MEIOSIS IN *NEUROSPORA CRASSA*. II. GENETIC AND CYTOLOGICAL CHARACTERIZATION OF THREE MEIOTIC MUTANTS

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ABSTRACT

Three recessive meiotic mutants, *asc(DL95)*, *asc(DL243)* and *asc(DL879)*, were detected by the abortion of many of their ascospores and were analyzed using both cytological and genetic methods. Even though *asc(DL95)*, *asc (DL243)* and the previously studied meiotic mutant, *mei-1* (SMITH 1975; LU and GALEAZZI 1978), complement one another in crosses, they apparently do not recombine (DELANGE and GRIFFITHS 1980). Thus, they may represent alleles of the same gene or comprise a gene cluster. Ascospore abortion in these mutants is caused by abnormal disjunction of meiotic chromosomes. In crosses homozygous for *asc(DL95)*, *asc(DL879)* or *mei-1*, both pairing of homologs and meiotic recombination frequencies are reduced. In each case, this primary defect is followed by the formation of univalents at metaphase I and their irregular segregation. The mutant *asc(DL243)* has a defect in ascus formation, and later in disjunction during the second meiotic and post-meiotic divisions. The first-acting defect before or during karyogamy results in the abortion of most cells. Some cells manage to proceed past this block. During the second meiotic division, most chromosomes of the few resulting asci are attached to only one of the two spindle-pole bodies. Disjunction at the post-meiotic division is also highly irregular. This mutant appears to be defective in the attachment of one spindle-pole body to a set of chromosomes. The defect may involve either a centromere-associated product or a spindle-pole body.

THE isolation of eight recessive, mutually complementing mutants with decreased fertility in *Neurospora crassa* has been reported elsewhere (DELANGE and GRIFFITHS 1980). Two mutants [*asc(DL131)* and *asc(DL400)*] produce barren perithecia; the remaining six mutants [*asc(DL95)*, *asc(DL243)*, *asc(DL711)*, *asc(DL879)*, *asc(DL917m)* and *asc(DL961)*] exhibit abortion of many ascospores.

That ascospore abortion may be a useful means for detecting mutants with a defect in the regular segregation of chromosomes has been suggested by studies done with the mutant *mei-1* (SMITH 1975). Approximately 90% of ascospores from crosses homozygous for *mei-1* abort. Abortion is evidently due to the irregular segregation of chromosomes, which is caused by the absence of pairing during the first meiotic prophase (LU and GALEAZZI 1978) and results in aneu-

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ploid products. The successful use of both genetic and cytological means of analysis of such mutants was demonstrated in those studies.

This paper reports genetic and cytological characterization of the three ascospore abortion mutants, *asc (DL95)*, *asc(DL243)* and *asc(DL879)* (see DELANGE and GRIFFITHS 1980), and some new observations on the *mei-1* mutant.

MATERIALS AND METHODS

Alleles: The alleles on linkage group I (LG I) used to select pseudo-wild type (PWT) colonies and to determine recombination and nondisjunction frequencies of that linkage group have been described elsewhere (DELANGE and GRIFFITHS 1980). Other alleles are: *ad-3A* (2-17-814)IR, *ad-3B* (2-17-128)IR, *aur* (34508)IR, *al-1* (Car-10)IR, *al-2* (74A-Y112-M38)IR, *arg-5* (27947)IIR, *acr-2* (KH5)IIIR, *pdx* (37803)IVR, *trp-4* (Y2198)IVR, *pan-1* (5531)IVR, *cot-1* [C102(t)]IVR, *inos* (37401)VR, *his-1* (K141)VR, *mei-1* (Abbott 4)IVR, *asc(DL95)*IVR, *asc(DL243)*IVR and *asc(DL879)*IIC. The allele $a^m(33)$ of the mating-type locus is heterokaryon-compatible with strains of *A* mating type and still permits crossing to such strains (GRIFFITHS and DELANGE 1978).

Strains: The recessive ascospore abortion mutants (*asc*) were isolated in PWT (disomic) strains that were heterozygous for LG I (*leu-3 a arg-1 ad-3B* and *un-3 A ad-3A nic-2 al-2*), but homozygous for the other linkage groups (see Figure 1; DELANGE and GRIFFITHS 1980). Ascospore isolates of genotypes *leu-3 a arg-1 ad-3B*; *asc* and *un-3 A ad-3A nic-2 al-2*; *asc* (each containing the *tol* mutant and the heterokaryon-compatibility alleles *C*, *d* and *e*) were obtained from crosses between the mutant PWT culture (*i.e.*, homozygous for an *asc* mutant) and wild-type strains OR-a and OR-A. These ascospore isolates were intercrossed and the LG I markers used to monitor crossover and nondisjunction frequencies (see Figure 1).

Cytological methods: Iron hematoxylin and aceto-orcein were used as stains. Only iron hematoxylin stains spindle-pole bodies and nucleoli. Staining with iron hematoxylin was done essentially as previously described (RAJU and NEWMEYER 1977; LU and GALEAZZI 1978; RAJU 1978). The method utilizing aceto-orcein is a combination of several methods (GRIFFITHS, DELANGE and JUNG 1974; M. BASL, personal communication). Perithecia were fixed in a solution of 6:3:1 absolute ethanol:chloroform:acetic acid. The fixed material was left at room temperature overnight, then stored at —20° for a period of 3 weeks to 6 months. This prolonged incubation helps to remove the fat globules from the cytoplasm. All other steps were as described previously (GRIFFITHS, DELANGE and JUNG 1974).

Methods used to obtain unordered asci (NEWCOMBE and GRIFFITHS 1972; PERKINS 1974) and other routine genetic manipulations (DAVIS and DESERRES 1970; DELANGE and GRIFFITHS 1980) have been previously reported.

RESULTS

Experimental design: Four recessive mutants, *asc(DL95)*, *asc(DL243)*, *asc-(DL879)* and *mei-1*, which show the abortion of some of their ascospores, were analyzed. This study was aimed at determining whether ascospore abortion in these mutants was the consequence of irregular segregation of chromosomes or of other abnormalities during meiosis. In Neurospora, irregular segregation of chromosomes can be detected by the presence of pseudo-wild type (PWT) progeny, which are disomics with complementing auxotrophic markers. In most cases, linkage group I (LG I) was used to monitor nondisjunction (see Figure 1). In the absence of exchange, nondisjunction at meiosis I (MI) will result in disomic ascospores PWT for all markers on LG I (auxo$^+$): *leu-3 a arg-1 ad-3B* + *un-3 A ad-3A nic-2 al-2*. In contrast, if there is a crossover, nondisjunction can

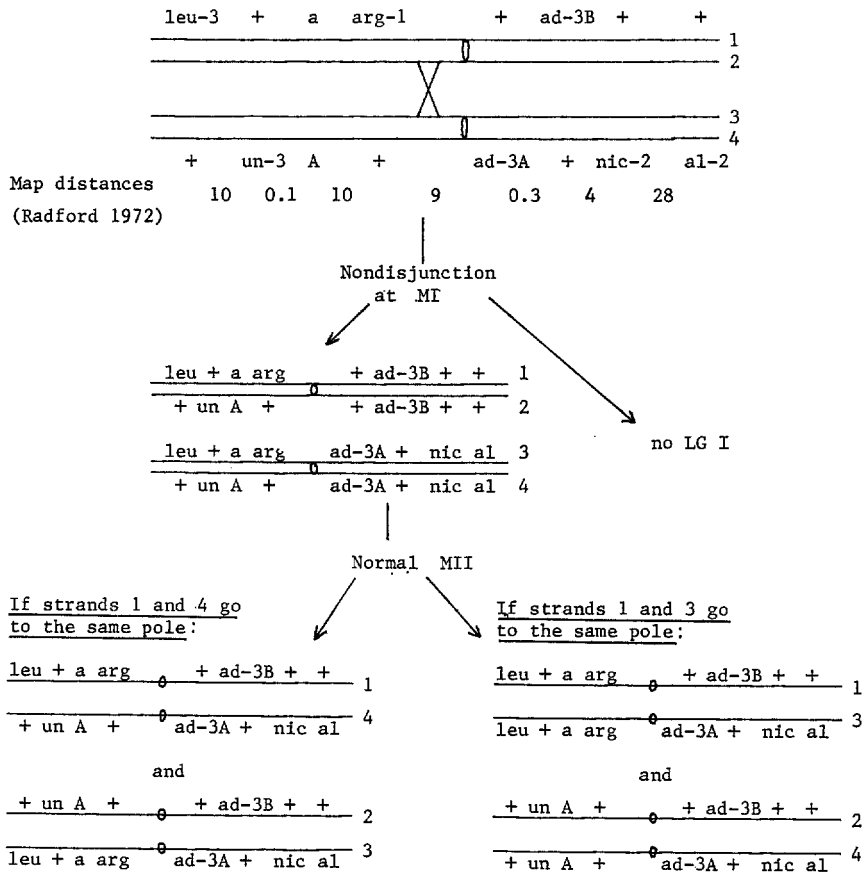

FIGURE 1.—PWT formation resulting from nondisjunction at the first meiotic division (MI). Providing appropriate markers are available, nondisjunction during meiosis I will produce characteristic progeny. The strains used to detect nondisjunction were heterozygous for several markers on LG I but homozygous for *tol* and a particular *asc* or *mei* allele (DELANGE and GRIFFITHS 1980). If non-exchange chromosomes nondisjoin at meiosis I, the progeny will be phenotypically wild type for all auxotrophic markers (auxo+) due to complementation of all markers on the two component chromosomes. Similarly, if a crossover has preceded the nondisjunction event (as illustrated in this figure), some progeny will be phenotypically wild type for markers on one part of the chromosome but mutant for the remaining part.

result in disomic ascospores PWT for all markers except those distal to the crossover (Figure 1).

Similarly, nondisjunction at MII can be detected through complementation of closely linked mutant alleles (in this study, *un-3/arg-1*, *ad-3A/ad-3B* and *al-1/al-2*). However, its detection requires a crossover between these alleles and the centromere (Figure 2). Consequently, detection will be more frequent the farther the complementing alleles are from the centromere.

Even though the detection of nondisjunction during both MI and MII depends on complementation between closely linked mutant alleles, these two events

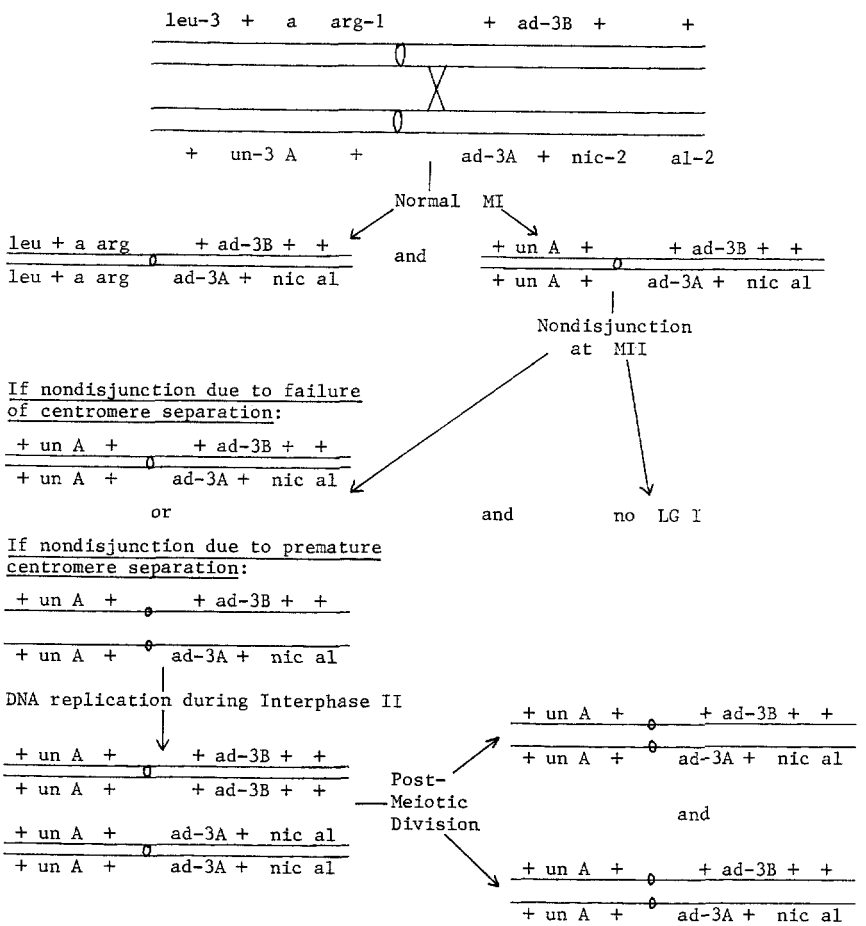

FIGURE 2.—PWT formation resulting from nondisjunction at the second meiotic division (MII). In order to detect nondisjunction during meiosis II, a crossover is required between the centromere and complementing mutant alleles. The resulting progeny will be heterozygous for markers distal to the crossover site, but homozygous for markers proximal and on the other arm of the chromosome.

can usually be distinguished if markers are present on both sides of the centromere. Disomics produced by nondisjunction at MII would usually be PWT for markers on one side of the centromere, but not the other (Figure 2). Nondisjunction at MI of certain crossover chromosomes would also produce disomics PWT for markers on only one side of the centromere (Figure 1). However, these would be far outnumbered by disomics PWT for markers on both sides of the centromere.

Whether produced by nondisjunction at MI or MII, disomic nuclei in young PWT ascospores soon haploidize (PITTENGER 1954). Therefore, these strains contain at least two complementing haploid nuclear types (see Figure 1 in DELANGE and GRIFFITHS 1980). The heterokaryotic nature of these PWT strains

was, in many cases, confirmed by testing the genotypes of individual conidial isolates.

An estimate of the percent recombination in PWT's was based on the frequency of albino auxo$^+$ PWT's. The production of these PWT's requires a crossover in the *nic-al* region. Since the reciprocal crossover class al^+/al^+ is not detected, the percent recombination in PWT's is twice the percent of albino auxo$^+$ PWT's.

asc(DL879)

Crosses homozygous for *asc(DL879)* generally resulted in about 70% ascospore abortion and a reduction in fertility.* The extent of spore abortion was quite constant in all crosses tested but fertility varied from low to medium. Initially, all strains carrying *asc(DL879)* grew at about one-third the rate of wild-type strains. It was found that this slow growth was due to an independent mutant allele (*slo*) linked to *asc(DL879)*. The *slo* allele, however, in no way affected the phenotype of *asc(DL879)*.

Percent recombination and nondisjunction: The analysis of ascospore cultures from four different crosses homozygous for *asc(DL879)* revealed a drastic alteration in the percent of both recombination and nondisjunction (Table 1). A high percent of PWT progeny, ranging from 16.4 to 37%, was recovered from all four crosses. The great majority of these were wild type for all auxotrophic loci on LG I. Therefore, it appears that these PWT cultures result from nondisjunction during the first meiotic division.

Three regions on LG I were monitored for percent recombination: *leu-un*, *un-nic* and *nic-al*. Compared to wild-type crosses, the values from all four crosses homozygous for *asc(DL879)* were reduced in all three regions (Table 1).

The nature of PWT progeny: To establish the nuclear composition of the PWT progeny from one of these crosses (879a13 × 879A15), the genotypes of conidial isolates from 10 PWT progeny were determined. In each case, the two parental types, *i.e.*, *un ad nic al* and *leu arg ad*, were recovered. Therefore, these PWT cultures were truly heterokaryotic, as would be expected if they were produced by nondisjunction at the first meiotic division. The additional detection of one crossover type among conidial isolates from two of the ten PWT cultures tested is consistent with somatic exchange events (PITTENGER and COYLE 1963).

The absence of crossover chromosomes from the 10 PWT cultures suggests that only nonexchange chromosomes fail to disjoin and are therefore included in the PWT progeny. To obtain a more quantitative measure of the frequencies of exchange chromosomes among PWT and non-PWT progeny, the percent recombination in the *nic-al* region was determined for both types of progeny from three crosses (Table 1). The value for PWT progeny (1.7% = 2 × frequency of albino PWT's; see section on Experimental Design) was much lower than that for non-PWT progeny (12.5%). It is therefore concluded that most nondisjunction involves nonexchange chromosomes.

* Fertility was always reduced due to the inviable aborted ascospores. However, in this paper, the term fertility is used to designate the total number of ascospores. An arbitrary measure of low, medium or high fertility is employed.

TABLE 1

Genetic analysis of four crosses homozygous for asc(DL879)*

Cross	Non-PWT progeny				PWT progeny					
	Total non-PWT progeny	Percent recombination			Total PWT progeny	PWT freq. (%)	Genotypes of PWT's			
		leu-un	*un-nic*	*nic-al*			auxo+	*al*	other	RF(*nic-al*)§
Q22–2 × Q22–8†	95	1.1	6.5	11.4	45	32.1	45	0	0	
Q8–1 × Q8–2†	63	3.2	7.9	12.7	37	37.0	35	1	1 (*leu arg*)	
Q35–1 × Q35–2†	81	2.5	6.2	13.6	33	29.0	33	0	0	
RF(*nic-al*) of 3 crosses combined:				12.5						1.7
879a13 × 879A15‡	61	6.5	0	3.3	12	16.4	12	0	0	
Wild type crosses		11–17	15–20	30–35		<0.1				

* Crosses were done on liquid medium; random ascospores were analyzed.
† *A* and *a* components from a PWT culture from a cross between strain I-34–8 (*un A ad nic al*) and 879A15 (*leu arg ad*; *asc (DL879)*).
‡ Ascospore isolates were derived as described in MATERIALS AND METHODS. LG I markers were identical for all four crosses (see Figure 1).
§ Percent recombination in the *nic-al* region among PWT progeny = 2 × percent albino PWT's (see Experimental design). The value was obtained by combining data from the first three crosses.

Ascus analysis: Unordered asci from a cross homozygous for *asc(DL879)* were analyzed in two ways. First, ascus abortion patterns were examined. Twenty-six out of 37 asci had an even number of black ascospores [8B:OW(1), 6B:2W(3), 4B:4W(2), 2B:6W(14) and OB:8W(6)], suggesting that the defect leading to ascospore abortion takes place prior to the post-meiotic division. However, the frequency of asci with odd numbers of black ascospores is too high (11/37) for complete regularity of this post-meiotic division. Second, the black ascospores from each ascus were germinated and the genotypes of the resulting cultures determined. Seven of 37 asci contained at least one PWT ascospore isolate. The presence of an odd number of PWT progeny in six of these asci strongly suggests that chromosome loss or secondary nondisjunction takes place during the post-meiotic division.

Percent recombination and nondisjunction involving chromosomes other than LG I: Thus far, nondisjunction has been recorded only for LG I. To investigate the degree of nondisjunction (and reduction in recombination) of other chromosomes, a cross [*A*; *asc(DL879)*; *cot-1*; *inos* × *a*; *asc(DL879)*; *pdx*; *his-1*] was analyzed, which permitted the simultaneous analysis of LG I, IV and V. LG I was tested for heterozygosity at the mating-type locus (*A/a*), LG IV for *pdx*$^+$ and *cot-1*$^+$, and LG V for *inos*$^+$ and *his-1*$^+$. In the latter two linkage groups, the percent recombination was approximated by the appearance of the double mutant (*e.g.*, *pdx cot-1*). The extreme rarity of such recombinants (1/165 *pdx cot-1* and 0/165 *inos his-1*) shows that recombination is reduced in all linkage groups (compare approximate map distances in wild-type crosses: *pdx-cot-1*: 20 mu; *inos-his-1*: 10 mu). In addition, this indicates that practically all *pdx*$^+$ *cot-1*$^+$ (71/165), and *inos*$^+$ *his-1*$^+$ (79/165) isolates are disomic for LG IV and V, respectively.

Table 2 shows the data on the simultaneous nondisjunction of these three

TABLE 2

Simultaneous nondisjunction of three linkage groups in two crosses homozygous for asc(DL879)*

Hyperploid for LG			Cross 129-75 × 128-15 Number of progeny		Cross 129-79 × 128-15 Number of progeny	
I	IV	V	Observed	Expected†	Observed	Expected†
+	+	+	28	14.5	8	2.7
+	+	—	9	12.7	3	4.3
+	—	+	11	15.9	6	5.0
—	+	+	5	12.2	3	5.4
+	—	—	9	14.0	3	8.0
—	+	—	8	10.7	7	8.6
—	—	+	12	13.4	6	10.0
—	—	—	23	11.7	24	16.0
			105	105.1	60	60.0

* Crosses were done on liquid medium.

† Determined from product of nondisjunction frequencies of individual linkage groups (LG I: 0.543, LG IV: 0.476, LG V: 0.533 for cross 129-75 × 128-15; and LG I: 0.330, LG IV: 0.350, LG V: 0.383 for cross 129-79 × 128-15).

chromosomes in two different crosses homozygous for *asc(DL879)*. The results reveal several aspects of nondisjunction in these crosses: (1) All three chromosomes tested show a high degree of nondisjunction. (2) The frequencies of nondisjunction of the three chromosomes in each particular cross are very similar. However, nondisjunction frequencies are different in different crosses (33.3, 35.0 and 38.3% in one cross and 54.3, 47.6 and 53.3% in the other). (3) The number of isolates either simultaneously PWT or non-PWT for all three linkage groups tested is significantly higher than expected ($p < 0.01$). This may mean that chromosomes do not disjoin independently of each other. Alternatively, it may reflect selection against isolates with some PWT and some non-PWT linkage groups. That the latter is quite plausible is shown by the percentage germination in these crosses (47.8, 50.0 and 57.5%).

Cytology: Cytological observations of crosses homozygous for *asc(DL879)* showed a drastic reduction in pairing of homologs at pachytene (Figure 3b). Some pairing was observed mainly near the tips of some chromosomes. Instead of seven bivalents, up to 14 univalents were detected on the spindle of metaphase I (Figure 3e). Even though about equal amounts of chromatin segregated during the first meiotic division, unequal amounts often segregated during the second and post-meiotic divisions leading to the different types of ascospore abortion.

In conclusion, the primary defect of mutant *asc(DL879)* appears to involve the pairing of homologs at the first prophase of meiosis. This results in a drastic decrease of recombination and the production of many univalents at metaphase I. The high frequency of auxo$^+$ PWT progeny suggests that nondisjunction takes place during the first meiotic division.

asc(DL243), DL393

Marked crosses homozygous for either *asc(DL243)* or *DL393* generally produced very few ascospores, and 90 to 98% of these were white (inviable). However, when no LG I markers were present, crosses homozygous for *DL393* produced 58 to 91% white ascospores. These results suggest that the phenotype of this mutant can be altered drastically by modifying genes.

The two noncomplementing mutant alleles of this locus are assumed to be homoalleles (of the same origin) because they behaved identically in genetic crosses (see below) and were recovered from the two ascospore isolates, DL243 and DL393, that were obtained from the same cross plate.

Percent recombination and nondisjunction: Random progeny analysis was performed on a number of crosses homozygous for *asc(DL243)* and *DL393*. Four types of progeny could not be explained as homokaryotic parental or crossover types (Table 3). These were apparently PWT cultures of genotypes auxo$^+$ (wild type for all auxotrophic markers), *un-3* (wild type for all markers except *un*-3), *ad-3* (*leu*$^+$ *arg*$^+$ *un*$^+$ *nic*$^+$) and *ad-3 nic-2* (*leu*$^+$ *arg*$^+$ *un*$^+$). In the pooled data, these four types represented 13.3% of all progeny.

Percentages of recombination in the three regions tested (*leu-un*, *un-nic* and *nic-al*) were similar to those obtained from wild-type crosses (Table 3). They

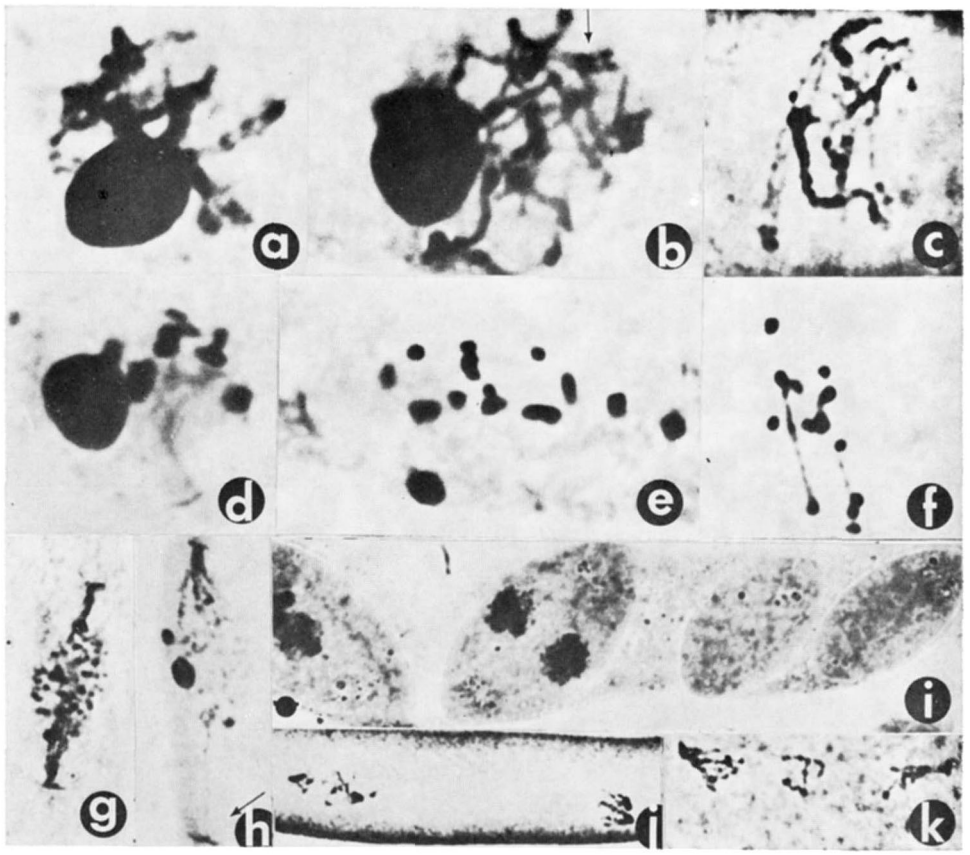

FIGURE 3.—Chromosome behavior in crosses homozygous for *asc(DL95)*, *asc(DL243)* and *asc(DL879)*. Preparations of *asc(DL95)* were stained with Feulgen and aceto-orcein; the others were stained with iron hematoxylin. The magnifications of Figures 3a-f, 3g-j and 3k are 3400×, 1300× and 1700×, respectively. The pairing of homologous chromosomes at pachytene is normal in crosses homozygous for *asc(DL243)* (a), drastically reduced for *asc(DL879)* (b) and incomplete for *asc(DL95)* (c). At diakinesis/metaphase I, seven bivalents appear for *asc(DL243)* (d), but up to 14 univalents were observed for *asc(DL879)* (e) and *asc(DL95)* (f). In crosses homozygous for *asc(DL243)*, many second-division figures were observed, with most chromatin attached to one spindle-pole body, but not the other (arrowed) (h); about equal amounts of chromatin may segregate in some asci (g). The enclosure of unequal amounts of chromatin into different ascospores was observed in many asci (i). The second meiotic division in crosses homozygous for *asc(DL95)* was also highly irregular: abnormal separation (j) and lagging of chromosomes (k) were observed frequently.

were determined as a proportion of the non-PWT progeny. Since most PWT's are not auxo$^+$ and since the percent recombination is not reduced, *asc(DL243)* does not behave as if its lesion is failure to pair and cross over at meiosis I.

The nature of PWT progeny: To determine the nature and origin of the PWT progeny, conidial isolates from 12 ascospore cultures representing all four types (auxo$^+$, *un-3*, *ad-3* and *ad-3 nic-2*) were tested. Table 4 shows genotypes recovered from these cultures. All cultures were heterokaryotic and most contained one

TABLE 3

Progeny analysis of crosses homozygous for asc(DL243) *and* DL393

Cross*	Non-PWT progeny: Total non-PWT progeny	Recombinants in region *leu-un*	*un-nic*	*nic-al*	PWT progeny: Total PWT progeny	Genotypes (*al* or *al*+) auxo+	*un*	*ad*	*ad nic*
243a32 × 243A18	16	3	5	6	1	1	0	0	0
243a32 × 243A28	17	1	4	3	3	0	1	1	1
243a23 × 243A27	17	3	2	4	3	0	0	2	1
243a31 × 393A35	18	3	1	6	3	2	1	0	0
4 comb. crosses DL243†	17	3	1	5	4	0	2	0	2
393a30 × 393A34	26	6	2	7	3	0	1	0	2
Total	111	19	15	31	17	3	5	3	6
Percent recombination									
Among non-PWT progeny		17.1	13.5	28.0	% PWT's = 17/128 = 13.3%				
In wild-type crosses (control)		11–17	15–20	30–35	% PWT's < 0.1%				

* Crosses between ascospore isolates that were derived from mutant strains DL243 and DL393 as described in MATERIALS AND METHODS (for genotypes, see Figure 1); these crosses were done on solid medium.

† Ascospore isolates from these crosses were obtained from shot asci; in the remaining crosses, random ascospore isolates were tested.

parental and one crossover chromosome. The *un*, *ad*, and *ad nic* genotypes were caused by the complementation of two nuclear types, one parental for LG I markers and one crossover in the *un-ad* region; two of the three auxo+ progeny contained two detectable crossover events in the *un-ad* region. Thus, all four

TABLE 4

Conidial isolates from representatives of all four types of apparently PWT progeny from crosses homozygous for asc(DL243)

Genotype of ascospore isolate	Conidial isolates: Parental (not including *al*) Genotype	No.	Crossover Genotype	No.	No. HK* genotype	Total no. tested
1 *ad*	*leu arg ad*	1	*un ad*	36	12	49
2 *ad*	*leu arg ad*	12	*un ad al*	20	17	49
3 *ad nic*	*un ad nic al*	15	*leu arg ad nic*	19	15	49
4 *un al*	*un ad nic al*	43	*leu un ad al*	4	2	49
5 *un*	*un ad nic*	13	*un ad*	24	12	49
6 *un*	*un ad nic al*	10	*un ad*	12	7	29
7 *un*	*un ad nic al*	3	*un ad*	13	11	27
8 *un*	*un ad nic*	0	*leu un ad al*	33	17	50
9 *un*	*un ad nic (al)*	0	*un ad*	22	5	27
10 auxo+	*un ad nic al*	4	*leu ad*	4	16	24
11 auxo+			*leu arg ad nic*	7	19	32
			un ad	6		
12 auxo+	*un ad nic al*	11			23	50
	leu arg ad	16				

* HK (= heterokaryon) isolates have the genotype of the original ascospore isolate.

types of PWT progeny appeared to be produced by the same event, each case involving at least one crossover event between *un-3* and *ad-3*. These crossovers were meiotic, rather than mitotic, since only one parental type was recovered from each PWT (see PITTENGER and COYLE 1963). The PWT's were apparently not caused by nondisjunction at meiosis I, following normal crossing over, since many more auxo$^+$ PWT's would have been expected. Instead, a high frequency of nondisjunction at meiosis II would explain the observed pattern of PWT progeny (see Figure 2; the auxo$^+$ types would be explained by two crossover events, one on each side of the centromere).

To rule out the remote possibility that nondisjunction at meiosis I involved only chromosomes with a crossover in the centromere region (the *un-ad* region spans the centromere of LG I), a cross between strains of genotype *leu-3 a arg-1 ad-3B al-1* and *un-3 A ad-3A nic-2 al-2* was analyzed. If nondisjunction at meiosis II was independent of exchange, a high frequency of orange (*al-1/al-2*) heterokaryotic progeny (parental + crossover in the centromere-*al* region) would result. Of a total of 65 isolates, 25 were heterokaryotic. This high frequency of heterokaryotic progeny suggested that nondisjunction at meiosis II involved both exchange and nonexchange chromosomes and thus was a general phenomenon not related to exchange.

Ascus analysis: Crosses homozygous for *asc (DL243)* and *DL393* produced similar ascospore abortion patterns. No asci with four or more black ascospores were ever detected. Most asci (99) contained eight white ascospores, but those with one (36) or two (31) black ascospores were not rare. Both black ascospores were germinated from eight 2B:6W asci. In each case, the pair of genotypes was identical, indicating that these constitute sister ascospores. The 1B:7W asci were apparently produced in part by chromosome loss or nondisjunction during the post-meiotic division. The high frequency of these 1B:7W asci shows that such loss is extensive.

Cytology: Crosses homozygous for *asc(DL243)* were examined cytologically. The first-acting defect of *asc(DL243)* takes place prior to or during karyogamy. The few asci that were produced showed normal pairing of homologs (Figure 3a). Seven bivalents condensed at diakinesis (Figure 3d) and divided in a normal fashion, distributing equal amounts of chromatin to the two poles. Most second-division figures show one spindle-pole body (SPB) with a large amount of chromatin attached and the other with little or no chromatin attached (Figure 3g,h). This differential attachment appears to be the cause of the extensive nondisjunction that was observed genetically. The amount of chromatin subsequently enclosed in the different ascospores is highly variable (*e.g.*, Figure 3i).

asc(DL95)

Crosses homozygous for *asc(DL95)* generally resulted in about 40% ascospore abortion; however, up to 70% abortion was detected in some crosses. The fertility of crosses on liquid medium appeared good. In some crosses made on solid medium, a nearly five-fold reduction in fertility was detected.

Percent recombination and nondisjunction: Random ascospore analysis of

three crosses homozygous for *asc(DL95)* revealed reduced recombination and increased nondisjunction frequencies (Table 5). The percent recombination was reduced in two of three regions examined; the amount of reduction appeared variable in both the *un-nic* and *nic-al* regions. This reduction was not due to peculiarities of the LG I chromosome used, since several independently derived LG I's were tested. However, the possibility that the reduction could be caused by a mutant other than *asc(DL95)* (*e.g.*, a *rec*-type mutant; see CATCHESIDE 1974) has not been rigorously ruled out.

No PWT progeny were detected among 239 progeny of a cross (Table 5, row 1) made on liquid medium. In contrast, all crosses made on solid medium produced a variable fraction (2.9 to 22.4%) of PWT progeny. The absence of PWT's from the one cross might have been due to the strains used, but was more likely due to the medium since PWT's were obtained from all 13 crosses made on solid medium. The majority of these PWT's were prototrophic for all LG I mutant markers. Therefore, these were apparently the result of nondisjunction during meiosis I.

The nature of PWT progeny: The nuclear composition of the eight PWT progeny recovered from cross 95–1 (Table 5) was determined by individually testing conidial isolates from these cultures. All eight cultures were heterokaryotic. Five of them were heterokaryotic for the two original nonexchange chromosomes, *leu-3 a arg-1 ad-3B* and *un-3 A ad-3A nic-2 al-2*. These were presumably the result of nondisjunction of nonexchange chromosomes at meiosis I. A single temperature-sensitive PWT (*un-3*) and an *ad-3A nic-2 al-2* isolate were both heterokaryotic for the original chromosome *un-3 A ad-3A nic-2 al-2* and a chromosome with a crossover in the *un-ad* region. These two PWT cultures may have resulted from nondisjunction during the first or second meiotic division (see Figures 1 and 2). The leucine-requiring PWT was heterokaryotic for the two crossover chromosomes *leu-3 a arg-1 ad-3B al-2* and *leu-3 un-3 A ad-3A nic-2*. This culture was apparently produced by the nondisjunction of these crossover chromosomes during the first meiotic division.

TABLE 5

Recombination and nondisjunction in three crosses homozygous for asc(DL95)

Cross*	Non-PWT progeny				PWT progeny			
	Total non-PWT progeny	Percent recombination			Total PWT progeny	PWT freq. (%) (*un* & auxo+)	Genotypes of PWT's	
		leu-un	*un-nic*	*nic-al*			auxo+	other
95A29 × 95a43†	239	15.9	2.1	16.3	0	0	0	0
95–1 (Y2 × X1)‡	154	16.9	9.8	22.7	8	3.7	5	3(*un, leu, ad nic al*)
95–2 (Y5 × X17)‡	94	18.1	10.6	11.7	10	9.6	10	0
Wild type crosses§		11–17	15–20	30–35				

* All strains used were ascospore isolates with genotypes as in Figure 1.
† This cross was made on liquid medium; the ascospores from shot asci were analyzed.
‡ These crosses were made on solid medium, and random ascospores were analyzed.
§ Recombination values are normally variable in Neurospora (CATCHESIDE 1974).

The nature of the leucine-requiring PWT isolate appeared to indicate that nondisjunction of crossover chromosomes may take place during meiosis I. To test the extent of such nondisjunction of crossover chromosomes, 263 PWT isolates from 10 crosses were obtained and scored for albino phenotype. The *nic-al* map distance among PWT progeny was estimated at 4.5 mu ($2 \times 6/263$; see Experimental design). In contrast, in three crosses examined, the percentages recombination in this region among non-PWT progeny were 16.3%, 22.7% and 11.7% (Table 5). These data provide strong evidence supporting the idea that, in this mutant, nondisjunction during meiosis I involves primarily nonexchange chromosomes, but that some crossover chromosomes fail to disjoin as well.

Ascus analysis: Many unordered asci from a cross (Table 5, row 1) homozygous for *asc(DL95)* have been analyzed. Most asci contained an even number of black ascospores: 8B:OW(41), 6B:2W(19), 4B:4W(22), 2B:6W(18), OB:8W(19) and 10 asci of all other types. The analysis of ascus patterns and of ascospores from these asci revealed several aspects of disjunction in this mutant. First, the percent of recombination in the *un-al* region was similar for 8B:OW asci (20%) and for all other asci combined (16.8%). Thus, the reduction in the percent of recombination is a general defect operative in all asci from this cross, not just an expression of a subgroup with increased ascospore abortion.

Second, the prevalence of asci with an even number of black ascospores suggests that the defect in disjunction takes place prior to the post-meiotic division, *i.e.*, during the first or second meiotic division. The absence of PWT progeny from the above-mentioned cross (Table 5, row 1) and the presence of large numbers of 8B:OW and 6B:2W asci suggest regular segregation of at least LG I during meiosis I. One might argue that ascospore abortion may be caused by nondisjunction of a linkage group other than LG I. In that case, the centromere region of only one homologous LG I (either *leu a arg ad* or *un A ad nic al*) would segregate with both copies of the nondisjoining chromosome(s). Therefore, all four viable products of 4B:4W asci would be either *leu a arg ad* or *un A ad nic al* (assuming no crossover had occurred). However, since seven of 10 asci had an MII pattern of segregation of LG I markers (*i.e.*, both types of chromosomes found in each 4B:4W ascus), it appears highly unlikely that conventional nondisjunction of any chromosomes during meiosis I could be the cause of the observed ascospore abortion.

Cytology: Three crosses homozygous for *asc(DL95)* were examined cytologically. The first defect was visible during the zygotene/pachytene stage when reduced pairing of homologous chromosomes is often evident (Figure 3c). Subsequently, a large number of metaphase I figures with up to 14 ($= 2N$) univalents were observed (Figure 3f). The segregation of about equal amounts of chromatin during meiosis I was usually followed by an irregular second meiotic division: spindle overlap, lagging of chromosomes, apparent slow separation of dividing nuclei and movement of segregating spindle-pole bodies to the same pole have been observed (*e.g.*, Figures 3j, k). These irregularities apparently lead to the distribution of unequal amounts of chromatin to the ascospores.

mei-1

During the present work, crosses homozygous for *mei-1* produced about 10% black ascospores when crossed on solid medium, as was observed previously (SMITH 1975), but 30% black spores when crossed on liquid medium. From a cross [*a^m(33) ad-3B*; *mei-1* × *un-3 A ad-3A nic-2*; *mei-1*] made on liquid medium, 22 of 24 ascospore cultures were adenine-independent, indicating that they were disomic for LG I. This value is as high as or higher than the value obtained by SMITH. Therefore, the initial defect resulting in the production of these high frequencies of PWT progeny was confirmed in this cross made on liquid medium.

Preliminary cytological observations of this cross confirmed the pairing defect and production of 14 univalents observed previously (LU and GALEAZZI 1978). However, the observations on subsequent stages of meiotic development differed significantly from those reported previously (*e.g.*, many asci were observed during the first interphase; irregularities such as spindle overlap and four-poled spindles were rare or absent). These preliminary observations suggest that the type of crossing medium may influence the pattern of segregation of univalents in Neurospora.

Interaction of mei-1 *and* asc (DL879)

Crosses homozygous for the double mutant *mei-1*; *asc(DL879)* produced about 70% spore abortion on liquid crossing medium, similar to values obtained from the two single mutants. The fertility of these crosses was good (*i.e.*, many spores were formed) although somewhat less than that of the wild type. Analysis of a cross homozygous for the double mutant, but heterozygous at the *ad-3* locus [*a^m(33) ad-3B*; *mei-1*; *asc(DL879)* × *un-3 A ad-3A nic-2*; *mei-1*; *asc(DL879)*], showed that 38 of 41 isolates (93%) were adenine-independent and therefore disomic for LG I. This value is similar to that of *mei-1* (80–90%), but different from that of *asc(DL879)* (20–55%). Therefore, these data suggest an epistatic dominance of *mei-1* over *asc(DL879)*. This epistatic relationship was confirmed cytologically; in such crosses there was an absence of pairing as in *mei-1*, rather than reduced pairing as in *asc(DL879)*.

DISCUSSION

Characteristics of four recessive meiotic mutants of *Neurospora crassa* are summarized in Table 6. The abortion of ascospores observed in crosses homozygous for each of the three newly isolated recessive mutants [*asc(DL95)*, *asc(DL243)* and *asc(DL879)*] was shown to be the consequence of abnormal disjunction of meiotic chromosomes. In two cases [*asc(DL95)* and *asc(DL879)*], the abnormal disjunction was apparently caused by a defect in the pairing of homologs during the first meiotic prophase. In the third mutant [*asc(DL243)*], a primary defect near karyogamy may have had a pleiotropic effect resulting in the nondisjunction observed during the second and post-meiotic divisions.

Correlation between pairing of homologous chromosomes and genetic ex-

TABLE 6

Characteristics of four recessive meiotic mutants in Neurospora crassa *with a defect in the regular disjunction of chromosomes*

Allele	Approx. % ascospore abortion	Crosses made on liquid or solid medium	Fertility*	Percent recombination *leu-un*	Percent recombination *un-al*	Pairing	Nondisjunction at MI	Nondisjunction at MII	Nondisjunction at PMD	Apparent initial defect
asc(DL95)	40–70	both	med-high	normal	reduced†	reduced	yes	yes	?	Pairing
asc(DL243)	90–98	both	very low	normal	normal	normal	no	yes	yes	Pre-ascus
asc(DL879)	70	both	low-med	reduced	reduced	much reduced	yes	?	yes	Pairing
mei-1	70	liquid	high	—	—	absent	yes	?	?	Pairing
	90	solid	high	absent‡		absent‡	yes‡	?	?	Pairing

* Total number of black and white ascospores produced.
† Reduction may at least in part be due to site-specific *rec* genes (see CATCHESIDE 1974).
‡ Data from SMITH (1975) and LU and GALEAZZI (1978); recombination was determined in regions of another chromosome (SMITH 1975).

change: The pairing defects of *asc(DL95)* and *asc(DL879)* are similar to those described for many asyndetic mutants in plants (reviewed in BAKER *et al.* 1976) and for the mutant *mei-1* in Neurospora (SMITH 1975; LU and GALEAZZI 1978). A reduction in pairing and chiasmata formation in higher plants is not always reflected in reduced genetic exchange. Thus, in *Lycopersicon esculentum*, three mutants with a reduced number of chiasmata produced normal or increased recombination frequencies (SOOST 1951; MOENS 1969). The disparity between the cytological and genetic observations may be a consequence of selective nonrecoverability of aneuploid gametes. In contrast, *ds* in *Hordeum vulgare* (ENNS and LARTER 1962) and *asc(DL95)*, *asc(DL879)* and *mei-1* in Neurospora show a good correlation between the extent of the pairing defect and the reduction in recombination. In Neurospora, this correlation may be explained by the apparent viability of many aneuploid products.

Correlation between reduced exchange and increased nondisjunction: Mutants with reduced pairing and exchange invariably exhibit abnormal patterns of disjunction at anaphase I. In recombination-defective meiotic mutants of Drosophila, only nonexchange chromosomes nondisjoin (BAKER and HALL 1976). Therefore, all bivalents that are held together by one or more chiasmata segregate in a normal fashion, and irregular segregation is due solely to univalents produced by a lack of chiasmata. In contrast, nondisjunction of some exchange chromosomes takes place in meiotic mutants of the nematode *Caenorhabditis elegans* (HODGKIN, HORVITZ and BRENNER 1979). Analysis of aneuploid progeny (PWT) produced by the mutants *asc(DL95)* and *asc(DL879)* of Neurospora showed that most, but not all, nondisjunction involves nonexchange chromosomes.

The absence of nondisjunction of exchange chromosomes in recombination-defective mutants of Drosophila may be the result of the action of the distributive pairing and disjunction system (*e.g.*, GRELL 1964), which may reduce the number of secondary abnormalities. In Neurospora, no evidence of a similar back-up disjunction system is available. Therefore, the low frequency of nondisjunction of exchange chromosomes may be a secondary effect of the production of nonexchange univalents. For example, the long duration of metaphase I observed in these mutants (see also PRAKKEN 1943) could have caused the precocious terminalization of the chiasmata and thus produced exchange univalents. Alternatively, lagging of chromosomes or spindle abnormalities induced by the abnormal nature of the chromosomes could have caused nondisjunction of some bivalents.

The nature of irregular disjunction: In the absence of a regular means of disjunction, univalents may move at random to either pole or divide equationally (by centromere division). These types of segregation have been encountered in higher plants (*e.g.*, CATCHESIDE 1939; SJODIN 1970); in many plant species, both types of segregation have been observed simultaneously (*e.g.*, PRAKKEN 1943). In Neurospora, some indirect evidence suggests that equational division of centromeres during anaphase I is a common means of segregation of univalents. Thus, a cross homozygous for the pairing-defective mutant *asc(DL95)* produced a high frequency of 6B:2W and 4B:4W asci that could not be explained by

regular nondisjunction at meiosis I of one or more chromosomes (see RESULTS). These asci and certain previously studied PWT-containing asci (THRELKELD and STOLZ 1970) could be best explained by the equational division of centromeres of some chromosomes. Direct proof for equational division awaits further cytological and genetic analysis of meiotic mutants. For example, it should be possible to observe the segregation of more than the haploid number of seven chromosomes at anaphase I.

Most genes that control pairing and exchange effect these processes in all chromosomes. The control of chiasma formation of a single chromosome has been observed only in *Hypochaeris radicata* (PARKER 1975). In *mei-1* (SMITH 1975) and *asc (DL879)* of Neurospora, both recombination and nondisjunction were affected drastically in several chromosomes tested.

A mutation that causes nondisjunction during meiosis II: The mutant *asc-(DL243)* appears to be unique. The primary defect takes place before the formation of asci. Most cells are apparently blocked prior to karyogamy. Since premeiotic DNA synthesis in Neurospora takes place just prior to karyogamy (IYENGAR *et al.* 1977), it is quite possible that the wild-type allele of *asc(DL243)* functions during or near this pre-meiotic S phase. Some cells, however, manage to proceed past this block and produce asci and ascospores. Such escape is not due to a mutational event, since $(A + a)$ PWT isolates from crosses homozygous for *asc(DL243)* produced the same crossing phenotype. Thus, the few asci produced in crosses homozygous for *asc(DL243)* are the consequence of leakiness. Regular pairing and disjunction at meiosis I is followed by an extremely high frequency of nondisjunction during meiosis II and chromosome loss or nondisjunction at the post-meiotic division. Nondisjunction during the second division apparently involves attachment of most chromosomes to one, but not the other, spindle-pole body (SPB).

In some respects, *asc(DL243)* resembles the *pal* and ca^{nd} mutants of Drosophila (see BAKER and HALL 1976). The mutant phenotype of *pal* is expressed only in males. Chromosomes of homozygous *pal* males are preferentially lost during the first zygotic cleavage division and perhaps during the meiotic divisions. Similar chromosome loss in ca^{nd} mutants occurs exclusively in females. In both cases, chromosomes are lost at one pole of the division. In these two mutants of Drosophila and the *asc(DL243)* mutant of Neurospora, a defect in the attachment of centromeres to SPB's causes either the loss (in Drosophila) or nondisjunction (in Neurospora) of a set of chromosomes.

A possible relationship between the defects at pre-ascus and meiosis II stages: It has been suggested that the wild-type alleles of *pal* and ca^{nd} in Drosophila specify a product that is a component of, or interacts with, the centromeric region of chromosomes and is necessary for the normal segregation of these chromosomes (BAKER 1975; BAKER *et al.* 1976). Alternatively, the phenotype of these mutants might be produced by a defective spindle-pole body. Similarly, in view of the timing of the first observable defect of *asc(DL243)*, it is possible that the corresponding wild-type gene product operates during the pre-meiotic S-phase and modifies the spindle-pole body or the centromere region of the newly syn-

thesized DNA. Either defect would generally cause a developmental block; however, the few calls that escape this block would lack the wild-type product necessary for regular segregation of chromosomes following meiosis I and thus would encounter problems during the second and subsequent divisions. One could speculate that this product may be necessary to reintroduce regular equational division after it was suppressed during the first division. A more definite assessment of the correlation between the two defects has to await more extensive analysis of this and similar mutants, *e.g.*, *mei-4*, in Neurospora (NEWMEYER and GALEAZZI 1978; RAJU and PERKINS 1978).

The nature of the defect of asc(DL243) *during meiosis II:* If the postulated abnormal centromere regions of *asc(DL243)* would align at random, one would

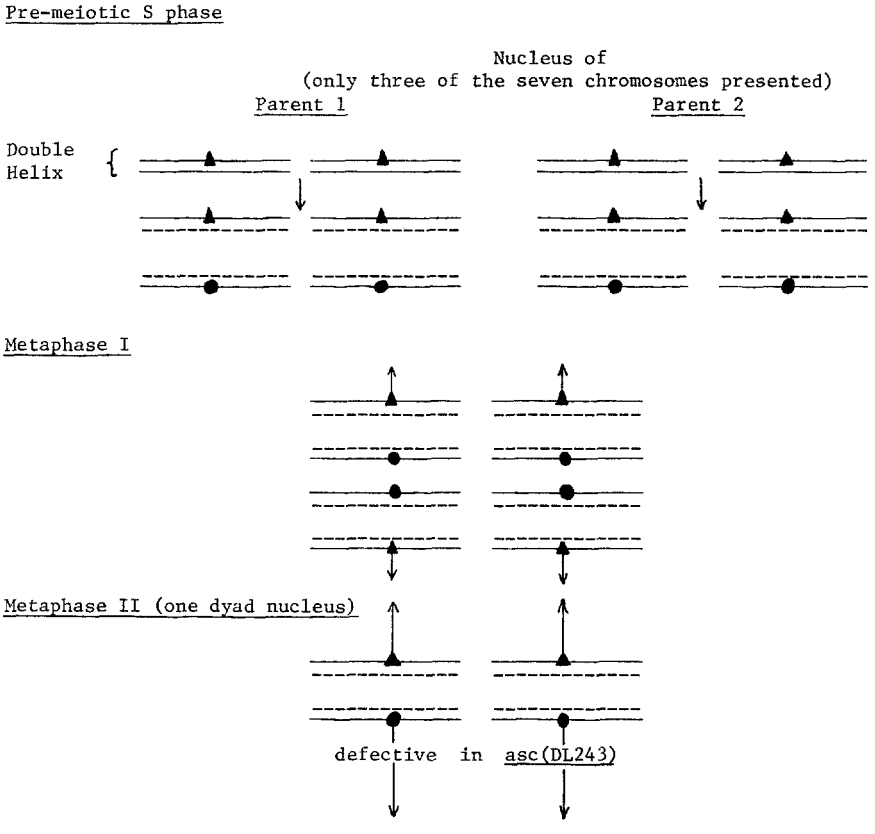

FIGURE 4.—Possible explanation for abnormal disjunction in crosses homozygous for *asc (DL243)*. Upon separation of DNA strands to enable pre-meiotic DNA replication, a centromere-associated protein (▲) is bound to one DNA strand. A novel protein (●) specific for meiosis would become attached to the other DNA strand. This protein might, for example, control some aspect of pairing of homologs and/or prevent regular centromere separation during the next division. This model requires that all chromatids with the newly synthesized (●) protein become aligned to the same pole during the second meiotic division. In the *asc(DL243)* mutant, this centromere-associated protein may be defective and thus prevent regular separation of chromatids at the second meiotic division (and likely at subsequent divisions).

expect random movement of each pair of chromatids of the seven chromosomes, and the formation of an extremely high frequency of aborted ascospores. Both cytological and genetic observations appear to contradict these expectations: (1) most chromosomes move to one pole, few or none to the other, and (2) many viable (black) ascospores are produced. Consequently, assuming a defect in the centromere regions, chromosomes could not align at random. Instead, centromeres that were synthesized at the same time (*e.g.*, during the pre-meiotic S phase) might normally align and segregate to the same pole (Figure 4). This type of preferential segregation has been observed in prokaryotic systems. (JACOB, RYTER and CUZIN 1966; LARK 1966) and proposed for some eukaryotic systems (*e.g.*, BAKER and HALL 1976). Even though the evidence for such alignment during mitotic divisions of eukaryotic cells is not convincing (*e.g.*, HEDDLE *et al.* 1967), such a mechanism may well operate during meiosis.

In conclusion, the present analysis has provided another demonstration of the usefulness of a combined cytological and genetic approach to the analysis of meiotic mutants in *Neurospora crassa*. Future studies should extend to the isolation of temperature-sensitive mutants and mutants that interact with existing meiotic mutants. In addition, study of the interaction between meiotic mutants may permit further assignment to specific pathways, *e.g.*, *mei-1* and *asc(DL879)* were thus assigned to the same pathway that is apparently involved in the establishment of pairing of homologs at prophase I.

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