MEIOSIS IN *NEUROSPORA CRASSA*. I. THE ISOLATION OF RECESSIVE MUTANTS DEFECTIVE IN THE PRODUCTION OF VIABLE ASCOSPORES

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ABSTRACT

A scheme has been devised for efficient isolation of recessive meiotic mutants of *Neurospora crassa*. These mutants were detected by their reduced fertility or by the abortion of ascospores. Their isolation involved the selection and screening of strains arising from ascospores disomic $(n + 1)$ for linkage group I (LG I), which bears the mating-type locus. These strains are self-fertile heterokaryons that contain two types of haploid nuclei of opposite mating type $(A + a)$. Selfings of these strains are homozygous for genes on all linkage groups except LG I and therefore allow the expression of recessive mutants with an altered sexual cycle. Using this selection procedure, three classes of mutants were detected. In one class, mutants had an early block in perithecial development (class I), and in another mutants had altered perithecia, but apparently unaltered fertility (class III). No recessive mutants were observed and all mutants tested (eight of class I and two of class III) were expressed only when used as the maternal parent. A third mutant class displayed normal production of perithecia, but defective formation of asci (class IIA), or black ascospores (class IIB). Four of 13 class IIA mutants were analyzed, and two of them [*asc(DL131)* and *asc(DL400)*] were definitely recessive. Analysis of 10 of 13 class IIB mutants disclosed six recessive, mutually complementing mutants: *asc(DL95)*, *asc(DL243)*, *asc(DL711)*, *asc(DL879)*, *asc(DL917m)* and *asc(DL961)*. Mutants *asc(DL95)*, *asc(DL243)* and the previously studied *mei-1* mutant (SMITH 1975) complemented one another in crosses, but did not recombine. These may be alleles of the same gene, or they may comprise a gene cluster.

DESPITE a detailed knowledge of the behavior of chromosomes during meiosis, little is known about the processes that control these events. This study was initiated to gain a better insight into such control by isolating and characterizing mutants with meiotic defects. Such mutants in which the phenotype of meiotic cells or their products is detectably abnormal will be referred to as meiotic mutants.

Many meiotic mutants have already been detected in a variety of organisms (for a review, see BAKER *et al.* 1976b). The systematic isolation of meiotic mutants has been initiated in Drosophila (SANDLER *et al.* 1968; BAKER and CARPEN-

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TER 1972), Saccharomyces (ESPOSITO and ESPOSITO 1969; ROTH and FOGEL 1971; ROTH 1976), Caenorhabditis (HODGKIN, HORVITZ and BRENNER 1979), Sordaria (ESSER and STRAUB 1958), Schizosaccharomyces (BRESCH, MULLER and EGEL 1968) and Podospora (SIMONET and ZICKLER 1972). Some of these mutants appear specific to meiosis, but many others also affect one or more vegetative properties, such as mutagen sensitivity (BOYD *et al.* 1976; BAKER *et al.* 1976a; PRAKASH and PRAKASH 1977), histidine sensitivity (NEWMEYER, SCHROEDER and GALEAZZI 1978), spontaneous mutation (ESPOSITO, BOLOTIN-FUKUHARA and ESPOSITO 1975; GOLIN and ESPOSITO 1977) and mitotic recombination (RODARTE-RAMON and MORTIMER 1972; RODARTE-RAMON 1972).

Even though meiotic mutants have been obtained in many organisms, the understanding of the defects is often in doubt because of a lack of means for genetic or cytological analysis. Various idiosyncracies of the life cycles of Podospora and higher plants complicate genetic analysis in these organisms. In contrast, even though genetic analysis of yeast and Drosophila is excellent, visualization of chromosomes by means of conventional cytological methods is not possible in yeast and is possible only after the pachytene stage in Drosophila (PURO and NOKKALA 1977). Therefore, it should be profitable to isolate and characterize meiotic mutants in an organism in which both genetic and cytological means of analysis are readily available. The ascomycete *Neurospora crassa* fits this description well.

In Neurospora, meiotic mutants that are dominant or expressed only when used as the conidial or protoperithecial parent can be directly obtained by screening strains resulting from mutagenized conidia. However, because of the heterothallic nature of Neurospora, induced recessive meiotic mutants cannot be expressed in the first generation of crossing. The technique described in this paper permits the rapid isolation of recessive meiotic mutants. This method involves the selection and screening of strains arising from ascospores disomic for linkage group I and heterozygous for the mating-type locus ($A + a$).

Meiotic mutants in *Neurospora crassa* can be detected by reduced fertility, due to a block in the formation of most or all asci, or by ascospore abortion. In Neurospora, aneuploidy results in the abortion of an ascospore if any part of the chromosome complement is missing. Therefore, the presence of aborted (white) ascospores is a good detection system for mutations that cause irregular segregation of chromosomes during meiosis (SMITH 1975).

During the initial screen, recessive mutations at six loci caused ascospore abortion, and recessive mutations at two loci resulted in an absence of ascospores.

MATERIALS AND METHODS

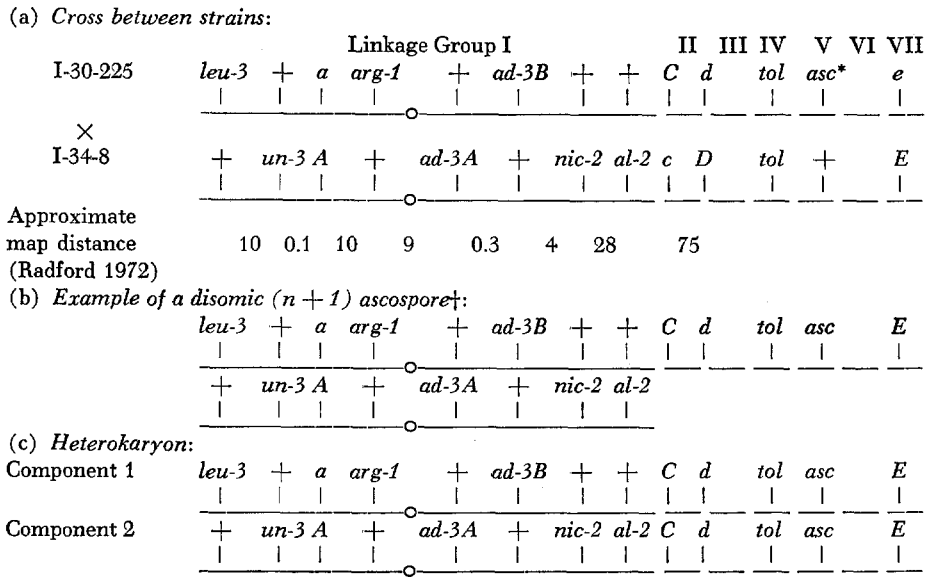
Alleles: The following alleles were used during this study: *leu-3* (R156), *un-3* (55701-t), *arg-1* (36703), *ad-3A* (2–17–814), *ad-3B* (2–17–114), *nic-2* (43002), *al-2* (74A-Y-112-M38), *tol* (N38) and 2 alleles at each of the 3 heterokaryon incompatibility loci: *C/c*, *D/d* and *E/e*.

Experimental design: Recessive meiotic mutants can be detected only in crosses homozygous for these mutants. A method for the homozygosis and detection of such mutants in *Neurospora crassa* has been developed (Figure 1). The method involves the isolation and testing of many strains, each arising from an ascospore disomic ($n + 1$) for chromosome *1* (LG I), which bears

the mating-type locus (A/a). The selection of ascospores disomic for LG I was made possible by the introduction of multiple markers into the parent strains (see Figure 1a for the location of these markers on their respective linkage groups). The following considerations were important in the choice of markers.

First, cultures arising from disomics for LG I constitute a heterokaryon with 2 complementing components of opposite mating type ($A + a$). Since the mating-type locus also acts as a heterokaryon incompatibility locus (BEADLE and COONRADT 1944; GARNJOBST and WILSON 1956), these disomic cultures grow very poorly. Therefore, the *tol* allele (N83), which suppresses this heterokaryon incompatibility without affecting crossing ability (NEWMEYER 1970), was introduced in both strains (I-30-225 and I-34-8 in Figure 1).

Second, 5 closely linked auxotrophic mutant alleles, *leu-3*, *arg-1*, *ad-3A*, *ad-3B*, *nic-2*, and the nonsupplementable heat-sensitive mutant allele *un-3*, which are all located within about 30 map units of each other on LG I, were used to select ascospores disomic for this linkage group. Disomics carrying complementing mutant alleles are called pseudo-wild type (PWT). A cross between *leu-3 arg-1 ad-3B* (strain I-30-225) and *un-3 ad-3A nic-2* (strain I-34-8) could produce 2 types of ascospore progeny capable of growth on minimal medium: multiple recombinants and PWT (= disomic) cultures. The arrangement of the closely linked markers virtually eliminates wild-type recombinant progeny. In fact, none were detected in this study. Thus, when ascospores are



* To be detectable by this method, a recessive meiotic mutation (*asc*) may be located on any linkage group except LG I.

† Only one of the eight possible combinations of *het* alleles is presented.

FIGURE 1.—General outline of a selective system used to isolate recessive meiotic mutants in *Neurospora crassa*. (a) A cross between strain I-34-8 (female) and the mutagenized strain I-30-225 (male) produces a low proportion of progeny disomic for LG I. These can be selected on minimal medium. (b) Each ascospore disomic ($n + 1$) for LG I contains two copies of this linkage group, but only one copy of the other linkage groups. (c) Subsequent haploidization in each culture derived from a disomic ascospore (PITTENGER 1954) produces two types of nuclei of opposite mating type. Such cultures, called pseudo-wild type (PWT), are capable of "selfing." All of the linkage groups except LG I are identical in both types of nuclei. Consequently, a recessive meiotic mutant allele on any linkage group except LG I would be detected in the "selfing" of the PWT culture.

plated on minimal medium, most colonies should result from ascospores disomic for LG I. Each of these PWT cultures contains two types of nuclei with complementing auxotrophic mutant alleles and opposite mating-type alleles.

Third, when ascospores are plated on minimal medium, the germ tubes of 2 adjacent ascospores may fuse. Such fusion products may produce a colony if the 2 nuclear types complement each other and are heterokaryon compatible. To minimize the frequency of colony-producing fusion products, 3 unlinked heterokaryon incompatibility loci (*C/c*, *D/d* and *E/e*) were utilized. The cross between strains I-30-225 and I-34-8 (see Figure 1) was made heteroallelic at each of these three loci. When ascospores produced by this cross are plated on minimal medium, only 1/8th of all fusion products with complementary auxotrophic requirements can form a colony. In fact, colonies produced by the fusion of 2 or more adjacent ascospores were practically eliminated when the plating concentration did not exceed 2×10^4 ascospores per 90 mm petri dish. Consequently, the plating of ascospores at concentrations up to 2×10^4 spores per plate allowed the virtually exclusive isolation of self-fertile PWT colonies.

Mutant induction and isolation: Conidia from 7-day-old cultures of strain I-30-225 were treated with 0.025 mM MNNG at 25° for 4, 5 or 6 hr (MALLING and DESERRES 1970). After termination of MNNG treatment with sodium thiosulphate at pH 8.0, the mutagenized conidial suspension was used as the fertilizing parent by pouring 10 ml of the suspension over 7-day-old mycelial growth of strain I-34-8. Ascospores produced by this cross were plated onto minimal medium at a concentration of approximately 10^4 per 90 mm petri dish (for further details on the plating procedure and exclusive use of purified agar in the medium, see GRIFFITHS and DELANGE, 1977). The frequency of colonies (self-fertile PWT's) thus produced was estimated at about 5×10^{-5}. Each of these cultures was assigned an isolation number with the prefix "DL" (*e.g.*, culture DL100 is PWT isolate number 100) and was isolated by 1 of 3 possible methods: (1) Using a dissecting microscope, I isolated very small colonies after 2 and 3 days of growth. At this time, the simultaneous transfer of unselected ascospores can be avoided. (2) After 4 or 5 days of growth, the agar-overlayer at the site of the colony was removed and part of the underlying agar, which included the mycelial growth of the colony, was transferred. This method is faster and, since all ascospores are in the overlayer agar, reliable. (3) Part of the mycelial growth or conidia were transferred after 8 days of growth on plates. The latter method is the fastest but may lack sensitivity since nongrowing germinated ascospores may be "rescued" by a poorly growing PWT culture. Each PWT culture was transferred to a slant of minimal medium in a 10×75 mm tube and allowed to grow for a week before being transferred to an 18×150 mm test tube containing 5 ml liquid minimal crossing medium and a strip of filter paper (NEWCOMBE and GRIFFITHS 1972). All selfs were incubated at 25°; some were also incubated at 16°. Incubation at both temperatures allowed the detection of temperature-sensitive mutants.

Cultures in which abnormal development of perithecia and/or ascospores resulted were crossed on liquid crossing medium with OR-A and OR-a wild-type strains. If aberrant development is caused by the expression of a recessive, rather than a dominant, mutant allele, crosses with both wild-type strains should produce normal perithecia and ascospores. Therefore, only strains with this behavior were classified as potential recessive mutants. Recessive mutants were distinguished from mutants whose phenotypes are expressed only when used as the male or female parent by means of reciprocal crosses between the mutant PWT culture and the OR-A and OR-a strains.

Meiosis appears to be initiated only after the major part of perithecial development has been completed. Thus, in screening for meiotic mutants, only those with well-developed perithecia have been analyzed further. Five *A* isolates of the cross between any given PWT and OR-A, and 5 *a* isolates of the cross between the PWT and OR-a were intercrossed in all combinations; if the mutant phenotype was detected, 1 mutant isolate of each mating type was used in the testing of all isolates from crosses between the PWT culture and the 2 wild type strains (OR-A and OR-a). Sometimes, all of these isolates were backcrossed to the original PWT culture. In either case, a 1:1 segregation of mutant and wild-type phenotypes confirmed the presence of a recessive mutant.

Media and routine manipulations were conventional for Neurospora (DAVIS and DESERRES 1970).

RESULTS

Using the selective system described in MATERIALS AND METHODS (see also Figure 1), 1,090 PWT cultures were isolated and allowed to self on liquid crossing medium. The 145 cultures that did not successfully complete the sexual cycle (*i.e.*, cultures that did not eject only viable black ascospores from mature perithecia) were crossed individually with OR-A and OR-a wild type strains. Forty-six mutants exhibited wild-type crossing ability with both OR-A and OR-a and thus were apparently not dominant (see Table 1). These strains, which may carry recessive mutant alleles or alleles whose phenotypes are expressed only in the female or male parent, were classified according to phenotype into three main groups: 16 strains with an early block in perithecial development (class I); 26 strains defective in the formation of asci or black ascospores (class II); and four strains with a miscellaneous developmental defect apparently not associated with reduced fertility (class III).

Class I: Mutants of this type produce either no perithecia at all (six strains), or few or incompletely developed perithecia (10 strains). Perithecia with a size intermediate between that of proto- and fully grown perithecia, and lacking a neck, are considered incompletely developed. To distinguish mutants that are expressed only when used as the female or male parent (female- or male-sterile mutants) from recessive mutants, four strains (DL205, DL349, DL406, DL700) with no perithecia, two strains (DL841, DL891) with few perithecia and two strains (DL186, DL434) with incompletely developed perithecia were crossed reciprocally with wild-type strains. (The remaining eight strains grew very poorly and were not tested). In each case, a mutant phenotype resulted when the mutant was used as the protoperithecial (female) parent, but not when the mutant was used as the conidial (male) parent. Thus, these strains are female sterile. Similar mutants have been previously isolated (MYLYK and THRELKELD 1974; JOHNSON 1978).

Class II-A: Thirteen strains produced perithecia that were either completely

TABLE 1

Initial characterization of 145 pseudo-wild type cultures with aberrant crossing behavior

	Type of crossing aberrancy	Number of PWT cultures *M*	*m*
Class I	A. Sterile (*i.e.*, no perithecia)	50	6
	B. Few or incompletely developed perithecia	3	10
Class II	A. Perithecia without spores	0	13
	B. More than 20% ascospore abortion	44	13
Class III	Defects not related to fertility (see text)	2	4
		99	46

Each culture was crossed with OR-A and OR-a wild-type strains; those producing normal perithecia and ascospores in both cases were classified as potential recessive mutants (*m*); reciprocal crosses of these mutants with the wild-type strains were needed to distinguish recessive mutants from mutants that are expressed only when used as the female or male parent; the defect in the remaining cultures was apparently caused by dominant determinant(s) and these were called *M*.

barren or contained very few black ascospores (about 10 to 20 spores per perithecium, as compared to hundreds in normal perithecia). This mutant class was subdivided into four phenotypes: eight strains with empty perithecia (DL246, DL308, DL373, DL400, DL446, DL741 and two additional strains that were discarded due to poor growth), two strains that produced only asci with eight little round bubbles, about one-quarter the size of regular ascospores, but no ascospores (DL314, DL423), one strain with empty asci (DL131), and two strains with few black ascospores (DL310, DL1163). Of four mutants tested, DL131 and DL400 were recessive; DL446 and DL314 apparently were not. DL446 produced barren perithecia when used as the protoperithecial parent, but not as the conidial parent. Although not analyzed further, this mutant appears similar to those in class I; instead of an early block (class I mutants), a late block in perithecial development may be involved. The defect in DL314 appears to have a complex pattern of inheritance. Whereas the original PWT culture produced only bubble asci, intercrosses of some isolates from a cross between DL314 and wild type produced empty perithecia, while others formed ascospores, most of which were not or were very slowly ejected from their perithecia. This mutant has not been examined further.

Class II-B: Thirteen strains displayed the abortion of many ascospores. The abortion in six of these strains (DL95, DL243, DL393, DL711, DL879, DL961) was found to be due to a single recessive mutation. No clear-cut pattern of inheritance was evident in strains DL165 and DL631, which displayed 50 to 80% ascospore abortion three weeks after crossing, but none after two months, and in DL1079, which produced only white inviable ascospores. Three other strains with about 20% ascospore abortion (DL117, DL285, DL768) were discarded because of scoring difficulties (the mutagenized inbred PWT strains have a wide range of spore abortion up to about 15 or 20%). Finally, the ascospore abortion in strain DL917 was apparently caused by a dominant spore-killer mutation (DELANGE, submitted for publication).

During the analysis of strain DL917, a recessive mutant allele that causes ascospore abortion and low fertility was detected. This allele was originally present in heterozygous condition and was therefore not detected in the original selfing of DL917. The spore-killer and abortion-type mutant alleles from this strain were designated DL917s and DL917m, respectively. A cross between strains 917A7 (*DL917m A*) and I-30–225 (*leu-3 a arg-1 ad-3B*) produced 30% (9/30) recombinant progeny between *leu-3* and *DL917m*. In addition, both *leu*$^+$ *a arg ad* recombinant progeny were mutant. These preliminary results indicate linkage of *DL917m* to the tip of the left arm of LG I.

The results of analysis of mutants DL95, DL243, DL393 and DL879 are reported elsewhere (DELANGE and GRIFFITHS 1980). These mutants appear to have a defect during meiosis. The wild-type allele of *DL917m* also appears to be necessary for meiosis, since preliminary cytological observations showed clustered nuclei at the interphase of the second meiotic division. Moreover, small and large ascospores were found interspersed in the same asci. The defect in DL711 is of a different nature. Crosses homozygous for this mutant produced many asci with

eight black ascospores and a variable number of asci with only white ascospores. The latter type of asci contained either four or eight spores. The amount of ascospore abortion varied widely between about 20 and 80%. A cross between two strains, each multiply marked for LG I, produced about 25% aborted ascospores. Progeny analysis of this cross indicated that both recombination and nondisjunction frequencies were normal.

Class III: The abnormal phenotypes of these four mutant PWT strains are apparently not related to fertility. Two strains discharged their spores very poorly and were not examined further. The mutant phenotype of each of the remaining two strains segregated as a single gene. Strain DL126, when used as the protoperithecial parent, produced orange instead of black perithecia; it resembles the previously described mutant, *per-1* (HOWE and JOHNSON 1976). Another mutant (DL413), when used as the protoperithecial parent, fails to form necks on the perithecia and is therefore unable to discharge ascospores. Since the wild-type locus apparently controls the formation of the *per*ithecial *n*eck, the mutant has been designated *pen-1*.

Finally, in a preliminary attempt to isolate temperature-sensitive mutants, an additional strain (DL709) was obtained that produces 4-spored asci at 16° and normal 8-spored asci at 25°. This cold-sensitive mutant is dominant and has been mapped at or near the centromere of LG I; it is designated *Fsp-2* (4-spored ascus). *Fsp-1* is not temperature-sensitive (RAJU 1977).

Recessive mutants altered in the formation of asci or viable ascospores: Nine recessive mutants (two of class II-A and seven of class II-B) that displayed the abortion of asci or ascospores have been identified. Since it is plausible that not all recessive mutants of this kind are meiotic (*mei*), the new locus designation *asc* will be used to refer to these mutants.

All nine *asc* mutants were tested for allelism to each other and to *mei-1*. Only *DL243* and *DL393* were allelic to each other. In addition, all *asc* mutants were nonallelic to *mei-1*. Table 2 shows the eight loci with their isolation numbers

TABLE 2

The phenotype of eight complementing recessive mutants, whose wild-type alleles are necessary for the formation of normal asci or black ascospores

Locus	Nature of defect	
	% Ascospore abortion	Other phenotype
asc(DL95)	40 – 70	Intermed. to high fertility*
asc(DL131)		Empty asci
asc(DL243), DL393	90 – 98	Very low fertility
asc(DL400)		Empty perithecia
asc(DL711)	20 – 80	Intermed. to high fertility
asc(DL879)	70	Low to intermed. fertility
asc(DL917m)	90	Low fertility
asc(DL961)	50	Intermed. to high fertility

* Fertility was defined as the total number of ascospores (black and white) that were ejected from the perithecia (see MATERIALS AND METHODS).

and comments on their phenotype. The locus designation *asc(DL243)* will be used for alleles of both DL243 and DL393.

Partial mapping of asc (DL95), asc (DL243) *and asc* (DL879)

asc(DL243): A cross between *mei-1* and *asc(DL243)*, originally made to obtain double mutants, produced no recombinants among 62 ascospore isolates: 25 were *asc(DL243) mei-1*$^+$ and 37 were *asc(DL243)*$^+$ *mei-1*. To enrich for recombinants between *mei-1* and *asc(DL243)*, crossovers were selected between closely linked markers on either side, using *trp-4*, 6.5 mu to the right of *asc(DL243)*, and *pdx*, 3.5 mu to the left of *mei-1*. Ascospores from the cross between *asc(DL243) trp-4 pan-1 a* and *pdx mei-1 A* were plated on minimal medium, and 300 wild-type recombinant colonies (*pdx*$^+$ *trp*$^+$) were separately transferred to vegetative medium. Testing of these cultures for meiotic phenotypes failed to reveal any recombinants of the two meiotic mutants: 80 were *mei-1* and 220 were *asc-(DL243)*. The *pdx*$^+$ *trp*$^+$ recombinants represent only about 5% of the progeny of this cross, so that testing 300 of them is equivalent to testing 6,000 progeny for a recombinant between *mei-1* and *asc(DL243)*. However, the reciprocal *mei-1/asc(DL243)* recombinant type that would be associated with a *pdx trp* recombinant would not be detected; thus, the true number of progeny tested was about 3,000. The recombination frequency between *mei-1* and *asc(DL243)* is therefore less than 0.1 mu.

asc(DL95): The mutant *asc(DL95)* was mapped close to *asc(DL243)*, and therefore to *mei-1*, since only *asc(DL95)* (45) and *asc(DL243)* (51) progeny were detected among 96 isolated from a cross between the two mutants.

asc(DL879): Ascus analysis of a cross between *asc(DL243)* and *asc(DL879)* established the close proximity of *asc(DL879)* to one of the centromeres since, in five out of seven asci, the mutant and its wild-type alleles segregated during the first division. In subsequent crosses to centromere-linked markers, *asc-(DL879)* was mapped on LG II, about 3 mu from *arg-5*.

DISCUSSION

A new system for the homozygosis of induced mutants has been used to isolate recessive mutants with a defect in the sexual cycle. The recovery of eight mutually complementing recessive mutants demonstrates the effectiveness of the selection procedure (Table 2). All recessive mutants were defective in the formation of asci (class IIA) or black ascospores (class IIB). Of the class IIA mutants, *asc(DL400)* completely lacked asci and *asc(DL131)* had aborted asci. Similarly, two types of class IIB mutants were recognized: (1) a defect during meiosis leading to ascospore abortion [*asc(DL95), asc(DL243), asc(DL879)* and probably *asc(DL917m)*]; and (2) abortion of all ascospores in some asci, but little or no abortion in the remaining asci [*asc(DL711)*].

That *mei-1*, *asc(DL95)* and *asc(DL243)* are mutually complementing, but very closely linked, raises the question of whether they are complementing alleles of the same gene or whether they comprise a gene cluster. Of the remaining mutants, only *asc(DL879)* could be definitely assigned to a new locus on LG II.

The other mutants might be allelic with previously described mutants with similar phenotypes, *e.g.*, *uvs-3*, *uvs-5* or *mei-4* (SCHROEDER 1975; NEWMEYER and GALEAZZI 1978). The mutants isolated during this study should become instrumental in the understanding of the formation of perithecia, asci and viable ascospores.

Development of perithecia in Neurospora: In all previously isolated mutants altered in the development of perithecia (WEIJER and VIGFUSSEN 1972; VIGFUSSEN and WEIJER 1972; MYLYK and THRELKELD 1974; JOHNSON 1978; GRIFFITHS and DELANGE 1978), the abnormal phenotype was expressed when only one of the parents in a sexual cross was mutant. In most cases, abnormal phenotype resulted when the mutants were used as the maternal parent, but some mutants were expressed when used as the paternal parent as well. The present method which enables the isolate of recessive mutants, should be especially useful since no recessive mutants would have been detected in previous mutant screens*. The finding that all eight class I mutants tested expressed the mutant phenotype only when used as the maternal parent emphasizes that at least the great majority of loci required for the development of perithecia act only in the protoperithecial parent.

Formation of asci and viable ascospores in Neurospora: In Neurospora, the development of asci is initiated after the premeiotic S phase and karyogamy (IYENGAR *et al.* 1977). Therefore, mutants that either lack asci or produce defective asci may have a meiotic defect. Such behavior has been detected among mutants defective in vegetative DNA repair, *e.g.*, *uvs-3*, *uvs-5*, *uvs-6* (SCHROEDER 1975) and *mei-3* (NEWMEYER, SCHROEDER and GALEAZZI 1978). Direct screening for ascus abortion should identify not only genes that are required for both meiotic and vegetative functions, but also those needed for meiosis alone. The mutant *asc(DL131)* might be meiosis-specific, since recent tests have shown a normal sensitivity to UV, MMS and histidine (unpublished).

In Neurospora, the failure of ascospore maturation may be caused by the absence of any part of the haploid chromosome complement (*e.g.*, due to chromosome rearrangements or nondisjunction) or by the presence of spore-killer or ascospore color mutants (for review, see PERKINS and BARRY 1977). In the present study, a novel type of mutant allele [*asc(DL711)*] that apparently does not affect the haploid chromosome complement causes the abortion of all ascospores in a number of asci without affecting the remaining asci.

Mutants with a defect in the regular segregation of chromosomes have been detected in many species (for review, see BAKER *et al.* 1976b). In Neurospora, the mutants *mei-1*, *Mei-2* (SMITH 1975; LU and GALEAZZI 1978) and *mei-4* (see PERKINS and BARRY 1977) have such defects. Such mutants often have a primary defect in recombination, the aberrant segregation merely being a consequence of univalents produced through a lack of exchange. The ascospore abortion mutants *mei-1*, *Mei-2* and the newly isolated *asc(DL95)* and *asc(DL879)*

* JOHNSON (1979) described a mutant that was dominant in that it was sterile as either the male or female parent, but became fully or partly recessive under certain conditions. Such behavior would not have been detected in the present screen.

appear to have exchange defects, but *mei-4* and the newly isolated *asc(DL243)* have defects in segregation that are not associated with reduced exchange (DELANGE and GRIFFITHS 1980).

Ascospore abortion of the recessive mutant *asc(DL711)* was apparently not due to aneuploidy of the inviable ascospores, but rather to an unknown type of inviability of all ascospores in a proportion of asci. The large degree of variation in expression of this mutant may be explained if a threshold amount of a substance within each ascus is required for the maturation of ascospores. A similar mode of action has been proposed to account for certain irregularities in the sperm dysfunction caused by *SD* mutants in Drosophila (MIKLOS and SMITH-WHITE 1971). The effect of *asc(DL711)* on whole asci and the great amount of variability of expression between different crosses is also reminiscent of bubble asci (see PERKINS and BARRY 1977). In each case, a certain proportion of asci fail to produce viable ascosopores — asci with aborted white spores for *asc-(DL711)* and asci with eight bubbles found in many wild type crosses — with no apparent effect on the remaining asci.

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