leu*-3 A strains in crosses with a wild-type strain.

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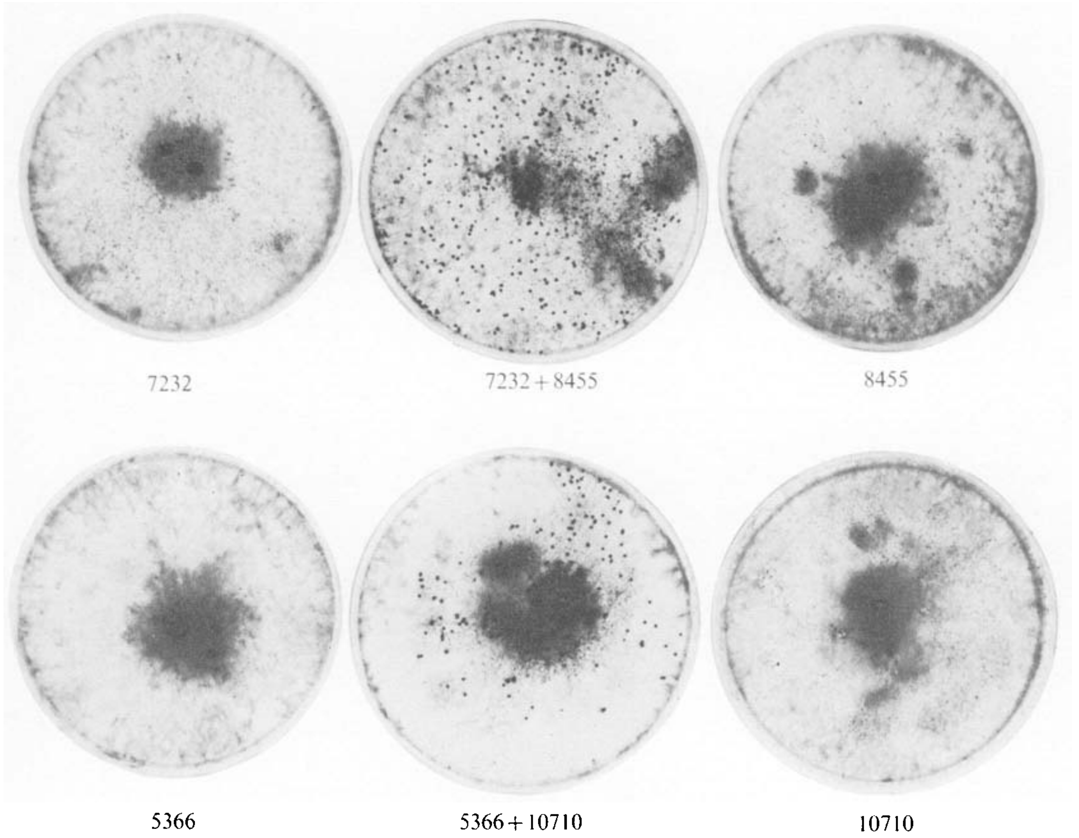

Photographs of crossing plates of two heterokaryons of male sterile mutants which show slight but obvious complementation in crosses with a wild-type strain.

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Table 1. *Complementation data of forced* (pan-*1 and* leu-*3*) *heterokaryons of male sterile mutants in crosses with wild-type St Lawrence strain*

	8455	8553	10299	10710	10718	5366	5538	16009	16044	P-D-12-1	10979	9312	9840	10402	10589	10598	10982
7232	+	+	−	−	−	+	−	−	+	−	+	0	+	0	0	0	0
8455		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8553			+	+	+	+	+	+	+	+	+	0	0	0	0	0	0
10299				0	−	0	−	−	+	+	+	+	0	+	0	+	+
10710					−	+	−	−	+	−	+	0	0	0	0	0	0
10718						0	−	−	−	−	+	+	+	+	+	+	+
5366							+	+	+	+	+	+	+	+	0	0	0
5538								−	+	+	+	0	0	+	0	+	0
16009									+	+	+	+	0	+	0	0	0
16044										−	−	−	−	−	−	−	−
P-D-12-1											−	−	−	−	−	−	−

0 = Successful heterokaryons not obtained.
+ = Occurrence of complementation.
− = Absence of complementation.

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Table 2. *Occurrence of recombination in different* mst × mst *crosses*

	8455	8553	10299	10710	10718	5366	5538	16009
7232	+	+	0	0	0	+	–	–
8455		+	+	+	+	0	+	+
8553			+	+	+	+	+	+
10299				0	–	0	–	0
10710					0	+	–	0
10718						0	–	–
5366							0	+
5538								0

0 = No offspring available.
\+ = Recombinants isolated.
– = No recombinants isolated.

4. DISCUSSION

Although complementation with respect to sexual function has been demonstrated in homothallic Ascomycetes (Wheeler & McGahen 1952; Carr & Olive, 1959; Esser & Straub, 1958), it is believed that the underlying study represents the first demonstration of complementation, with respect to sexual development, in a heterothallic Ascomycete. In evaluating complementation in this system it is important to consider the stage of sexual cycle which appears to be blocked. Backus (1939) demonstrated the fusion of the conidium with the trichogyne and the subsequent entry of at least a portion of the conidial protoplasm. The nucleus (or nuclei) are assumed to travel down the trichogyne into the ascogonium. Since there is no reason to believe that only one of the nuclei from the macroconidium enters the trichogyne and migrates toward the ascogonium, one can suppose that all (or most) of them do so. Normally, however, only one (Sansome, 1947; Weijer & Dowding, 1960) enters the ascogonium and becomes associated with the female nucleus.

Thus, two mutant male sterile (*mst*) nuclei in a heterokaryotic conidium acting as the spermatium could complement one another (with respect to loss of function) only up to a certain stage in sexual development. This point is the time at which one male nucleus (destined for fertilization) becomes dissociated from the rest; probably this point is reached at the time of entry of the male nucleus into the ascogonium or the time of association of the male and female nuclei in the ascogonium. From this point on in sexual development, complementation between two male nuclei can no longer take place. However, very shortly hereafter the male and female nuclei become associated in the ascogonium. Complementation should then be possible between the male and female nuclei during all stages of sexual development beyond this point. This has been demonstrated in some homothallic Ascomycetes (e.g. Esser & Straub, 1958).

From the outline present above one would expect that, for those mutants blocking the sexual cycle prior to the stage of separation of one male nucleus from the rest of the male macroconidial nuclei, complementation results should be easily comprehensible. This is the case for all of the nine 'early' mutants (mutants 7232/16009, Table 1). As the data in Table 1 indicate, complementation takes place

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in some combinations of these mutants but not in others. It is also evident that, for these mutants, no complementation can be attributed to the wild-type female nucleus because, under those circumstances, it cannot be expected that a particular mutant (A) complements in one combination (A + B) but not in another (A + C). Since all crosses are made with a fertile female strain, further confirmation of this derives from the fact that these mutants are infertile in crosses with a wild-type female strain. Consequently, there is no complementation from the female nucleus or all of the mutations are dominant.

The results of the complementation tests for the rest of the mutants tested (16044/10982, Table 1) are somewhat less perspicuous. These mutants appear to block at later stages of sexual development, probably at karyogamy and later (Weijer & Vigfusson, 1972). Hence, in a heterokaryon (of two mutants blocking stages of karyogamy or later) which functions as a *male* strain in a cross, complementation of function between the two components of the heterokaryon cannot be expected to take place because at this point only one of the components (nuclei) of the male conidium is involved. At karyogamy, however, the male nucleus becomes associated with the wild-type female nucleus and consequently complementation from the female nucleus is expected to take place leading to the completion of the sexual cycle. From observation it is apparent that crosses between 'late' mutants and a wild-type female strain do not behave in the expected manner but remain infertile. This is not unexpected since the selection procedure employed a cross with a wild-type female strain and therefore only mutants which would not complement with the female nucleus would be isolated. Whether this is due to dominance of the mutant gene or whether it suggests a deficiency which resides within the nucleus itself (e.g. a specific male function) is unknown at this time.

On the basis of the complementation data presented in Table 1, it appears that four mutant genes are responsible for the early blocks in the sexual cycle. Strains 5366, 8455 and 8553 represent, respectively, three of these genes while strains 7232, 10299, 10710 10718, 5538 and 16009 represent the fourth gene. The intergenic recombination data (Table 2) are compatible with this classification. Although these data are not complete and the sample size upon which the data was based was small, there are no cases where the data are in disagreement with the complementation results. It is not unexpected that four or more genes should be found controlling the stages of sexual development up to the point of association of the male and female nuclei in the ascogonium. From similar studies in related organisms and by *a priori* reasoning, at least six different steps in this early part of the sexual cycle in *Neurospora* can be defined. These steps involve (entirely or at least partially) functions which one would expect to be those of the male nucleus. These are: (*a*) production of a diffusible substance by the conidium; (*b*) attachment of the conidium to the trichogyne; (*c*) dissolution of the cell walls of the conidium and trichogyne; (*d*) entry of the conidial cytoplasm and nuclei into the trichogyne; (*e*) migration of the male nucleus (nuclei) to the ascogonium; and (*f*) association of the male and female nuclei in the ascogonium. The existence of the first step is in some doubt (Weijer & Vigfusson, 1972).

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Esser & Straub (1958) found eight different genes in *Sordaria macrospora* which blocked sexual development prior to ascus formation. Five of these, however, are clearly female functions and have no counterpart in this portion of the study. The other three, *pl*, *f* and *z*, are described by Esser and Straub as blocking somewhere during the dikaryotic phase, karyogamy and ascus formation. Wheeler (1954) reported six genes which block plasmogamy in *Glomerella cingulata*. Three of these, arg^1, bi^1 and th^1, behave phenotypically much the same as the mutants discussed in the present study in that they produce 'an abundance of perithecial initials, but these never develop' (Wheeler, 1954, p. 344). No symbols have as yet been assigned to these four genes.

Classification of the remainder of the mutants (those which block at later stages of sexual development) is speculative at this point. The complementation tests (Table 1) are incomplete, due to the difficulty in obtaining successful heterokaryons. Moreover, recombination tests have not as yet been carried out. Mutant 9312 (as well as 10233 and 10589) produces in crosses with a wild-type strain abundant brown perithecia which are empty and show no ostiole development. Cytological observation of the contents of these perithedia reveals croziers and very young asci which are devoid of any spore-like structures, suggesting a genetic block in sexual development just prior to or shortly after karyogamy similar to the pattern exhibited by the B^2 gene in *G. cingulata* (Wheeler, 1954) which produces perithecia containing croziers. According to Wheeler the block is believed to occur just prior to karyogamy.

Other 'late' mutants, in crosses with a fertile wild type *fl*, produce abundant perithecia which are normally pigmented but still immature as judged by the development of the ostiole (Weijer & Vigfusson, 1972). These perithecia are empty although some do contain a few spores. Cytological observation of the contents of these perithecia reveals asci containing four irregularly shaped bodies. The indicated genetic block in the sexual cycle is in one of the stages from meiosis up to the time of spore wall formation. These mutants behave in a manner very similar to the dw^1 gene of *G. cingulata* (Wheeler & Driver, 1953), which produces a variety of effects from morphologically abnormal ascospores to 70–80 % ascus abortion. According to Wheeler & Driver (1953), the dw^1 gene blocks meiosis, Similarly, Esser & Straub (1958) reported three genes in *Sordaria macrospora* – *min* and *pa*, which block meiosis, and gene *s*, which blocks shortly after meiosis.

A large number of mutants produce only wild-type progeny in crosses with a wild-type female strain (Weijer & Vigfusson, 1972). These would appear to involve a gene or genes which confer a form of male sterility which is lost in the first division of meiosis.

As stated earlier, classification of late-acting genes is not possible at this point as data (Table 1) are limited and the data that are available are not in total agreement with previously obtained linkage data (Weijer & Vigfusson, 1972). It is expected that the 'late' mutants will ultimately be found to involve several genes blocking the various stages from karyogamy to spore maturation.

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