A MUTANT AFFECTING MEIOSIS IN NEUROSPORA[1]

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ABSTRACT

Many mutants affecting meiosis increase the occurrence of aneuploid meiotic products. In Neurospora, mutants of this type cause ascospore abortion which is reflected by an increase in the proportion of ascospores failing to develop black pigment. The usefulness of the criterion *white-ascospore-production* as a signal for the presence of a mutant affecting meiosis is demonstrated by the recovery of several such mutants. One of these is *mei-1* (meiotic-1), a recessive mutant on linkage group IV. Crosses homozygous for *mei-1* produce 90% white ascospores (*vs.* 5% in wild-type crosses). Viable ascospores, invariably black, are always disomic for one or more linkage groups; the chromatids assorted into viable ascospores do not engage in crossing over in meiosis. The distribution of viable ascospores in individual asci suggests that all meioses are defective in the first meiotic division, and that most meioses are defective in both divisions.

MUTANTS affecting meiosis were subjects of study relatively early in the development of genetic methodology. For example, GOWAN (1928, 1933) analyzed a Drosophila mutant, *c3G*, that causes a failure of synapsis, crossing over and normal disjunction (see HALL 1972). BEADLE studied a number of maize mutants, including one that results in asynapsis and nondisjunction (1930), another resulting in "sticky chromosomes" and abnormal disjunction (1932a) and another resulting in failure of cytokinesis (1932b).

In spite of these and other early studies, it was not until recently that mutants affecting meiosis were sought out systematically for use as tools to probe the processes of meiosis (e.g., LINDSLEY *et al.* 1968 in Drosophila; BRESCH MÜLLER and EGEL 1968 in Schizosaccharomyces; ESPOSITO *et al.* 1970 in Saccharomyces; SIMONET and ZICKLER 1972 in Podospora). The rationale common to each of these studies is that meiosis is a gene-controlled system and that it is therefore possible to obtain mutants of genes with meiosis-specific functions (see especially SANDLER *et al.* 1968). Mutants of this type are labeled *meiotic mutants*.

The studies cited above have revealed that many meiotic mutants cause an increase in nondisjunction and, subsequently, in aneuploidy in the meiotic products. This observation proves to be of some technical advantage in Neurospora because hypohaploid ascospores are white (aborted), while reciprocal, hyperhaploid ascospores develop normal black pigmentation and have good germination and viability (see, for example, MCCLINTOCK 1945; PERKINS 1974 for

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segmental aneuploidy; COYLE and PITTENGER 1965 for whole chromosome aneuploidy). It has been possible to exploit this property of ascospore pigmentation as the basis of a procedure for detecting meiotic mutants.

One of the several mutants initially recognized, by this criterion has been examined in some detail. This mutant, *mei-1* (meiotic-1) is a recessive on linkage group IV; crosses homozygous for *mei-1* produce about 90% white, inviable ascospores. Viable ascospores are disomic for one or more linkage groups as if generated by meiosis I nondisjunction of non-crossover chromatids. Portions of these results have been previously reported in abstracts (SMITH and PERKINS 1972; SMITH 1973).

MATERIALS AND METHODS

Strains: The allele of *mei-1* used in these studies was extracted from the translocation strain *T(I;IV)cut* A (Culture No. 176 of the Fungal Genetics Stock Center [FGSC], California State University, Humboldt, Arcata, California 95521). The translocation is apparently inseparable from the osmotic-sensitive *cut* mutant which was obtained by KUWANA (1953) in wild type 4A (probably Abbott 4A) following exposure to UV. Strains with *mei-1* in normal chromosome sequence have been deposited with FGSC.

The markers used in crosses 1 and 2 are described in the legend to Figure 1. Each of these is also available from the FGSC.

Strains for crosses 3 and 4 (see Table 4 legend) were constructed by crossing a strain with the closely linked markers *pyr-1 pdx-1+ mei-1*, to *multicent* which has linkage group IV markers *pyr-1+ pdx-1 mei-1+* in addition to a marker at each of the other six centromeres (PERKINS 1971). Ascospores were isolated on medium lacking pyridoxine but containing uridine so that those which grew were predominantly *pyr-1 mei-1*; these were then scored for multicent markers on other linkage groups. Several strains with various combinations of markers were each crossed to *mei-1* and progeny of these *mei-1* homozygous crosses scored for somatic segregation of the multicent markers involved. This allows a determination of effects of *mei-1* homozygosity on all seven centromeres.

Scoring somatic segregation: Segregation of markers in mitotic divisions occurs primarily by haploidization of disomic nuclei to produce a haploid *heterokaryon.* This process is detected by plating conidia from the heterokaryon, isolating individual colonies which arise from these conidia and testing them for recessive markers not apparent in the heterokaryon. All platings were done on media containing sorbose (formula of BROCKMAN and DE SERRES 1963) which restricts Neurospora's normally spreading growth to small colonies (TATUM, BARRATT and CUTTER 1949). This reduces the chance of unnoticed colony overlap, which can result in production of heterokaryons with properties similar to those of disomics.

Scoring for presence of mei-1: Strains to be tested for *mei-1* were crossed in a 10 × 75 mm tube to a six-day-old culture of a known *mei-1* stock of opposite mating type. After 12 days the wall of the tube was examined for ascospores and the strain characterized as *mei-1* if approximately 10% of ascospores were black, or as *mei-1+* if approximately 95% of ascospores were black.

The use of microconidial strains: Cross 1 (Figure 1) was homozygous for the linkage group II mutants *pe* (peach: Y8743m) and *fl* (fluffy: L). The double mutant produces no macroconidia, but only microconidia, about 99% of which are uninucleate. (Wild-type strains produce mostly the multinucleate macroconidia.) Thus, *pe fl* strains are technically advantageous when dealing with cultures giving somatic segregation of recessive markers.

Procedure for examination of unordered asci for patterns of ascospore abortion: For determination of ascus patterns, crosses homozygous for *mei-1* were made on petri dishes. After 12 days at 25°, in the dark, the plate was placed inverted over a 3 cm × 6 cm 4% agar-water slab so that the ostioles were approximately 1 mm from the slab. After an appropriate length of time,

depending upon the rate of discharge of ascospores from the perithecia, the slab was examined for clusters of eight ascospores. These cluster were characterized for number of black and white ascospores. For a detailed description of this technique, including special precautions, see PERKINS (1974). Other methodology will be found in DAVIS and DE SERRES (1970) and in SMITH (1974).

RESULTS

Recovery of mei-1: The effects of *mei-1* were first noticed in association with the osmotic mutant, *cut* (SMITH and PERKINS 1972). The particular *cut* allele involved is inseparable by recombination from one of the breakpoints of a reciprocal translocation (*T*) involving linkage groups I and IV [*T(I; IV) cut*]. Fertility of newly discovered translocations is routinely tested in isosequential $T \times T$ crosses, which if fertile are expected to yield euploid meiotic products. Therefore, allowing for some spontaneous abortion that results in production of white ascospores, 90% to 95% of ascospores from $T \times T$ crosses should be black. Some *T(I;IV)cut* × *T(I;IV)cut* crosses, however, unexpectedly gave 10% black ascospores. This behavior proved to be the result of homozygosity for a recessive mutant, *mei-1*, on the right arm of linkage group IV and separable from the translocation. Based upon one crossover in 500 progeny, *mei-1* is just distal to *arg-2*.

Asci produced by mei-1 *homozygosity*: Approximately 90% of ascospores shot from perithecia of *mei-1* homozygous matings are white (aborted). Some information about the nature of the event responsible for abortion should be revealed by the distribution of white ascospores among asci. This information can be obtained using STRICKLAND's (1960; PERKINS 1974) method of collecting shot, unordered asci. These groups of eight ascospores, each group representing one ascus and therefore one meiosis, are then scored for numbers of black and white ascospores. Table 1 gives the results of examining unordered asci in crosses homozygous for *mei-1* in (a) homozygous normal chromosome sequence and in (b) homozygous translocation sequence. For comparison, the result of having *mei-1* heterozygous is shown in crosses (c) homozygous for normal chromosome sequence and (d) heterozygous for the *T(I;IV)cut* translocation.

TABLE 1

Tetrads from mei-1 *crosses*

Number of ascospores (black:white) in unordered asci from crosses either homozygous or heterozygous for *mei-1* and either homozygous normal chromosome sequence (N/N), homozygous translocation sequence [*T(I;IV)cut*] (T/T) or heterozygous sequence (N/T).

Type of cross		Ascus classes					
mei-1 genotype	Sequence	8:0	6:2	4:4	2:6	0:8	Other*
mei-1/mei-1	T/T	0	0	8	11	21	27
mei-1/mei-1	N/N	0	0	15	21	31	14
+/*mei-1*	N/T	128	2	15	1	85	0
+/*mei-1*	N/N	70	4	2	0	0	0

* Mostly 1 black:7 white and 3 black:5 white.

The pattern of black and white ascospores in crosses homozygous for *mei-1* suggests that no meioses are normal (a normal meiosis would be signaled by an 8 black : 0 white ascus) and that the primary event is one which affects the first meiotic division and, in most meioses, persists through meiosis II. Thus, a small number of 4:4 asci might represent meioses in which nondisjunction, in the first division only, generated four aborted and four normal (pigmented) ascospores.

Progeny of mei-1 *homozygous crosses*: The first indication of disomy in progeny of *mei-1* homozygous crosses was the recovery of mating-type heterozygotes; in addition to reacting with testers of both mating types, these progeny usually display a distinctive "Dark Agar" phenotype (NEWMEYER and TAYLOR 1967). Mating-type heterozygotes had previously been shown to result from disomy for linkage group I, the mating-type chromosome. The disomy may be generated by nondisjunction even in the absence of a meiotic mutant (SMITH 1974). The analysis of progeny from the crosses shown in Figure 1 confirms that most viable progeny from *mei-1* homozygous crosses are disomic.

The top half of Table 2 shows the genetically pure *haploid* genotypes recovered by plating microconidia from the culture of a representative ascospore from cross 1 (Figure 1). These genotypes can be accounted for by assuming that this ascospore was originally multiply disomic, containing homologs with parental marker arrays. In subsequent mitotic divisions, and prior to haploidization, somatic crossing over in the centromere region (e.g., between *cys-10* and *pyr-1* on linkage group IV) generated new marker arrays (recombinants in Table 2). This interpretation is in accord with the findings of previous studies of haploidization and somatic crossing over in Neurospora disomics and disploids (COYLE and PITTENGER 1965; THRELKELD and STOLTZ 1970; SMITH 1974).

A total of 35 cultures from cross 1, each derived from a single ascospore, were examined for haploid genotypes present. The results are summarized at the

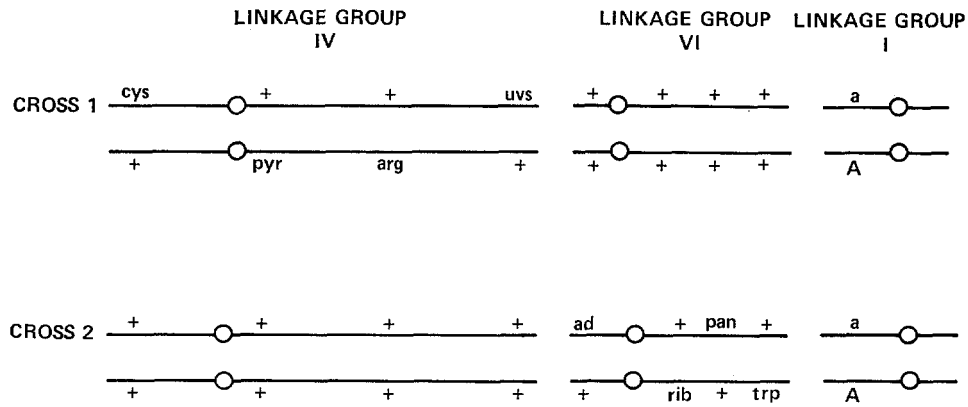

FIGURE 1.—Crosses 1 and 2 are shown as diploid genotypes. Markers and isolation numbers are *cys-10* (cysteine; 39816), *uvs-2* (ultraviolet-sensitive; no number), *pyr-1* (pyrimidine; H263), *arg-2* (arginine; 33442), *ad-1* (adenine; 3254), *pan-2* (pantothenate; Y154M64), *rib-1* (riboflavin; 51602t) and *trp-2* (tryptophan; 45302). Both crosses are homozygous for *pe* (peach; Y8743m) and *fl* (fluffy;L). *A* and *a* are mating types.

TABLE 2

Analysis of ascospores from a cross homozygous for mei-1

Top: Haploid genotypes recovered from the culture of a single ascospore from cross 1 (Figure 1). Bottom: Summary of results of examining 35 ascospores from cross 1. Group IV marker-array-types are as shown in the top of the table.

Genotype					
Linkage group IV				Linkage group I	Group IV marker array
cys-10	*pyr-1*+	*cot-1*+	*uvs-2*	A	Type 1 (Parental)
cys-10+	*pyr-1*	*cot-1*	*uvs-2*+	A	Type 2 (Parental)
cys-10+	*pyr-1*	*cot-1*	*uvs-2*+	a	Type 2
cys-10	*pyr-1*	*cot-1*	*uvs-2*+	A	Type 3 (Somatic recombinant)
cys-10+	*pyr-1*+	*cot-1*+	*uvs-2*	A	Type 4 (Somatic recombinant)

Group IV marker arrays recovered	Mating type heterozygosity	Number of ascospores giving this result
1,2,3,4	Yes	3
1,2,3	Yes	2
1,2,3	No	1
1,2,4	Yes	12
1,2	Yes	10
1,2	No	5
1	Yes	2

bottom of Table 2. Thirty-three of these cultures originated from ascospores which were disomic for linkage group IV. Each of these 33 contained the two types of group IV chromatids with parental marker arrays. The single genotype, with respect to group IV markers, present in each of the two haplo-IV progeny was also a parental type. In contrast, the map distance from *cys-10* to *uvs-2* is about 100 map units in control crosses. Eighteen of the 33 diplo-IV progeny also contained at least one of the marker arrays generated by somatic crossing over in the *cys-10* to *pyr-1* interval.

Twenty-nine of these progeny, including the two haplo-IV progeny, showed somatic segregation for mating type and were thus presumably disomic for linkage group I. In sum, 28 were disomic for both groups I and IV, five were disomic for IV only (haplo-I) and two were disomic for I only (haplo-IV).

Cross 2 was used to determine whether this behavior in *mei-1* homozygous crosses also affects linkage group VI. Eight progeny from that cross were all disomic for group VI and contained group VI homologs with parental marker arrays. Five of the eight were heterozygous for mating type, implying group I disomy. No attempt was made to detect products of somatic crossing over.

The combined results of crosses 1 and 2 suggest that a majority of viable progeny from a cross homozygous for *mei-1* receive two, noncrossover, homologs for all linkage groups. The occurrence of somatic crossing over may result in new (nonparental) marker arrays with the exchange point always near the centromere. Somatic assortment of nonhomologs is independent, as established in previous studies of multiple disomics in Neurospora (SMITH 1974).

Evidence that mei-1 *affects all linkage groups*: Direct evidence for disomy of linkage groups not marked in crosses 1 and 2 has been obtained by crossing *mei-1* into the *multicent* background and using the derived strains in *mei-1* homozygous crosses. Most ascospores from these crosses were disomic for one or more chromosomes. These results, summarized in Table 3, indicate that the effects of *mei-1* extend to all chromosomes.

Map location of mei-1: The location of *mei-1* in linkage group IV first became apparent from crosses carried out to separate it from *T(I;IV)cut*. The data placing it very close to *arg-2* and probably distal, came from the cross *mei-1* × *col-4 arg-2*. Only one crossover was found among 500 progeny tested.

An historical note on mei-1: The *cut* strain from which *mei-1* was extracted was obtained by KUWANA (1953) as a mutant following UV treatment of a wild type identified by KUWANA as 4A. Strain 4A had been obtained from G. W. BEADLE, and was in all probability Abbott 4A, although this was not realized until the work reported here was nearly complete. Some Abbott strains are known to carry a recessive factor which causes ascospore abortion when homozygous (EMERSON and CUSHING 1946; BARRATT 1954). The Abbott factor had been mapped by D. R. STADLER near *col-4* on the right arm of linkage group IV (D. R. STADLER, 1955 personal communication to D. D. PERKINS). Once it was realized that *mei-1* and the Abbott factor have both map location and phenotypic features in common, a *mei-1* strain was crossed to Abbott 4A (FGSC No. 1228). This cross gave about 90% white ascospores, as is typical for *mei-1* homozygous crosses, implying that *mei-1* and the Abbott factor are identical. A stock of Abbott 12a (FGSC No. 1758) does not contain *mei-1*.

The Abbott factor, regarded as a nuisance in the past (BARRATT 1954), has apparently come of age.

Other meiotic mutants in Neurospora: The white-ascospore screening procedure has led to the recognition of several additional mutants. In this collection, most is known about *mei-2*, a dominant which maps very near *inos* in linkage group V. Crosses heterozygous or homozygous for *mei-2* give extensive nondisjunction of all linkage groups. Crossing over has been tested in linkage group IV

TABLE 3

Markers showing somatic segregation in progeny of mei-1 *homozygous crosses*

Cross no.*	Linkage group I	II	III	IV	V	VI	VII
1	*mt*	—	—	*cys,pyr, cot,uvs*	—	—	—
2	*mt*	—	—	—	—	*ad,pan, rib,tryp*	—
3	*mt*	—	*acr*	—	—	*ylo*	*wc*
4	*mt*	*bal*	—	—	*at*	*ylo*	*wc*

Crosses 1 and 2 are illustrated in Figure 1. Cross 3 was heterozygous for mating type (*mt*; Linkage group I), acriflavin-2 (*acr*; III), yellow-1 (*ylo*; VI) and white collar (*wc*; VII). Cross 4 was heterozygous for mating type, balloon (*bal*; II), attenuated (*at*; V), yellow and white collar.

and is much reduced, apparently in nonpolar fashion. Cytologically, chromosome pairing is much reduced in crosses involving *mei-2* (B. C. Lu, personal communication).

A note on the recognition of meiotic mutants: Compound or complex rearrangements can simulate meiotic mutants in producing predominantly white, variable ascospores (Table 1 and see Table 3 in Perkins 1974). This occurs only when the rearrangement sequence is *heterozygous*, however. Homozygous rearrangement-sequence crosses produce balanced genomes and predominantly viable black ascospores. This is not true of meiotic mutants, whether dominant or recessive.

DISCUSSION

A large proportion of the meiotic mutants now known in several organisms affect the disjunction process in some way (e.g., Lindsley *et al.* 1968 in Drosophila). Therefore, a technique which is based upon the recognition of the resulting aneuploid products would appear to allow the recovery of a broad spectrum of mutant types. The recovery of *mei-1* and other mutants demonstrates that the technique is a useful one in Neurospora.

The study of *mei-1*, and preliminary results with *mei-2*, also confirm that aberrant meiotic products in Neurospora are readily analyzed and that the results of analysis allow inferences about the nature of the meiotic defect. For *mei-1* homozygous crosses, aneuploidy and the absence of meiotic crossover products in viable progeny point to an abolition of crossing over or to lethality of crossing over. While these possibilities cannot be resolved by the genetic data, preliminary cytological observations of Dr. B. C. Lu (unpublished) suggest that the problem is one involving both synapsis and distribution of meiotic products in the ascus. In many pachynema figures homologous chromosomes are unpaired; in others pairing seems to be normal. Many anaphase II figures reveal overlapping spindles, and telophase II nuclei contain greater than a haploid chromosome complement. [Singleton (1948) made preliminary cytological observations on a cross between Abbott 4A and a strain derived from it, and reported numerous meiotic abnormalities.] One hypothesis to account for these results is that *mei-1* results in the production of a defective component for synapsis (*or termination of synapsis*) that fails, in some cells, to promote synapsis (or terminates it too soon); in other cells the product functions so that pairing and crossing over can occur but the chromatids cannot separate (*or the product functions too late in termination*). Whether the product functions in achievement or termination of synapsis, its *normal* function is evidently a precondition to proper spindle behavior. Perhaps further resolution will come with fine structure and biochemical studies.

The apparent identity of *mei-1* with the Abbott factor implies that this meiotic mutant was present in the wild population in Louisiana from which Abbott collected his material. In this regard, it is of interest to note that Sandler *et al.* (1968) found a high frequency of meiotic mutants in natural populations of Drosophila. A collection of wild Neurospora of wide geographic origin is now available for similar testing (Perkins, Turner and Barry 1975).

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