THE ISOLATION AND CHARACTERIZATION OF RECESSIVE MEIOTIC MUTANTS IN NEUROSPORA CRASSA

by

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The study of the genetic control of meiosis has been initiated in *Neurospora crassa* by the isolation of recessive meiotic mutations. These mutations were detected by their reduced fertility or by the abortion of ascospores. To allow their expression, recessive meiotic mutations were made homozygous by selecting (n + 1) disomic ascospores. Cultures produced by each of these ascospores contain two types of nuclei with identical genes (including mutations) on all chromosomes except linkage group (LG) I, which contains the mating type locus. The simultaneous presence of these two types of nuclei allows the initiation of the sexual cycle, and therefore the detection of recessive mutations affecting the sexual cycle, including meiosis, on any chromosome except LG I. Using this method, three major classes of mutants have been detected. First, eleven mutants affecting perithecial development were expressed only as the maternal parent. Second, thirteen mutants produced perithecia with few or no ascospores. The infertility in two of these mutants was definitely caused by recessive mutations (*asc-2* and *asc-4*). Finally, the abortion of many ascospores was detected in thirteen mutant strains. Among these strains, six recessive mutations (*asc-1*, *asc-3*, *asc-5*, *asc-6*, *asc-7*, and *asc-8*) caused the abortion of many ascospores. The dominant mutation *SK(ad-3A)* was detected in this screen for recessive mutations, because it caused ascospore abortion when crossed with an *ad-3A* mutant but not with a wild type strain. This mutation, apparently allelic to *ad-3A*, caused the abortion of all *ad-3A*-containing ascospores.
The three ascospore abortion-type mutants asc-1, asc-3, and asc-6 were analyzed in more detail using both cytological and genetic methods. Ascospore abortion in these mutants was caused by abnormal disjunction of meiotic chromosomes. In mutants asc-1 and asc-6, the primary defect in pairing of homologs during the first meiotic prophase was followed by the formation of univalents at metaphase I. Observations on these mutants and on the mei-1 mutant (previously isolated; see Smith, 1975) suggested equational centromere division of many univalents at anaphase I. Subsequent irregular and prolonged separation of chromosomes at the second meiotic division appeared to be a secondary effect of the abnormal first division. The asc-3 mutant had 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 resulted in the abortion of most cells. Some cells managed to proceed past this block. During the second meiotic division most chromosomes of the few resulting asci were attached to only one of the two spindle-pole bodies. Disjunction at the post-meiotic division was also highly irregular. This mutant appeared to be defective in the attachment of one spindle-pole body to a set of centromeres. The defect may involve either a centromere-associated product or a spindle-pole body.
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PREFACE

This thesis is composed of four parts plus an introduction. The first three chapters are presented in a form suitable for subsequent publication. Chapter I describes the use of a new system in *Neurospora crassa* that enables the isolation of recessive mutants defective in the production of viable ascospores. The genetic and cytological characterization of some of these mutants has been described in Chapter II. Chapter III describes the study of an ascospore abortion-type mutant which specifically aborts ad-3A-containing ascospores. The final chapter is a general discussion and concentrates on the future approaches and uses of these types of mutants of *Neurospora*.
INTRODUCTION
This study was initiated to gain a better insight into the genetic control of meiosis. The fungus *Neurospora crassa* appeared ideally suited to conduct such research because both cytological and genetic means of analysis are excellent. However, this organism was lacking in one main aspect, namely the availability of a selection system that would enable the isolation of recessive meiotic mutations. Therefore, I have devised a system that enables the rapid screening of selected cultures for such mutations. The successful use of this system will be described in Chapter I of this thesis. A combination of cytological and genetic analyses of these mutations should be instrumental in gaining a more complete understanding of the genetic control of meiosis. The principal features that render the characterization of meiotic mutants so effective in *Neurospora* are given below.

**Cytological Analysis**

The chromosomes, nucleoli, spindles and spindle-pole bodies can be effectively followed during all stages of meiotic development, starting before karyogamy until ascospore formation (McClintock, 1945; Singleton, 1953; Raju and Newmeyer, 1977; Lu and Galeazzi, 1979). All fourteen (= 2N) chromosomes individualize shortly after karyogamy. During zygotene and pachytene of the first prophase, these chromosomes elongate and the homologs pair. This is usually followed by two rapid meiotic divisions, a post-meiotic division, and finally the enclosure of each nucleus in an ascospore. The chromosomes individualize
at each prophase. Thus it is often possible to determine the number of chromosomes per nucleus just prior to each division, and therefore any defect (for example, caused by a mutation) in the regular segregation of chromosomes.

Genetic Analysis

Several aspects of the life cycle of Neurospora crassa render it ideal for both the detection and genetic characterization of meiotic mutations:

(1) Whole populations of asci can be quickly scanned and all individual products of meiosis (i.e., ascospores) are recoverable.

(2) Most viable and inviable ascospores can be distinguished on the basis of color: viable ascospores are invariably black, whereas inviable ascospores are often white. This distinction is especially useful because it enables the detection and characterization of mutants with a defect in the disjunction of chromosomes. Such disjunction defects would produce ascospores which either miss one or more chromosome(s) or have extra chromosomes. The former type of ascospore would be white and the latter black (McClintock, 1945; Coyle and Pittenger, 1965).

(3) The apparent viability of aneuploid products (n + 1, n + 2, etc.) greatly facilitates the study of mutants with disjunction defects. Aneuploid products can be detected by the complementation of mutations (usually auxotrophic) on two homologous copies of a chromosome. They are called pseudo-wild type, or PWT, because, even though they contain mutant nuclei, they
appear to be wild type. Nondisjunction can be detected at both the first and second division provided the chromosome tested is appropriately marked (a more detailed description of this is given in Materials and Methods of Chapter II of this thesis).

(4) All products (ascospores) from each particular meiosis can be studied. The patterns of white and black ascospores in each ascus, and the analyses of genotypes of the viable black ascospores may provide information on the nature of irregular disjunction of chromosomes.

(5) The availability of special linkage tester strains makes the mapping of new mutations a relatively fast procedure in *Neurospora crassa* (Perkins and Bjorkman, 1979). Mapping of mutations with known defects would allow the detection of gene clusters of related functions.

The use of the genetic and cytological analysis of meiotic mutants appears excellent in *Neurospora*. However, even though such analysis would provide information on the control of the development and movement of macromolecular aggregates (e.g., chromosomes, chromatids, and spindle-pole bodies), it would probably not provide any detailed understanding of molecular processes that control the action of these aggregates. The understanding of these processes would require analysis by means of electron microscopy and biochemistry. The present work should therefore be seen as a framework from which more detailed analyses could be carried out. For example, the pairing of homologous chromosomes during the first prophase of meiosis has already been
successfully studied in *Neurospora* using electron microscopy (Gillies, 1972). Thus far, the characteristics of the life cycle of *Neurospora* have made biochemical analysis of meiosis difficult but such analysis will probably become feasible in the near future.

Before proceeding to discuss meiotic mutations in general and the isolation of them in *Neurospora* (see Chapter I), a description of some critical processes of meiosis should serve to focus on the main problems that need to be solved.

The major landmark events of the meiotic cycle are schematically represented in Figure 1. This cycle appears to represent a modification of the mitotic cycle. The following critical processes are characteristic of meiosis: the initiation of meiosis, recombination of genes, the reductional division, and the return to the mitotic cycle.

**Initiation of Meiosis**

The initiation of the meiotic division cycle appears to take place during the G1 period. Nuclei that have been committed to meiosis can be distinguished by their subsequent characteristic pattern of DNA replication (see next section). However, even though the nuclei are committed to some early meiotic events, they are not yet irreversibly committed to complete the meiotic cycle. Instead, they may, in the absence of the appropriate genetic or environmental controls, revert back to the mitotic cycle. The processes which irreversibly commit the different meiotic events will be discussed in the following sections.

The initiation of the meiotic cycle may be controlled by environmental conditions, internal signals generated by development in multicellular organisms, or a combination of these. In some unicellular
Figure 1. Representation of meiosis as a specialized form of the mitotic cycle. The mitotic cycle consists of mitosis (M), G1, DNA synthesis (S), and G2. At a specific point during the G1 phase, meiosis is initiated. A choice is made here between continuing the mitotic cycle or initiating meiosis. This choice depends on environmental clues. A series of subsequent steps ultimately lead back into the mitotic cycle.
premeiotic

S

initiation of meiosis

G1

S

M

reductional division

G2

pairing and recombination
microorganisms, meiosis is initiated when the substrate (food source) is depleted. In fact, in the budding yeast *Saccharomyces cerevisiae*, the initiation of meiosis is repressed by nitrogen and glucose. That this repression is under genic control has been demonstrated by the isolation of mutants which are insensitive to it (Dawes, 1975). In yeast, following the initiation signal, mating hormones are produced which arrest nuclei at a point during the G1 period (see Fig. 1). Subsequently, meiosis of most cells is initiated in a synchronous manner.

In many multicellular organisms, a number of (germ) cells are apparently especially programmed to undergo meiosis. In these cases, the state of development of the individual will generate the required signals that are necessary for the initiation of meiosis. Since these organisms are generally protected from environmental changes by internal homeostatic control, initiation should not be dependent on environmental conditions. In contrast, initiation in a number of microorganisms, e.g., many fungi and cellular slime molds, depends on environmental and developmental signals. In general, nutritional deficiency signals the initiation of the formation of fruiting bodies which involves a series of developmental steps that culminate in the induction of meiosis. In many fungi, specific mating type genes are involved in the production of fruiting bodies (e.g., Fincham and Day, 1971). However, it is not

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1 This stage may correspond to *G₀* in many multicellular organisms since cells may spend a long time at these points without losing their viability. In contrast, when the cycle is arrested at any other point, e.g., by means of a temperature-sensitive cell cycle mutant, viability rapidly declines as a function of incubation at the restrictive temperature.
known whether these genes are also involved in the initiation of meiosis. The study of mutations with defects prior to meiosis itself may become instrumental in the understanding of the genetic control of the initiation of meiosis.

**Meiotic Recombination**

Recombination is the central component of the meiotic process. The amount of recombination can be varied in two ways: a change in chromosome number, or a change in crossing-over between homologous chromosomes (i.e., intrachromosomal recombination). The independent assortment of chromosomes during the meiotic divisions generates new combinations of chromosomes from the two parent nuclei. Thus, an increase in chromosome number increases the number of possible new combinations of genes. Intrachromosomal recombination, involving gene conversion and crossing-over or exchange, requires a highly coordinated sequence of events which recur during each meiotic cycle. The frequency of crossing-over depends on the amount of accurate pairing of homologous stretches of DNA within the chromosomes and the efficiency of the exchange events. The recovery of a large number of mutations with aberrant pairing or exchange (for review, see Baker et al., 1976a), indicate that these processes are under strict genetic control.

The temporal stages that are essential for the control of pairing and exchange can be investigated by interfering with specific stages of more or less synchronously dividing populations of meiotic cells. It has been possible to interfere with meiosis using temperature shock, temporary application of inhibitors, explantation of meiotic cells from germ tissue to synthetic medium (e.g., Stern and Hotta,
1967; 1973), or temperature-sensitive mutations (e.g., Grell, 1978). The effect of these manipulations on pairing and exchange has shown that processes taking place just prior to the pre-meiotic S phase until pachytene are required for normal pairing and exchange.

The earliest stage in which normal function is necessary for the occurrence of regular exchange has been detected in *Chlamydomonas* (Chiu and Hastings, 1973). Recombination was increased after treatment with phenethyl alcohol or mitomycin C just prior to the pre-meiotic S phase. The reason for this effect is not clear since these inhibitors have quite different specificities.

There are several lines of evidence suggesting the involvement of pre-meiotic DNA synthesis in the recombination process. First, a function unique to meiosis is suggested by the extended period of time required to complete this pre-meiotic S phase. This is apparently due to a reduced number of initiation sites for DNA replication (Callan, 1973). The importance of the pre-meiotic S phase is also clear from the analysis of mutations that are defective in the process in *Saccharomyces*. None of these mutants develop synaptonemal complexes, although axial cores may be produced. It appears that these mutants are never committed to pairing and meiotic recombination. It follows that pre-meiotic DNA synthesis is either required for central element assembly into synaptonemal complexes or that both of these events are subject to common genetic control (Moens et al., 1976).

Second, in *Lilium*, 0.3% of DNA synthesis is delayed until early zygotene when pairing takes place (Hotta, Ito and Stern, 1966; Hotta and Stern, 1971; Stern and Hotta, 1977). The inhibition of this delayed
replication at early zygotene completely blocks pairing of homologs, suggesting that this replication is necessary for the pairing process in *Lilium*.

Third, temporary application of x-irradiation or inhibitors of DNA synthesis during the pre-meiotic S phase of *Chlamydomonas* resulted in increased exchange. In addition, temperature shocks at this stage caused altered chiasma and cross-over frequencies in several species (reviewed by Stern and Hotta, 1973). Even though the extent and direction of the effect on exchange varies in different species, the results clearly suggest an involvement of the pre-meiotic S phase in the recombination process.

Fourth, in *Saccharomyces*, commitment to exchange involves single-strand scissions of chromosomal DNA and apparently is a reversible cellular stage achieved during the pre-meiotic S phase (Silva-Lopez et al., 1975; Jacobsen et al., 1975).

Fifth, the sensitive period of a temperature-sensitive recombination deficient mutant in *Drosophila* occurs during the pre-meiotic S phase (Grell, 1978).

Shortly after the pre-meiotic S phase, cells in *Lilium* and *Saccharomyces* become committed to meiosis. Meiocytes of *Lilium* that are explanted onto synthetic medium after this commitment step proceed through an extended prophase characteristic of meiosis (Stern and Hotta, 1967). When meiocytes are explanted shortly after this commitment step, little or no pairing results at the next prophase. In addition, most chromosomes in meiocytes that have been explanted near or at leptotene will undergo unstable pairing. In either case, univalents are generally
produced at metaphase I. Thus it appears that both the achievement and stabilization of pairing of homologous chromosomes during the meiotic prophase require the activity of genes or their products well before the actual pairing process. Other components necessary for proper pairing and exchange are only required during these processes. For example, DNA synthesis is required during zygotene. The inhibition of DNA synthesis at early zygotene causes cell abortion by preventing the delayed semiconservative replication of 0.3% of total DNA needed for pairing of homologs. Inhibition at mid-zygotene causes extensive fragmentation of chromosomes at later stages, while at late zygotene it produces chromatid breaks which are mainly observed as bridges and breaks at the second meiotic division (Stern and Hotta, 1967).

The phenotypes obtained in these studies have also been observed in mutant meiocytes of many species. Mutants with pairing defects in plants are referred to as asyndetic. These may be asynaptic if a defect in the achievement of pairing is involved, or desynaptic if chromosome pairs are unstable and subsequently fall apart to form univalents at metaphase I. Other mutants affect chromosome integrity. These may involve gene products that are necessary for DNA synthesis during zygotene. In Drosophila, mutations with defects in pairing or exchange of homologs have been distinguished by their pattern of reduced exchange. The polarity of the pairing process suggests that if preconditions to exchange (e.g., pairing) were affected, the decrease in exchange would take place in a non-uniform manner along the length of each chromosome (Sandler et al., 1968). The majority of mutants with
altered exchange fell into this category. In contrast, a defect in the exchange process \textit{per se} should result in a uniform reduction of exchange in all regions. Only one gene appeared to behave in this manner (for review, see Baker \textit{et al.}, 1976a).

The above mentioned studies indicate that an extended period is needed to prepare and complete normal pairing and exchange and that many genes are probably involved in this process. In addition, because of the concurrence of reduced pairing and exchange and increased numbers of univalents, it may be anticipated that mutants with defects in pairing and exchange will simultaneously have increased nondisjunction. This has been verified with mutants from many species (see next section; see also Baker \textit{et al.}, 1976a).

The Two Meiotic Divisions

Haploidization of the chromosome complement requires two nuclear divisions without an intervening round of DNA replication. In most organisms this is achieved by the segregation of homologous chromosomes at the first division (reductional) followed by a regular division of centromeres at the second division (equational). This appears to be the most economical way to assure haploidization of all chromosomes. Besides the absence of the intervening DNA replication, there are two distinct deviations from regular mitotic divisions. First, the centromeres have to remain undivided until anaphase of the second division. In lily meiocytes, the potential for centromeres to divide during the next division is suppressed shortly after the pre-meiotic S phase (the centromeres of cells that have been explanted onto synthetic medium after this commitment step cannot divide during the next division).
That the delay of centromere division until anaphase II involves genetically specified events is clear from the existence of mutants in which separation occurs precociously (Clayberg, 1959; Lamm, 1944; Johnsson, 1944; Davis, 1971). Second, a virtually foolproof method of assuring segregation of homologs is essential for the regular segregation and haploidization during meiosis. The regular segregation of homologs invariably involves some pairing or alignment process. In most species, chiasmata are used to hold homologs together until they segregate at anaphase I. This relationship between exchange and subsequent segregation of homologs has been established by characterization of mutants with reduced intrachromosomal recombination. The homologs in mutant meiocytes are often not held together due to a lack of chiasmata; the resultant univalents move at random to the two poles or they divide by centromere division. The process by which homologs are held together at their chiasmata until anaphase I thus appears to be one of the main factors which assures the regular segregation of homologs at the first division of meiosis. In addition, controlled terminalization of chiasmata is critical to this type of segregation. The control of the reductive division may also involve other components since several mutants in Drosophila have a defect in disjunction of chromosomes at the first division, even though recombination is normal. The mutant ca^nd has been most

\(^2\)Regular segregation can be assured in different ways. For example, Drosophila males which have no crossing-over seem to utilize chromosome specific control of segregation which apparently involves pairing (see Baker and Hall, 1976). In Drosophila females, the segregation of non-exchange chromosomes is assured by the process of alignment called distributive pairing (Grell, 1964, 1969).
extensively studied (Baker et al., 1976a). Cytological analysis revealed spindle distortion with consequent nondisjunction of many chromosomes at the first division and chromosome loss at subsequent divisions.

In conclusion, studies on meiosis in a variety of organisms have shown that many processes operating from before pre-meiotic S phase until pachytene are essential for the regular occurrence of pairing and exchange. In addition, normal pairing and exchange is usually required to ensure the regular segregation of chromosomes during the meiotic divisions. However, these studies provide little insight into the nature and control of these processes. The identification and cytological and genetic characterization of mutations in most or all genes that are required for the successful completion of meiosis should be the logical first step to a full understanding of the meiotic process. This kind of approach has been followed in several organisms but it appears that a good means for the detection of mutants and of their cytological and genetic characterization render Neurospora particularly suitable to study meiosis in this manner.
CHAPTER I

MEIOSIS IN *NEUROSPORA CRASSA*.

I. THE ISOLATION OF RECESSIVE MUTANTS DEFECTIVE

IN THE PRODUCTION OF VIVABLE ASCOSPORES
INTRODUCTION

The normal sequence of chromosome behavior during meiosis has been determined by a combination of cytology and transmission genetics. A number of features of this division cycle are unique and distinguish meiosis from mitosis. First, pre-meiotic DNA synthesis involves fewer replication initiation sites, and some synthesis may be delayed until the following prophase (Stern and Hotta, 1973). Second, the first meiotic prophase lasts unusually long and involves the pairing of all homologous chromosomes. Genetic recombination that takes place at this time is orders of magnitude higher than that observed during mitotic division (Parag and Parag, 1975). Third, homologous chromosomes generally segregate from each other during the first meiotic division. Fourth, no DNA replication takes place during the interphase following this division.

Despite this 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 defects during meiosis. Such mutants in which the phenotype of meiotic cells or their products are detectably abnormal will be referred to as meiotic mutants. There are two types of meiotic mutants; they can be distinguished on the basis of their behavior during the vegetative phase. The first type has no apparent effect on any vegetative function and is called meiosis-specific. The second type of meiotic mutant is also defective in vegetative growth or maintenance.
Meiosis-Specific Mutants

Meiotic mutants which have no detectable defect in any vegetative functions have been detected in many organisms, including higher plants, fungi, and Drosophila3. (for a review, see Baker et al., 1976a). The defect in these mutants may cause a readily detectable meiotic phenotype such as altered recombination, disjunction, or fertility (see later section). Alternatively, the change of phenotype of such mutants may be subtle. The rec-type mutants controlling region-specific recombination in Neurospora and Schizophyllum are examples of the latter type (Catcheside, 1974). In this paper I will only deal with mutations which affect recombination, disjunction, or fertility, i.e., those with a visibly abnormal phenotype.

Mutants With Defects During Both the Vegetative and Sexual Phases

Two types of mutants with defects in vegetative functions have been associated with corresponding defects in meiotic processes. First, all temperature-sensitive cell cycle mutations of Saccharomyces that have been tested also caused meiotic abnormalities (Simchen, 1978). It appears that both types of cell division are under a certain amount of common control. Second, some genes required for vegetative DNA metabolism (e.g., repair, mutation, somatic recombination), are presumably also required for meiotic exchange or associated processes. Mutations in these genes have been isolated on the basis of their sensitivity to

3However, some mutants of Drosophila with no apparent defect of somatic functions were shown to affect specifically differentiated somatic cells at well-defined periods (Baker et al., 1978).
UV, ionizing radiation, or the alkylating agent methyl methane sulphonate (MMS). Others were obtained by their altered frequencies of mitotic gene conversion or spontaneous mutation.

Many mutants that are sensitive to DNA-damaging agents have been isolated and studied in the yeast **Saccharomyces** (Prakash and Prakash, 1977), and in **Drosophila** (Boyd et al., 1976a,b; Boyd and Setlow, 1976). In yeast, three repair pathways were identified on the basis of cross-sensitivity to mutagenic agents. Class I mutants are sensitive to nitrogen mustard (HN₂) and UV; class II to MMS and x-rays; and class III to all four agents. It was found that many mutants of classes II and III have an effect on meiosis (Baker et al., 1976a; Prakash and Prakash, 1977). A similar overlap of gene functions required for both repair to MMS-induced damage and meiosis (e.g., recombination) is apparent from the analysis of mutants selected in **Drosophila** on the basis of increased sensitivity to MMS (Baker et al., 1976b). Mutations in five complementation groups cause sensitivity to HN₂, UV, MMS, and x-rays. These are analogous to the class III mutants in yeast. Mutations in three and possibly four of these complementation groups reduce the frequency of meiotic exchange. Of these, two (**mei-41** and **mus-101**) are defective in post-replication repair, and one (**mei-9**) is defective in the repair of UV-induced damage. Mutations in at least two genes cause sensitivity to MMS and x-rays, and thus resemble the yeast class II mutants; these two have an apparent defect in meiosis. From these results it is clear that gene functions involved in certain pathways of DNA repair in somatic cells are also required during meiotic recombination. Therefore, the identification and characterization of various repair pathways will provide
information that may lead to the understanding of mechanisms involved in the process of meiotic recombination and associated processes. Conversely, the isolation of meiotic mutants may stimulate the study of repair pathways.

Gene functions involved in DNA metabolism in somatic cells can also be identified by screening for mutants with altered induced mitotic gene conversion frequencies (e.g., Rodarte-Ramon and Mortimer, 1972; Rodarte-Ramon, 1972). Three out of four mutants thus isolated had sporulation defects.

The mutations spo-7 (Esposito et al., 1975) and rem-1 (Golin and Esposito, 1977) in Saccharomyces resulted in meiotic defects and an altered frequency of spontaneous mutation. Other meiotic mutants were sensitive to histidine (e.g., uvs-3, uvs-4, uvs-5, uvs-6, and mei-3 in Neurospora crassa; Newmeyer et al., 1978). In three histidine-sensitive mutants examined the increased sensitivity was accompanied by meiotic blockage, UV sensitivity, and increased instability of duplications.

The Detection of Meiotic Mutants

The systematic isolation of meiotic mutants has been initiated in several organisms. Two types of criteria were used to detect these mutants. First, mutants with altered recombination and/or disjunction frequencies were recovered in Drosophila (Sandler et al., 1968; Baker and Carpenter, 1972), Saccharomyces (Roth and Fogel, 1971; Roth, 1976), and Caenorhabditis (Hodgkin et al., 1979). Second, complete or partial sterility has been used as a criterion for the isolation of meiotic mutants in Sordaria (Esser and Straub, 1958), Schizosaccharomyces (Bresch et al., 1968), Saccharomyces (Esposito and Esposito, 1969), and
Podospora (Simonet and Zickler, 1972). Since reduced fertility may be caused by many defects not directly associated with meiosis, cytological and/or genetic observations are required to confirm the isolation of meiotic mutants among sterility mutants.

Meiotic Mutants in Neurospora

Even though meiotic mutants have been obtained in many organisms, the understanding of the defects is often in doubt because of a lack of genetic or cytological means of analysis. Various idiosyncrasies 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 of Drosophila (Pura 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 good. The ascomycete Neurospora crassa fits this description well.

In Neurospora, meiotic mutations which are dominant or expressed only in 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 mutations cannot be expressed in the first generation of crossing. It is possible to detect such mutations by intercrossing a number of isolates from each cross involving a potential mutant, but this is very time-consuming. The technique described in this thesis enables the rapid isolation of recessive meiotic mutations in Neurospora.
This method involves the selection of strains arising from ascospores disomic for linkage group I and heterozygous for the mating type locus \((A + a)\). Such strains are self-fertile and homozygous for all linkage groups except LG I. Therefore, each of these cultures can be directly tested for the presence of a recessive meiotic mutation.

Meiotic mutations in *Neurospora crassa* can be detected as reduced fertility due to a block in the formation of most or all asci, or as 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 such aborted (white) ascospores is a good detection system for mutations which cause irregular segregation of chromosomes during meiosis (Smith, 1975).

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

**MATERIALS AND METHODS**

**Strains**

The following alleles were used during this study: \(\text{leu-3 (R156)}\); \(\text{un-3 (55701-t)}\); \(\text{arg-1 (36703)}\); \(\text{ad-3A (2-17-814)}\); \(\text{ad-3B (2-17-114)}\); \(\text{nic-2 (43002)}\); \(\text{al-2 (74A-Y-112-M38)}\); \(\text{tol (N83)}\); and two alleles at each of the three heterokaryon incompatibility loci C/c, D/d, and E/e. The location of these mutations on linkage groups and the approximate map distances (Radford, 1972) between linked mutations are shown in Fig. 1a.
Fig. 1 General outline of a selective system used to isolate recessive meiotic mutants in *Neurospora crassa*. For details, see Materials and Methods.

(a) A crossover between strain I-34-8 (female) and the mutagenized strain I-30-335 (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 produces two types of nuclei of opposite mating type in each culture produced by a disomic ascospore; such cultures are capable of "selfing." The genes and therefore newly induced meiotic mutations are identical in both types of nuclei. Consequently, if a recessive meiotic mutation were present in the disomic ascospore, it would be detected in the "selfing" of the resulting PWT culture.
(a) Cross between strains:

<table>
<thead>
<tr>
<th>Linkage Group I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>leu-3 + a arg-1 + ad-3B +</td>
<td>C d</td>
<td>tol</td>
<td>asc</td>
<td>e</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I-30-225

x

| I-34-8 | + un-3 A + ad-3A + nic-2 al-2 | c D | tol | + | E |

Approximate map distance (Radford, 1972)

10 0.1 10 9 0.3 4 28 75

(b) Example of a disomic (n + 1) ascospore:

leu-1 + a arg-1 + ad-3B + | C d | tol | asc | E |

+ un-3 A + ad-3A + nic-2 al-2

(c) Heterokaryon:

Component 1

leu-3 + a arg-1 + ad-3B + | C d | tol | asc | E |

Component 2

+ un-3 A + ad-3A + nic-2 al-2 | C d | tol | asc | E |

* An induced meiotic mutation may be located on any linkage group except LG I. Mutations of this kind, isolated during this study, were designated asc (see Results).

† Only one of the eight possible combinations of het alleles is presented.
Mutant Induction and Isolation

Recessive meiotic mutants can only be detected in crosses homozygous for these mutants. A method for the homozygosis and detection of such mutants in Neurospora crassa has been developed (Fig. 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 \((A/a)\). Disomic nuclei in Neurospora are inherently unstable (Pittenger, 1954). They usually haploidize quickly by losing, at random, one or the other homologous chromosome. Since the selected ascospores carry disomic nuclei which are heterozygous for the mating type locus, the resulting colonies are heterokaryons with two haploid nuclear types of opposite mating type \((A + a)\). In addition, since each disome contains a haploid complement of all chromosomes except LG I, genes on these chromosomes will be identical in the two nuclear types produced during haploidization.

The simultaneous presence of both mating types in these heterokaryons will, under the appropriate conditions of nitrogen starvation (Westergaard and Mitchell, 1947), induce perithecial formation and meiosis. The mating reaction of these heterokaryons will be referred to as "selfing." This resulting "self" will be heterozygous for LG I and therefore the mating type locus, but homozygous for genes on the other chromosomes. Therefore, any induced recessive meiotic mutation which is located on one of these chromosomes will be present in homozygous condition. Thus, the prerequisite for their detection has been met.
Strains Selective for Self-Fertile Pseudo-Wild Type (PWT) Cultures

The selection of ascospores disomic for LG I was made possible by the introduction of various markers onto the selective parent strains (see Fig. 1a for the location of these markers on their respective linkage groups).

First, cultures arising from disomics for LG I constitute a heterokaryon with two 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 will grow very poorly. Therefore, the tol mutation which suppresses this heterokaryon incompatibility without affecting crossing ability (Newmeyer, 1970) was introduced in both strains (I-30-225 and I-34-8 in Fig. 1).

Second, the closely linked auxotrophic mutations leu-3, arg-1, ad-3A, ad-3B, nic-2, and the nonsupplementable heat-sensitive mutation 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. 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 two types of ascospore progeny capable of growth on medium containing no leucine, arginine, adenine, or nicotinic acid (i.e., minimal medium): multiple recombinants and PWT 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 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 mutations and opposite mating type alleles.

Third, when ascospores are plated on minimal medium, the germ tubes of two adjacent ascospores may fuse. Such fusion products may produce a colony if the two nuclear types complement each other and are heterokaryon compatible. To minimize the frequency of colony-producing fusion products, three 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 Fig. 1) was made heteroallelic at each of these three loci. When ascospores produced by this cross are plated on minimal medium, only $\frac{1}{8}$th of all fusion products with complementary auxotrophic requirements can form a colony. In fact, colonies produced by the fusion of two or more adjacent ascospores were practically eliminated when the plating concentration did not exceed $2 \times 10^4$ ascospores/90 mm petri dish. Consequently, the plating of ascospores at concentrations up to $2 \times 10^4$ spores per plate allowed the virtually exclusive isolation of self-fertile PWT colonies.

The Isolation of Induced Recessive Mutations Which Affect Meiosis

Conidia from seven-day old cultures of strain I-30-225 were treated with 0.025 mM MNNG at 25°C for four, five, or six hours (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 seven-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 $5 \times 10^{-5}$. Each of these cultures was assigned an isolation number with the prefix "P" for PWT (e.g., culture P100 is PWT isolate number 100), and was isolated by one of three possible methods. First, using a dissecting microscope, very small colonies were isolated after two and three days of growth. At this time the simultaneous transfer of unselected ascospores can be avoided. Second, after four or five 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, is reliable. Third, part of the mycelial growth or conidia were transferred after eight days of growth on plates. The latter method is the fastest but may lack sensitivity since non-growing germinated ascospores may be "rescued" by a poorly growing PWT culture. A subsequent self of such a rescued fast-growing culture would be heterozygous at many loci.

Each PWT culture was transferred to a slant of minimal medium in a 10 x 75 mm tube, and allowed to grow for a week before being transferred to an 18 x 150 mm test tube containing 5 ml liquid minimal crossing medium and a strip of filter paper (Newcombe and Griffiths, 1972). All selves were incubated at 25°C, some also at 16°C. 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 minimal 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, mutation, crosses with
both wild type strains should produce normal perithecia and ascospores. Therefore, only cultures with this behavior were classified as potential recessive mutants. Recessive mutants were distinguished from mutants that are expressed only when present in the male or female parent, by means of reciprocal crosses between the mutant PWT culture, and the OR-A and OR-a strains.

Some of these mutations may affect perithecial formation while others are more directly involved with the production of viable ascospores. Among mutants with a defect in perithecial development, no asci or ascus initials have ever been reported. Therefore, it appears plausible that meiosis is 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 further analyzed. Five A isolates of the cross between any given PWT and OR-A, and five a isolates of the cross between the PWT and OR-a were intercrossed in all combinations; if the mutant phenotype was detected, one mutant isolate of each mating type was used in the testing of all isolates from crosses between the PWT culture and the two wild type strains (OR-A and OR-a). Sometimes, all these isolates were backcrossed to the original PWT culture. In either case, a 1:1 segregation of mutant and wild type phenotype confirmed the presence of a recessive point mutation.

Media and routine manipulations were conventional for Neurospora (Davis and de Serres, 1970).
RESULTS

Using the selective system described in MATERIALS AND METHODS (see also Fig. 1), 1090 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., eject only viable black ascospores from mature perithecia, were screened against dominant mutations by crossing them individually with OR-A and OR-a wild type strains. Table I shows that 46 strains exhibited wild type crossing ability with both OR-A and OR-a. These strains, which may carry recessive mutations or mutations that are expressed only in the female or male parent, were classified according to phenotype into three main groups: 16 strains with a defect in the perithecial development (class I); 26 strains defective in the formation of asci or black ascospores (class II); and 4 strains with a miscellaneous developmental defect apparently not associated with reduced fertility (class III).

Class I

Mutants of this type either produce no perithecia at all (6 strains), or few or incompletely developed perithecia (10 strains). Perithecia with a size in between proto- and fully grown perithecia, and lacking a neck, are considered incompletely developed. To distinguish mutants that are expressed only in the female or male parent (female or male sterile mutants) from recessive mutants, 4 strains (P205, P349, P406, P700) with no perithecia, 2 strains (P841, P891) with few perithecia, and 2 strains (P186, P434) with incompletely developed perithecia were crossed reciprocally with wild type strains.
TABLE I. Initial characterization of 145 pseudo-wild type cultures with aberrant crossing behavior*

<table>
<thead>
<tr>
<th>Type of Crossing Aberrancy</th>
<th>Number of PWT Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
</tr>
<tr>
<td><strong>Class I</strong></td>
<td></td>
</tr>
<tr>
<td>A: Sterile (i.e., no perithecia)</td>
<td>50</td>
</tr>
<tr>
<td>B: Few or incompletely developed perithecia</td>
<td>3</td>
</tr>
<tr>
<td><strong>Class II</strong></td>
<td></td>
</tr>
<tr>
<td>A: Perithecia without spores</td>
<td>0</td>
</tr>
<tr>
<td>B: More than 20% ascospore abortion</td>
<td>44</td>
</tr>
<tr>
<td><strong>Class III</strong></td>
<td></td>
</tr>
<tr>
<td>Defects not related to fertility (see text)</td>
<td>2</td>
</tr>
</tbody>
</table>

*Each culture was crossed with OR-A and OR-a wild type strains; those producing normal perithecia and ascospores with both strains were classified as potential recessive mutants (m); the defect in the remaining cultures was apparently caused by dominant determinant(s) and these were called M.
(the remaining 8 strains grew very poorly and were not tested). In each case, mutant phenotype resulted when the mutant strain 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 which were either completely barren or contained very few black ascospores (about 10-20 spores per peritheciurn as compared to hundreds in normal perithecia). This class of mutants was subdivided into 4 phenotypes: 8 strains with empty perithecia (P246, P308, P373, P400, P446, P741, and 2 additional strains that were discarded due to poor growth); 2 strains that produced only asci with 8 little round bubbles, about one-quarter the size of regular ascospores, but no ascospores (P314, P423); 1 strain with empty asci (P131); and 2 strains with few black ascospores (P310, P1163). The cause of the defect in 4 strains was further investigated. In 2 cases (P131 and P400) a recessive point mutation was involved. However, the empty perithecia of P446 and the bubble asci of P314 were apparently not caused by a recessive mutation. One out of 5 ascospore isolates obtained from a cross between P446 and OR-A wild type 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 P314 appears to have a more complex pattern
of inheritance. Whereas the original PWT culture produced only bubble asci, intercrosses of some isolates from a cross between P314 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 were characterized by their pattern of ascospore abortion. The abortion in 6 of these strains (P95, P243, P393, P711, P879, P961) was found to be due to a single recessive mutation. The fertility (total number of black and white ascospores) of strains P243, P393, and P879 was very low. These mutants are discussed further in the following section or in Chapter II. The ascospore abortion in another strain (P917) was apparently caused by a dominant spore-killer mutation. This mutant is described in Chapter III. Strain P1079 produced only white inviable ascospores whereas strains P165 and P631, with 50-80% ascospore abortion 3 weeks after crossing, contained mostly black ascospores after 2 months. No clear-cut pattern of inheritance was evident in strains P165, P631, and P1079. The remaining 3 strains with about 20% ascospore abortion (P117, P285, P768) 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, during the analysis of strain P917 (see Chapter III), a recessive mutation which caused ascospore abortion and low fertility was detected. This mutation was originally present in heterozygous condition and was therefore not detected in the original selfing of P917. A cross between strains 917A7 (mutant A ascospore isolate from
the cross P917 x OR-A) and I-30-225 (leu-3, a, arg-1, ad-3B) produced 9/30 recombinant progeny between leu-3 and the mutation. In addition, both a, arg, ad recombinant progeny were mutant. These preliminary results indicate linkage of this recessive mutation to the tip of the left arm of LG I.

Class III

The abnormal phenotypes of these 4 mutant PWT strains are apparently not related to fertility. Two strains discharged their spores very poorly and were not examined further. The 2 remaining strains affected the phenotype of the peritheciurn. Strain P126 produced orange instead of black perithecia. This phenotype was controlled by a point mutation expressed only in the protoperithecial parent. Since this phenotype is apparently only produced by mutations at the per-1 locus (Howe and Johnson, 1976), it is plausible that P126 is an allele of this locus. Another point mutation (in P413), similarly expressed only in the protoperithecial parent, prevents the formation of necks on the perithecia. This defect prevents the discharge of ascospores from their perithecia. Since the wild type locus apparently controls the formation of the perithecial neck, the mutation will be designated pen-1.

Finally, in a preliminary attempt to isolate temperature-sensitive mutants, a strain (P709) was found to produce 4-spored asci at 16°C, and the normal 8-spored asci at 25°C. This cold sensitive mutant is dominant and has been mapped at or near the centromere of LG I. The mutant will be designated Fsp-2 (4-spored ascus). Fsp-1 is not temperature-sensitive (Raju, 1977).
Recessive Mutations that Affect the Formation of Asci or Viable Ascospores

A total of 9 recessive mutations (2 of class II-A and 7 of class II-B) affecting ascus or ascospore production have been positively identified during this study. In the past such mutations have been detected among mutants that were UV-sensitive (uvs-3, -5, and -6) or caused increased instability of duplications (mei-3). Some other mutations (mei-1, mei-4) were detected directly by their ascospore abortion (for review see Perkins and Barry, 1977).

Both meiotic and other developmental processes are required to complete the sexual cycle and, thus, to produce asci and viable ascospores. Therefore, a defect in the production of asci or viable ascospores could be caused by a defect not directly associated with meiosis. Consequently, not all recessive mutations of this kind would be meiotic (mei) mutations. The new locus designation asc which is introduced here refers to all recessive mutations resulting in the absence of asci or the abortion of asci or ascospores.

All 9 asc mutations were tested for allelism to each other and to mei-1. It was found that only P243 and P393 were allelic to each other. In addition, all asc mutations were non-allelic to mei-1. Table II shows the 8 loci with allele or isolation numbers and comments on their phenotype.

The results of analysis of asc-1, asc-3 and asc-6 are reported in Chapter II. These appear to have a defect during meiosis. The wild type gene of asc-7 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
TABLE II. The phenotypes of recessive class II mutations at eight loci, whose wild type alleles are necessary for the formation of normal asci or black ascospores

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Isolation No.</th>
<th>% Ascospore Abortion</th>
<th>Nature of Defect</th>
<th>Other Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>asc-1</td>
<td>P95</td>
<td></td>
<td>40 - 70</td>
<td>Intermed to high fertility*</td>
<td></td>
</tr>
<tr>
<td>asc-2</td>
<td>P131</td>
<td></td>
<td></td>
<td>Empty asci</td>
<td></td>
</tr>
<tr>
<td>asc-3</td>
<td>P243, P393</td>
<td></td>
<td>90 - 98</td>
<td>Very low fertility</td>
<td></td>
</tr>
<tr>
<td>asc-4</td>
<td>P400</td>
<td></td>
<td></td>
<td>Empty perithecia</td>
<td></td>
</tr>
<tr>
<td>asc-5</td>
<td>P711</td>
<td></td>
<td>20 - 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>asc-6</td>
<td>P879</td>
<td></td>
<td>70</td>
<td>Low to intermed fertility</td>
<td></td>
</tr>
<tr>
<td>asc-7</td>
<td>P917</td>
<td></td>
<td>90</td>
<td>Low fertility</td>
<td></td>
</tr>
<tr>
<td>asc-8</td>
<td>P961</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fertility was defined as the total number of ascospores (black and white) that were ejected from the perithecia (see MATERIALS AND METHODS).
ascospores were found interspersed in the same asci. The defect of the asc-5 mutation is of a different nature. Crosses homozygous for this mutation produced many asci with 8 black ascospores and a variable number of asci with only white ascospores. The latter type of asci contained either 4 or 8 spores. The amount of ascospore abortion varied widely between about 20 and 80%. A range of 50-80% was encountered in 11 crosses involving ascospore isolates. A cross between 2 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. Finally, the mutant asc-8 was not analyzed further.

DISCUSSION

A new system for the homozygosis of induced mutations has been used to isolate recessive mutations with a defect in the sexual cycle. Selected PWT cultures were screened for defects in the formation of perithecia, asci and viable ascospores. The initial screen would also detect, in addition to recessive mutations, those that are only expressed in the maternal or paternal parent. Among 1090 PWT cultures screened, the development of perithecia was affected in 18 strains (16 class I and 2 class II). In addition, 28 strains (26 class II and 2 class III) produced apparently normal perithecia but the production of asci or viable ascospores was affected. The defect in perithecial development of all 8 class I mutant strains tested was only expressed in the protoperithecial parent. The stage of the defect of protoperithecial development of these mutants has not been pursued in this study. Of the two class III mutants with a defect in perithecial
development, 1 strain defective in perithecial color (per-1) and 1 defective in neck formation (pen-1) were also expressed only in the maternal parent. Even though the class II mutant P446 produced normal-looking perithecia, the absence of asci and its expression in the maternal parent appear very similar to the class I type mutants. Possibly the only difference between this mutant and the class I mutants is the stage of perithecial development at which the defect is expressed.

Defects in the production of viable ascospores in 9 strains, representing 8 loci (asc-1 through asc-8) were due to recessive mutations. In addition, even though the screen distinguished between dominant and recessive mutations, the recovery of 1 dominant mutation (SK) among cultures screened for recessive mutations was a consequence of its specific effect on ad-3A. A cross between SK and ad-3A produced ascospore abortion but neither SK nor ad-3A did so when crossed with wild type tester strains (see Chapter III).

The recovery of recessive mutations at 8 different loci demonstrate the effectiveness of the selection procedure. Four different types of mutations were classified: (1) complete absence of asci (asc-4); (2) aborted or empty asci (asc-2); (3) defect during meiosis leading to ascospore abortion (asc-1, asc-3, asc-6 and probably asc-7); (4) abortion of all ascospores in some asci but little or no abortion in the remaining asci (asc-5). 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

The mutant phenotype in all previously isolated mutants affecting the development of perithecia (Weijer and Vigfussen, 1972; Vigfussen and Weijer, 1972; Mylyk and Threlkeld, 1974; Johnson, 1978; Griffiths and DeLange, 1978) was expressed in a dominant fashion, i.e., when only one of the parents in a sexual cross was mutant. While some were expressed in either parent or in the paternal parent, most of these mutants were expressed only when used as the maternal parent. The present method which enables the isolation of recessive mutants should be especially useful since no such mutants would have been detected in previous mutant screens. Even though recessive mutants would be detected in the present screening procedure, none were identified among 8 mutants tested. The mutant effect in all 8 strains was expressed only in the maternal parent. This analysis, therefore, emphasizes that at least the great majority of loci required for the development of perithecia act only in the protoperithecial parent. However, the recent recovery and characterization (Johnson, 1979) of a mutant which is sterile as the female or the male parent indicates that some recessive mutations may be found in this class. This mutation became partially recessive when both components of the cross were heterokaryon compatible. Such a mutation would not have been detected in the present screen because the tester strains OR-A and OR-a, used to distinguish between dominance and recessiveness, were not heterokaryon compatible with the components of the PWT strains tested. Even if the PWT carried the same alleles as OR-A and OR-a at the 3 incompatibility loci C/c, D/d and E/e (true in only about one-eighth of PWT strains),
the mating type incompatibility would still be present. To get around the latter problem it would be necessary to use tester strains carrying tol (Newmeyer, 1970) or a mating type mutation conferring compatibility with the opposite mating type (Griffiths and DeLange, 1978).

Formation of Asci in Neurospora

In *Neurospora* the development of asci is initiated after the pre-meiotic S phase and karyogamy (Iyengar et al., 1977). Therefore, mutants which either lack asci or produce defective asci may have their defect during meiosis. The absence or early abortion of asci (i.e., the perithecia are barren) is characteristic of several recessive meiotic mutations in *Neurospora* which are simultaneously defective in vegetative DNA repair (*uvs-3, uvs-5, uvs-6, mei-3*). Similar relationships, especially in yeast and *Drosophila*, have been well-documented and illustrate an overlap in functions required during somatic maintenance (e.g., repair of DNA damage) and meiotic recombination or related processes.

The detection of mutants on the basis of their ability to produce asci should become a powerful tool because it enables the identification of genes which are required only during meiosis, and of those which are also required for vegetative functions. In addition, cytological analysis and the use of temperature-sensitive mutants would facilitate the detection of temporal stages of activity and final blockage points of such mutants.

Formation of Viable Ascospores in Neurospora

The failure of the maturation and viability of ascospores may be caused by the absence of any part of the haploid chromosome complement
or by the presence of a mutant gene in these ascospores (for review, see Perkins and Barry, 1977). Examples of the former kind are deficiencies of part of chromosomes due to rearrangements, and deficiencies of whole chromosomes due to nondisjunction (e.g., mei-1, mei-4). The failure of maturation of ascospores may also be due to ascospore color mutants or spore killer mutants. In the present study a third type of mutant (asc-5) was detected which was recessive and caused 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 already been instrumental in understanding the relationship between meiotic exchange and the disjunction of homologous chromosomes during the first meiotic division. Recessive mutations of this kind have been detected in many species (for review see Baker et al., 1976a). Two such mutations (mei-1, mei-4; Perkins and Barry, 1977) have been detected previously in Neurospora crassa. Many of these mutants have a primary defect in recombination, the aberrant segregation merely being a consequence of univalents produced through a lack of exchange. In Neurospora, exchange is virtually eliminated in crosses homozygous for mei-1 (Smith, 1975) due to an almost complete lack of pairing of homologs (Lu and Galeazzi, 1979). Some of the newly isolated spore abortion mutations (asc-1 and asc-6) have similar effects on recombination, and the asc-3 mutation causes defects in segregation that are not associated with reduced exchange. These mutants have been analyzed more extensively and the results will be described in Chapter II.

Ascospore abortion of the recessive asc-5 mutation 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 amount of variation in expression of this mutation 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 mutations in Drosophila (Miklos and Smith-White, 1971). The effect of asc-5 on whole asci and the large amount of variability of expression between different crosses is also reminiscent of bubble asci (Perkins and Barry, 1977). In each case, a certain proportion of asci fail to produce viable ascospores--asci with aborted white spores for asc-5 and asci with 8 little bubbles found in many wild type crosses--with no apparent effect on the remaining asci. It is not known whether the bubble asci may superimpose on the white-spored asci.

Ascospore color mutants and spore killer mutants can also cause ascospore abortion. In the case of ascospore color mutants, the mutant ascospores abort whereas spore killer mutations cause the abortion of ascospores that would be viable in other crosses. In all cases, heterozygous crosses result in 50% ascospore abortion. The mutant SK(ad-3A) was isolated during this study and appears to have characteristics of both a spore killer mutant and an ascospore color mutant. This mutant has been analyzed in more detail and is described in Chapter III.

Finally, future studies should include the improvement of the selection system to allow for faster isolation of PWT cultures. For example, meiotic mutations with increased PWT frequencies could be used for this purpose. In addition, emphasis will be put on the isolation of conditional mutants.
CHAPTER II

MEIOSIS IN *NEUROSPORA CRASSA*.

II. GENETIC AND CYTOLOGICAL CHARACTERIZATION

OF FOUR MEIOTIC MUTANTS
INTRODUCTION

The isolation of eight recessive mutually complementing mutations which affect fertility in *Neurospora crassa* has been reported in Chapter I. Mutations in two loci (asc-2 and asc-4) resulted in barren perithecia; the remaining six mutations (asc-1, asc-3, asc-5, asc-6, asc-7 and asc-8) caused the abortion of many ascospores.

That ascospore abortion may be a very useful means of detecting mutations with a defect in the regular segregation of chromosomes has been suggested by studies done with the *mei-1* mutation (Smith, 1975). Approximately 90% of ascospores from crosses homozygous for this mutation were aborted. This abortion was evidently due to the irregular segregation of chromosomes which was caused by the absence of pairing during the first meiotic prophase (Lu and Galeazzi, 1979) and resulted in aneuploid products. The successful use of both genetic and cytological means of analysis of such mutants was also demonstrated in those studies.

This paper reports the genetic and cytological characterization of the three ascospore abortion mutations asc-1, asc-3 and asc-6 (see Chapter I) and some new observations on the *mei-1* mutation. The segregation of chromosomes during meiosis was shown to be defective in crosses homozygous for each of these mutations. In each case, the segregation defect appeared to be a secondary consequence of a prior abnormality. A defect in the pairing of homologous chromosomes in crosses homozygous for asc-1, asc-6 or mei-1 resulted in defective segregation of chromosomes during the first and second divisions of meiosis (and possibly
the post-meiotic division). In contrast, the block in the development of most asci in crosses homozygous for asc-3 was followed by segregation irregularities, for the few asci formed, during the second and post-meiotic divisions.

MATERIALS AND METHODS

Alleles Used

The alleles on LG I used to select PWT colonies and to determine recombination and nondisjunction frequencies of that linkage group, have been described in Chapter I. Alleles used to study multiple disomy in crosses homozygous for asc-6 are: ad-3A (2-17-814); aur (34508); al-2 (74A-Y112-M38); acr-2 (KH5); pdx (37803); cot-1 (C102(t)); inos (37401); his-1 (K141). The location of these loci on their linkage groups and map distances between loci, were applicable, are illustrated in Fig. 2. Other loci are: al-1 (Car-10) on LG IR; arg-5 (27947) on IIR; trp-4 (Y2198) and pan-1 (5531) on IVR. The meiotic mutants mei-1 (Smith, 1975), asc-1 (P95), asc-3 (P243) and asc-6 (P879) have been previously described (Chapter I). The ad-3B allele (2-17-128) was used in crosses homozygous for mei-1 or mei-1;asc-6. Finally, the mutation 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 (asc) mutations were isolated in PWT strains which 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 Fig. 1 and Chapter I). Ascospore isolate strains of genotypes
Fig. 1  The two nuclear components of PWT cultures in which asc mutations were recovered.
<table>
<thead>
<tr>
<th>Component 1</th>
<th>centromere</th>
<th>leu-3</th>
<th>+</th>
<th>a</th>
<th>arg-1</th>
<th>+</th>
<th>ad-3B</th>
<th>+</th>
<th>tol</th>
<th>asc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component 2</td>
<td>+</td>
<td>un-3</td>
<td>A</td>
<td>+</td>
<td>ad-3A</td>
<td>+</td>
<td>nic-2</td>
<td>al-2</td>
<td>tol</td>
<td>asc</td>
</tr>
<tr>
<td>Approximate</td>
<td>10</td>
<td>0.1</td>
<td>10</td>
<td>9</td>
<td>0.3</td>
<td>4</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>map distances</td>
<td>(Radford, 1972)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Approximate map distances (Radford, 1972)
Fig. 2 A cross homozygous for asc-6, designed to test nondisjunction of three linkage groups (LG I, IV, and V) simultaneously.
Approximate map distances (Radford, 1972)
leu-3, a, arg-1, ad-3B; asc and un-3, A, ad-3A, nic-2, al-2; asc (each containing the tol mutation and the heterokaryon compatibility loci C, d and e) were obtained from crosses between the mutant PWT culture (i.e., homozygous for an asc mutation) 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 Fig. 1).

Cytological Methods

The two stains iron-hematoxylin and aceto-orcein were used with about equal success. The methods differed mainly in their ability to visualize spindle-pole bodies and nucleoli (iron-hematoxylin stains both structures whereas aceto-orcein stains neither). Staining with iron-hematoxylin was done essentially as described previously (Raju and Newmeyer, 1977; Lu and Galeazzi, 1979) with a slight modification. Following hydrolysis, perithecia were washed overnight at room temperature in a solution of 3:1:1 absolute ethanol:acetic acid:chloroform (Carnoy's solution). This procedure removed the fat globules from the cytoplasm and therefore allowed the visualization of chromosomes at all stages of meiosis.

The method utilizing aceto-orcein is basically a combination of several methods (Griffiths et al., 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 and then incubated at -20°C for a period of 3 weeks to 6 months. This prolonged incubation helps to remove the fat globules from the cytoplasm. The fixed perithecia were then washed in water, hydrolyzed in 1 N HCl for 4-5 minutes at 60°C, washed again in ice water and immersed in leuco-basic
fuchsin for about 40 minutes at room temperature. Subsequent methods of staining, viewing and photography were described previously (Griffiths et al., 1974).

Methods used to obtain clusters of 8 ascospores, i.e., asci (Newcombe and Griffiths, 1972; Perkins, 1974) and other routine genetic manipulations (Davis and deSerres, 1970; Chapter I) have been reported previously.

Detection of Chromosome Segregation Defects During the First Meiotic Division (i.e., During MI)

In most cases, nondisjunction of LG I was measured. This linkage group was multiply marked to allow the detection of ascospore isolates that contain both complementing copies of LG I. Strains in which complementation of mutations on any part of a chromosome occurs are apparently wild type for these markers and are therefore called pseudo-wild type (PWT).

Nondisjunction of non-crossover chromosomes, assuming regularity of subsequent divisions, would result in ascospores PWT for all mutations on LG I (auxo⁺): leu-3, a, arg-1, ad-3B + un-3, A, ad-3A, nic-2, al-2. In contrast, if crossover chromosomes are involved, the following phenotypes will be expected: leu (leucine requiring) and auxo⁺ (wild type) if nondisjunction followed a single crossover event in the leu-un region (CO leu-un); leu, un and auxo⁺ (CO un-arg); leu arg, un and auxo⁺ (CO arg-centromere); ad nic al, ad and auxo⁺ (CO centromere-ad); nic al and auxo⁺ (CO ad-nic); al and auxo⁺ (CO nic-al). The disomic nuclei in young PWT ascospores soon haploidize (Pittenger, 1954; Chapter I). Therefore, these strains contain at least two complementing haploid
nuclear types. The heterokaryotic nature of such strains was, in many cases, confirmed by testing the genotypes of individual conidial isolates.

Even though all of the above mentioned heterokaryotic strains are PWT for at least some auxotrophic mutations, only those PWT progeny in which all auxotrophic mutations on a particular linkage group complement (i.e., auxo⁺) were included in the estimation of PWT frequencies in crosses homozygous for asc-1, asc-6 and mei-1. The PWT frequencies were determined in one of two ways: (i) by testing individual ascospore isolates, or (ii) by plating ascospores on both minimal (only auxo⁺ PWT strains grow) and on medium supplemented with leucine, arginine, adenine and nicotinic acid (all viable strains grow).

In one case (crosses homozygous for asc-6) the simultaneous nondisjunction of more than one chromosome was determined. Complementing mutations on LG I (A and a mating type alleles), LG IV (pdx and cot), and LG V (inos and his) were used to detect PWT progeny for these linkage groups (see Fig. 2; aur, al-2, ad-3A and acr mutations in the strains used are not relevant in the present study).

Detection of Chromosome Segregation Defects During the Second Meiotic Division (i.e., During MII)

Nondisjunction at MII can be detected through complementation of closely linked mutations. Two such complementing mutations can only be present on the same dyad chromosome (i.e., just prior to MII) following an exchange event between these mutations and the centromere. Consequently, nondisjunction at MII can only be detected following such an exchange event and will be detected more frequently the further the
complementing mutations are from the centromere.

Even though the detection of nondisjunction during both MI and MII depends on the complementation between closely linked mutations, these two types of events can usually be distinguished if mutant markers are present on both sides of the centromere. In the case of nondisjunction at MII, mutant markers on one side of the centromere, but not the other, complement. In contrast, nondisjunction at MI usually results in the complementation of mutations on both sides of the centromere (Fig. 3).

Recombinant Frequency (RF) in Crosses with a High Amount of Nondisjunction

Nondisjunction Mainly at the First Meiotic Division (MI). The frequency of recombinants is generally equated to the proportion of recombinant chromatids. This value is readily determined among haploid progeny by testing for the presence of specific marker mutations. However, the detection of recombinant chromatids in PWT progeny is often more complicated. To determine this value, the \nic-al region (30-35 \mu) was used. Asci with a crossover event in this region, followed by nondisjunction at MI, would produce one-fourth albino (al) and three-fourths orange (al+) PWT progeny (assuming regular subsequent divisions). Similar asci in which chromosomes disjoin in a regular manner would produce one-half recombinant progeny (for the \nic-al region). Therefore, the frequency of recombinant chromatids for PWT progeny would be twice the frequency of albino PWT progeny (= 2x al PWT/total PWT).

The frequency of recombinant chromatids is usually identical to the crossover frequency in a particular region. This is particularly so if, as in \textit{Neurospora}, all chromatids from each meiosis are recovered from
Fig. 3  Expected PWT progeny resulting from nondisjunction during the first meiotic division (a), and a crossover followed by nondisjunction during the second meiotic division (b).
(a) leu arg ad-3B
       un ad-3A nic al

MI

leu arg ad-3B
       un ad-3A nic al

MII

leu arg ad-3B
       ad-3A nic al

(PWT)

Note: some crossover types are also recovered (see Materials and Methods)

(b) leu arg ad-3B
       un ad-3A nic al

MI

leu arg ad-3B
         leu arg ad-3A nic al

and

un ad-38
         un ad-3A nic al

MII

Nondisjunction

leu arg ad-3B
         ad-3A nic al

or

un ad-38
         ad-3A nic al

(un)

Note: exchange between the centromere and arg-1 will produce nondisjunction progeny ad-3B and ad-3A, nic, al.
a sexual cross. However, in crosses where nondisjunction takes place, a fraction of ascospores is inviable and many chromatids will not be recovered. In such cases, the frequency of recombinant chromatids may not be the same as crossover frequency, since crossover or non-crossover chromatids might be preferentially included in the viable progeny. For example, a defect in pairing of chromosomes at the first prophase of meiosis generally results in their irregular disjunction (see DISCUSSION). Since the lack of pairing excludes the possibility of crossing-over, it should be mainly the non-crossover chromatids that would be involved in irregular disjunction and, therefore, may be preferentially excluded from the viable progeny. Thus, in these cases, the recombinant frequency would be higher than the actual crossover frequency. Since there appears to be no evidence suggesting that crossover chromosomes might be preferentially excluded from the viable progeny, the recombinant frequency in a cross with nondisjunction during MI should be considered an overestimate of the crossover frequency. Consequently, any reduction in recombinant frequency should represent a real decrease in crossover frequency.

The overall frequency of recombinant chromatids from both PWT and non-PWT progeny could be determined by combining the individual RF values of these two types of progeny. However, the relative contribution of the frequency of recombinant chromatids, which is different for PWT and non-PWT progeny (see RESULTS), is in doubt. The original nuclei disomic for LG I, giving rise to PWT progeny, and those haploid for LG I, producing non-PWT progeny, many be differentially included into viable ascospores. Such differential viability may produce a frequency of
recombinant chromatids which does not correctly reflect crossover frequency. Therefore, the overall recombinant frequency was not determined.

**Nondisjunction During the Second Meiotic Division (MII).** The recombinant frequency (RF) in any region equals the proportion of recombinant chromatids. In crosses with a high frequency of nondisjunction at MII, the large number of resultant disomic progeny prevent the identification of some recombinant chromatids. In addition, since the frequency of disomic nuclei is unknown, it is not possible to directly determine the total number of chromatids among the progeny from such a cross. Therefore, to obtain a more appropriate estimate of RF, only asci containing a crossover in a well-marked region should be considered. The frequency of disomic and haploid progeny from such asci can be estimated since all disomic nuclei produce distinguishable heterokaryotic (HK) products and half the haploid nuclei are recombinant (CO). In the present study, the RF value was determined in a region which was marked on one side by the closely linked mutations *un-3* and *arg-1*, and on the other side by *ad-3A* and *ad-3B* (Fig. 3). Deviations produced by crossovers between *un-3* and *arg-1* (1-2 mu)\(^4\) or *ad-3A* and *ad-3B* (0.3 mu) should be considered very minor since the *arg-ad* region spans 15-20 mu.

The subgroup of asci with a crossover in the *arg-ad* region produces ascospores which are haploid for LG I, and those that are disomic for LG I. Half the haploid ascospores will be recombinant (CO), and the disomics will carry a parental and a crossover chromosome (P + CO). The

\(^4\)This low RF value was consistently obtained during this study. The deviation from the value previously reported (Radford, 1972) in Fig. 1 may well be due to rec-type genes (see e.g., Catcheside, 1974).
resultant CO and HK progeny can be identified. In these asci, the frequency of HK progeny equals the frequency of disomic nuclei. This value equals HK/HK + 2 CO (or disomic/disomic + haploid). Assuming that this frequency of disomic nuclei is the same in all types of asci, regardless of crossover events in specific regions, this frequency would apply to all progeny from the cross. Therefore, the total number of disomic ascospore isolates would be:

\[
\left( \frac{HK}{HK + 2 \text{CO}} \right) \times \text{total progeny.}
\]

Similarly, the total number of haploid ascospore isolates would be:

\[
\left( \frac{2 \text{CO}}{HK + 2 \text{CO}} \right) \times \text{total progeny.}
\]

These values enable the estimation of the total number of chromatids obtained from a cross:

\[
2 \times \text{(disomics)} + \text{haploids} = \left( \frac{2 \text{HK}}{HK + 2 \text{CO}} + \frac{2 \text{CO}}{HK + 2 \text{CO}} \right) \times \text{total progeny}
\]

\[
= \left( \frac{2(\text{HK} + \text{CO})}{HK + 2 \text{CO}} \right) \times \text{total progeny.}
\]

Subsequently, the RF value in a region that allows the distinction between CO and HK progeny would be:

\[
\text{RF} = \frac{\text{CO + HK}}{2(\text{HK} + \text{CO})} \times \text{total progeny (}= \text{total chromatids)}.\]

\[
= \frac{\text{HK} + 2 \text{CO}}{2 \times \text{total progeny}}.
\]

In the present study, the CO value was obtained using the un-nic region. However, HK progeny could only be detected following a crossover in the
slightly smaller arg-ad region. Therefore, since a low estimate for HK was used, the RF value of the un-nic region obtained by this method would be a slightly low estimate.

RESULTS

Four recessive mutations (asc-1, asc-3, asc-6 and mei-1) which cause the abortion of some of their ascospores, were analyzed. In each case, the aborted ascospores were white instead of black, and inviable. The fertility of crosses homozygous for each recessive mutation was naturally always reduced due to the inviable ascospores. However, in this chapter, the term "fertility" is used to designate the total number of ascospores (black or white). An arbitrary measure of low (when very few spores are produced), medium, or high fertility is employed. As will be shown, the ascospore abortion in all four mutations is caused by irregular segregation, or nondisjunction, of chromosomes. In Neurospora, such irregular segregation of chromosomes can be detected by the presence of PWT progeny (see MATERIALS AND METHODS). PWT progeny are detected through their complementation of some or all auxotrophic mutations.

The three newly-isolated asc mutations (see Chapter I) will be discussed in order of their apparent increasing complexity and stage of defect in the segregation of chromosomes: asc-6 (first division); asc-3 (second division); and asc-1 (first and second divisions). Finally, some new observations of the mei-1 mutation will be reported.

asc-6 (P879)

CROSSES homozygous for this recessive mutation generally resulted in about 70% ascospore abortion and a reduction in fertility. The amount
of spore abortion was quite constant in all crosses tested but fer-
tility varied from low to medium (Table I).

All initial strains carrying asc-6 grew about three times as
slowly as wild type strains. It was found that this slow growth was
due to a point mutation (slo) linked to the meiotic mutations (see sec-
tion on mapping). The slo mutation, however, in no way affected the
phenotype of asc-6.

Recombination and Nondisjunction Frequencies. The analysis of
ascospore cultures from four different crosses homozygous for asc-6 re-
vealed a drastic alteration in both RF and nondisjunction frequency
(Table I). A high frequency 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 mutations on LG I. It therefore appears
that these PWT cultures are the result of nondisjunction during MI.

Three regions on LG I were monitored for RF: leu-un, un-nic
and nic-al. Compared to wild type crosses, the RF values from all four
crosses homozygous for asc-6 were reduced in all three regions (Table I).

The Nature of PWT Progeny. To establish the nuclear composi-
tion of the PWT progeny from these crosses, the genotypes of conidial
isolates from ten PWT progeny, recovered from the cross between strains
879a13 and 879A15, 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. The additional detection of a few crossover
types among conidial isolates from two PWT cultures is consistent with
somatic exchange events (Pittenger and Coyle, 1963).
Table I. Genetic analysis of four crosses homozygous for asc-6

<table>
<thead>
<tr>
<th>Cross</th>
<th>Total non-PWT progeny</th>
<th>Recombination frequency (%)</th>
<th>PWT progeny</th>
<th>Genotypes of PWT's</th>
<th>Fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>leu-un</td>
<td>un-nic</td>
<td>nic-al</td>
<td>Total</td>
</tr>
<tr>
<td>Q22-2 x Q22-8*</td>
<td>95</td>
<td>1.1 (1)</td>
<td>6.5 (6)</td>
<td>11.4 (11)</td>
<td>45</td>
</tr>
<tr>
<td>Q8-1 x Q8-2*</td>
<td>63</td>
<td>3.2 (2)</td>
<td>7.9 (5)</td>
<td>12.7 (8)</td>
<td>37</td>
</tr>
<tr>
<td>Q35-1 x Q35-2*</td>
<td>81</td>
<td>2.5 (2)</td>
<td>6.2 (5)</td>
<td>13.6 (11)</td>
<td>33</td>
</tr>
<tr>
<td>RF(nic-al) of 3 crosses combined:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>879a13 x 879A15†</td>
<td>61</td>
<td>6.5 (4)</td>
<td>0 (0)</td>
<td>3.3 (2)</td>
<td>12</td>
</tr>
<tr>
<td>Wild type crosses</td>
<td>11-17</td>
<td>15-20</td>
<td>30-35</td>
<td>&lt; 0.1</td>
<td></td>
</tr>
</tbody>
</table>

* A and a components from a PWT culture from a cross between strain 1-34-8 (un, A, ad, nic, al) and 879A15 (leu, a, arg, ad; asc-6).
† Ascospore isolates derived as described in Materials and Methods. LG I markers were identical for all 4 crosses (see Fig. 1).
‡ RF(nic-al) among PWT progeny = 2x freq. of albino PWT's (see Materials and Methods).
The absence of crossover chromosomes from the ten PWT cultures may suggest that only non-exchange 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 RF values in the nic-al region were determined for both types of progeny from three crosses (Table I). The value for PWT progeny (1.7% = 2 x frequency of albino PWT's; see MATERIALS AND METHODS) was much lower than that for non-PWT progeny (12.5%). It is therefore concluded that most nondisjunction involves non-exchange chromosomes.

**Ascus Analysis.** Unordered asci from a cross homozygous for asc-6 were analyzed in two ways. First, all types of ascus abortion patterns were recovered (Table II). The prevalence of asci with an even number of black ascospores (26/37) suggests 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 out of 37 asci contained at least one PWT ascospore isolate. These seven asci and the genotypes of their ascospore cultures are shown in Table III. 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.

**Recombination and Nondisjunction Frequencies Involving Chromosomes Other Than LG I.** Thus far, nondisjunction has only been recorded for LG I. To investigate the degree of nondisjunction (and reduction in
Table II. Ascus analysis of a cross homozygous for asc-6 (879a13 x 879A15)

<table>
<thead>
<tr>
<th>Types of Asci (black:white ascospores)</th>
<th>Number of Asci Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 : 0</td>
<td>1</td>
</tr>
<tr>
<td>6 : 2</td>
<td>3</td>
</tr>
<tr>
<td>4 : 4</td>
<td>2</td>
</tr>
<tr>
<td>2 : 6</td>
<td>14</td>
</tr>
<tr>
<td>0 : 8</td>
<td>6</td>
</tr>
<tr>
<td>other (mostly 1:7, 3:5)</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
</tr>
</tbody>
</table>
Table III. Isolates obtained from asci, produced by a cross (879a13 x 879A15) homozygous for asc-6, which contain at least one PWT culture

<table>
<thead>
<tr>
<th>Type of Ascus (B:W)</th>
<th>No. Isolates Germinated</th>
<th>PWT</th>
<th>Genotype of Isolates leu, arg, ad</th>
<th>un, ad, nic, al</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 : 6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 : 5</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3 : 5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4 : 4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4 : 4</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 : 3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
recombination) of other chromosomes, a cross was analyzed which enabled
the simultaneous analysis of LG I, IV and V (Fig. 2). 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 frequency of recombination was approximated by the appearance of
the double mutant (e.g., pdx, cot-1). The extreme rarity of such recom-
binants (1/165 pdx, cot-1, and 0/165 inos, his-1) shows that recombina-
tion 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 addi-
tion, 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 IV shows the data on the simultaneous nondisjunction of
these three chromosomes in two different crosses homozygous for asc-6.
The results reveal several aspects of nondisjunction in these crosses:
i) All three chromosomes tested show a high degree of nondisjunction.
ii) The frequencies of nondisjunction of the three chromosomes in each
particular cross are very similar. However, nondisjunction fre-
cuencies 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 cross).
iii) The number of isolates either simultaneously PWT or non-PWT for
all three linkage groups tested is significantly higher than ex-
pected (p < 0.01). This may mean that chromosomes do not disjoin
independently of each other. Alternatively, it may reflect selec-
tion against isolates with some PWT and some non-PWT linkage
groups. That the latter is quite plausible is shown by the per-
centage germination in these crosses (47.8, 50.0 and 57.5%). In
### Table IV. Simultaneous nondisjunction of three linkage groups in two crosses homozygous for asc-6

<table>
<thead>
<tr>
<th>Hyperploid for LG</th>
<th>Cross 129-75 x 128-15</th>
<th>Cross 129-79 x 128-15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Progeny</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Observed (O)</td>
<td>Expected* (E)</td>
</tr>
<tr>
<td></td>
<td>(O-E)^2/E</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Observed (O)</td>
<td>Expected* (E)</td>
</tr>
<tr>
<td>+ + +</td>
<td>28 14.5 12.6 8 2.7</td>
<td></td>
</tr>
<tr>
<td>+ + -</td>
<td>9 12.7 1.1 3 4.3</td>
<td></td>
</tr>
<tr>
<td>+ - +</td>
<td>11 15.9 1.5 6 5.0</td>
<td></td>
</tr>
<tr>
<td>- + +</td>
<td>5 12.2 4.2 3 5.4</td>
<td></td>
</tr>
<tr>
<td>+ - -</td>
<td>9 14.0 1.8 3 8.0</td>
<td></td>
</tr>
<tr>
<td>- + -</td>
<td>8 10.7 0.7 7 8.6</td>
<td></td>
</tr>
<tr>
<td>- - +</td>
<td>12 13.4 0.1 6 10.0</td>
<td></td>
</tr>
<tr>
<td>- - -</td>
<td>23 11.7 10.9 24 16.0</td>
<td></td>
</tr>
</tbody>
</table>

\[ \chi^2_{df \gamma} = 32.9 \]

* Determined from product of nondisjunction frequency of individual linkage groups (LG I: 0.543, LG IV: 0.476, LG V: 0.533 for cross 129-75 x 128-15; and LG I: 0.33, LG IV: 0.350, LG V: 0.383 for cross 129-79 x 128-15).
combination with data on colony-forming ability obtained from the mutant asc-1 (see below, and Table X), the selective hypothesis seems most appealing (see also DISCUSSION).

**Cytology.** Cytological observations of crosses homozygous for asc-6 show a drastic reduction in pairing of homologs at pachytene (Fig. 4a, b). Some pairing was observed mainly near the tips of some chromosomes. This reduction is reflected in a drastic decrease in the number of recombinants obtained from these crosses.

An apparent consequence of reduced pairing and exchange is the production of univalents during diakinesis and metaphase I. Fig. 4c shows two separate chromosomes attached to the nucleolus at diakinesis. These appear to be two univalents of the same chromosome. Instead of seven bivalents, up to 14 univalents can be detected on the spindle of metaphase I (Fig. 4e). Subsequent division distributes roughly equal amounts of chromatin to opposite poles and, at prophase II, up to 14 (instead of the usual seven) chromosomes materialize in each dyad nucleus. Fig. 4f, g). This observation is compatible with the equational division of many univalents during the first division (see DISCUSSION). The second division takes a long time to complete; whereas figures at this stage of division were observed only rarely in wild type crosses, they were quite common in crosses homozygous for asc-6 (Fig. 4h-k). Finally, unequal amounts of chromatin often segregate during this and the post-meiotic division leading to the different types of ascospore abortion.

In conclusion, the primary defect of mutant asc-6 appears to be a defect during the pairing of homologs at the first prophase of meiosis. This results in a drastic decrease of recombination and the production
Fig. 4  Chromosome development in crosses homozygous for asc-6. Pictures (f), (g), and (h) were stained with Feulgen and aceto-orcein; all other preparations were stained with iron-haematoxylin. Most chromosomes fail to pair at pachytene (a,b) x3400; some regions can be seen to pair though ((a) arrowed). At diakinesis, the two homologs of the nucleolar chromosome may be separately attached to the nucleolus (c) x3400; figures (c) and (d) represent one ascus at two different foci. At metaphase I, up to 14 univalents may be seen (e) x3400, and a similar number of chromosomes (7-14) individualize during the following prophase in each dyad nucleus (f,g) x 1300. A long period necessary to complete the second division was suggested by the unusually large number of nuclei at this stage of division (i,j,k; spindle-pole bodies are arrowed) x2400; this may sometimes cause some spindle overlap (j). That the material closest to the spindle-pole bodies is DNA, was shown by preparations made with aceto-orcein stain which only stains DNA (h) x1300.
of many univalents at the first metaphase. The high frequency of PWT progeny suggests that nondisjunction takes place during the first meiotic division. The mechanism of such nondisjunction may be, at least in part, the equational separation of univalents. The subsequent irregular and extended second division of meiosis and abnormal segregation at the post-meiotic division account for a large amount of the ascospore abortion.

asc-3 (P243, P393)

Crosses homozygous for this recessive mutation generally produced very few ascospores and, of those produced, 90-98% were white and incapable of germination and growth. Crosses homozygous for mutant ascospore isolates obtained from strain P393 produced between 9 and 42% black ascospores. Since these crosses were wild type for LG I markers, recombination and nondisjunction frequencies could not be determined. These high frequencies of black ascospores were never obtained in similar crosses with marked homologs of LG I. However, the results appear to suggest that the phenotype of this mutant can be altered drastically by modifying genes.

The two non-complementing mutations 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 P243 and P393 which were obtained from the same cross plate.

Recombination and PWT Frequencies. Random progeny analysis was performed on a number of crosses homozygous for asc-3. Four types of progeny could not be explained as homokaryotic parental or crossover types (Table V). These were apparently PWT cultures of genotypes auxo+
Table V. Progeny analysis of crosses homozygous for asc-3 (P243 or P393)

<table>
<thead>
<tr>
<th>Cross*</th>
<th>Non-PWT Progeny</th>
<th>PWT Progeny</th>
<th>Genotypes (al or al+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No.</td>
<td>No. Recombinants in Region</td>
<td>Total No.</td>
</tr>
<tr>
<td></td>
<td>Non-PWT Progeny</td>
<td>leu-un</td>
<td>un-nic</td>
</tr>
<tr>
<td>243a32 x 243A18</td>
<td>16</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>243a32 x 243A28</td>
<td>17</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>243a23 x 243A27</td>
<td>17</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>243a31 x 393A35</td>
<td>18</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4 comb. crosses</td>
<td>17</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>P243</td>
<td>26</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>19</td>
<td>15</td>
</tr>
</tbody>
</table>

Overall RF (%) among non-PWT progeny:
17.1  13.5  28.0
PWT freq. = 17/128 = 13.3%

Wild type crosses, RF values:
11-17  15-20  30-35
PWT freq. <0.1%

* Crosses between ascospore isolates which were derived from mutant strains P243 and P393 as described in Materials and Methods (for genotypes, see Fig. 1).
† Ascospore isolates from these crosses were obtained from shot asci.
(wild type for all auxotrophic marker mutations), un-3 (wild type for all marker mutations except un-3), ad-3 (leu$^+$, arg$^+$, un$^+$, nic$^+$) and ad-3, nic-2 (leu$^+$, arg$^+$, un$^+$). In the pooled data, these four types of progeny represented 13.3% of all progeny.

RF in the three regions tested (leu-un, un-nic and nic-al) were similar to those obtained from wild type crosses (see Table V). They were determined as a proportion of the non-PWT progeny. More appropriately, corrected values will be presented in a later section.

The Nature of PWT Progeny. To determine the nature and origin of the PWT progeny, conidial isolates from twelve ascospore cultures representing all four types (auxo$^+$; un-3; ad-3; and ad-3, nic-2) were tested. Table VI shows genotypes recovered from these cultures. All cultures were heterokaryotic and most contained one parent and one crossover chromosome. The un, the ad, and the 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 out of the three auxo$^+$ progeny contained two detectable crossover events in the un-ad region (either a parental and double crossover component, or two single crossover components). Thus, all four 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 data are best explained by postulating an unusually high frequency of nondisjunction during the second division of meiosis (see Fig. 5), especially since such nondisjunction in these crosses will only be detected as heterokaryotic progeny if a crossover event in the un-ad region had preceded such irregular segregation (Fig. 3, MATERIALS AND METHODS).
<table>
<thead>
<tr>
<th>Genotype of Ascospore Isolate</th>
<th>Parental (not inc. al)</th>
<th>Conidial Isolates</th>
<th>Crossover</th>
<th>No. HK* Genotype</th>
<th>Total No. Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype</td>
<td>No.</td>
<td>Genotype</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>1-ad</td>
<td>leu, arg, ad</td>
<td>1</td>
<td>un, ad</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>2-ad</td>
<td>leu, arg, ad</td>
<td>12</td>
<td>un, ad, al</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>3-ad nic</td>
<td>un, ad, nic, al</td>
<td>15</td>
<td>leu, arg, ad, nic</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>4-un al</td>
<td>un, ad, nic, al</td>
<td>43</td>
<td>leu, un, ad, al</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5-un</td>
<td>un, ad, nic</td>
<td>13</td>
<td>un, ad</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>6-un</td>
<td>un, ad, nic, al</td>
<td>10</td>
<td>un, ad</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>7-un</td>
<td>un, ad, nic, al</td>
<td>3</td>
<td>un, ad</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>8-un</td>
<td>un, ad, nic</td>
<td>0</td>
<td>leu, un, ad, al</td>
<td>33</td>
<td>17</td>
</tr>
<tr>
<td>9-un</td>
<td>un, ad, nic, (al)</td>
<td>0</td>
<td>un, ad</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>10-PWT</td>
<td>un, ad, nic, al</td>
<td>4</td>
<td>leu, ad</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>11-PWT</td>
<td></td>
<td></td>
<td>leu, arg, ad, nic</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>un, ad</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-PWT</td>
<td>un, ad, nic, al</td>
<td>11</td>
<td>leu, arg, ad</td>
<td>16</td>
<td>23</td>
</tr>
</tbody>
</table>

*HK (= heterokaryon) isolates have the genotype of the original ascospore isolate.
Fig. 5  The origin of PWT progeny from crosses homozygous for asc-3. The heterokaryotic progeny recovered from crosses homozygous for asc-3 are readily explained by one (or more) crossover event(s) near the centromere, followed by a nondisjunction at the second meiotic division.

(a) A crossover between arg-1 and the centromere will, following a regular first meiotic division, generate two nuclei, each heterokaryotic for the region to the left of the crossover; subsequent nondisjunction at the second division will leave the resulting nuclei heterokaryotic for this region. In this manner, both ad and ad, nic, (al) progeny can be produced. Similarly, a crossover event between ad and the centromere will render nuclei heterokaryotic for the region to the right of the crossover; this will produce either leu, arg or un progeny.

(b) If two crossover events, one to the left and the other to the right of the centromere, take place, PWT progeny will result. The data in Table VI are compatible with this interpretation of the origin of the (auxo+) PWT isolates; the 2-strand double crossover shown in this figure was apparently involved in two PWT isolates (see Table VI, isolates 10 and 12). (c) This 3-strand double crossover was responsible for the remaining PWT.
(a)

\[
\begin{align*}
\text{leu} + \text{arg} & \quad \text{ad-3B} + + \\
\text{leu} + \text{arg} & \quad \text{ad-3B} + + \\
\text{leu} + \text{arg} & \quad \text{ad-3B} + + \\
\text{leu} + \text{arg} & \quad \text{ad-3B} + + \\
\text{leu} + \text{arg} & \quad \text{ad-3B} + + \\
\text{leu} + \text{arg} & \quad \text{ad-3B} + +
\end{align*}
\]

\[\text{pachytene}\]

\[\text{+ un} + \quad \text{ad-3A nic al}\]

\[\text{leu} + \text{arg} \quad \text{ad-3B} + + \]

\[\text{+ un} + \quad \text{and}\]

\[\text{interphase I}\]

\[\text{leu} + \text{arg}\]

\[\text{+ un} + \quad \text{ad-3A nic al}\]

\[\text{+ un} + \quad \text{nondisjunction at 2nd meiotic division}\]

\[\text{ad-3B (un, ad + leu, arg, ad)}\]

or: \[\text{ad-3A, nic-2, al-2 (un, ad, nic, al + leu, arg, ad, nic, al)}\]

(b)

\[\text{leu} + \text{arg} \quad \text{ad-3B} + + \]

\[\text{leu} + \text{arg} \quad \text{ad-3B} + + \]

\[\text{1} \quad \text{2} \quad \text{1} \quad \text{2}\]

\[\text{+ un} + \quad \text{ad-3A nic al}\]

\[\text{leu} + \text{+} \quad \text{ad-3B} + + \]

\[\text{+ un} + \quad \text{ad-3A nic al}\]

\[\text{see Table VI line 10}\]

or

\[\text{leu} + \text{arg} \quad \text{ad-3B} + + \]

\[\text{+ un} + \quad \text{ad-3A nic al}\]

\[\text{see Table VI line 12}\]
(c)

\[
\begin{array}{cccc}
\text{leu} & + & \text{arg} & + \text{ad-3B} & + \\
\text{un} & + & \text{ad-3A} & \text{nic} & \text{al} \\
\text{leu} & + & \text{arg} & + \text{ad-3A} & \text{nic} \\
\text{un} & + & \text{ad-3B} & + & + \\
\end{array}
\]

see Table VI, line 11
To rule out the remote possibility that nondisjunction only involved 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. In this cross, nondisjunction at the second meiotic division would be detected as heterokaryotic progeny if exchange in the un-al region had taken place. Out of a total of 65 isolates, 25 were heterokaryotic. This high frequency of heterokaryotic progeny suggested that nondisjunction at the second meiotic division involved both exchange and non-exchange chromosomes, and thus was a general phenomenon not related to exchange.

Ascus Analysis. Table VII shows the types of asci produced by four crosses homozygous for the P243-derived asc-3 mutation and by one cross homozygous for the P393-derived mutation. No asci with four or more black ascospores were ever detected. Most asci contained eight white ascospores but those with one or two black ascospores were not rare. Both black ascospores were germinated from eight 2B:6W asci. In each case, the pair of genotypes was identical to each other, 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.

Correction of Recombination Frequencies (RF). Since in crosses homozygous for asc-3 some crossover chromosomes are detected as homokaryotic crossover products and others are present in heterokaryotic products, the RF values cannot be determined in the usual manner. To
Table VII. Ascus analysis of crosses homozygous for asc-3*

<table>
<thead>
<tr>
<th>Cross</th>
<th>Types of Asci (black:white ascospores)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 : 5</td>
</tr>
<tr>
<td>P243-derived strains:</td>
<td></td>
</tr>
<tr>
<td>243a26 × A18</td>
<td>0</td>
</tr>
<tr>
<td>243a26 × A28</td>
<td>2</td>
</tr>
<tr>
<td>243a32 × A18</td>
<td>0</td>
</tr>
<tr>
<td>243a32 × A13</td>
<td>1</td>
</tr>
<tr>
<td>Combined data</td>
<td>3</td>
</tr>
<tr>
<td>P393-derived strain:</td>
<td></td>
</tr>
<tr>
<td>393a30 × A34</td>
<td>2</td>
</tr>
</tbody>
</table>

*Four crosses involving ascospore isolates derived from mutant strain P243 and one cross between ascospore isolates derived from P393.
determine the RF value of a region it should be possible to distinguish all crossover and heterokaryotic progeny resulting from asci with a crossover in the region. The modified RF value for such a region equals \((HK + 2 CO)/2 \times \text{total progeny}\) (\(HK\) = the number of heterokaryotic progeny produced by a crossover in the region followed by nondisjunction at MII; \(CO\) = the number of homokaryotic cross-over products in the region; see MATERIALS AND METHODS). Using this equation, the modified RF value in the un-nic region from the nine crosses of Table V should be \((17 + 2 \times 15)/2 \times 128 = 18.3\%\). This is probably a slight underestimate since the value for \(HK\) was determined for the smaller arg-ad region. Using the same criteria for a cross between the strains leu-3, a, arg-1, ad-3B, al-1 and un-3, A, ad-3A, nic-2, al-2, a value of \((8 + 2 \times 8)/2 \times 65 = 18.4\%\) was obtained. In the latter cross, a modified RF value in the ad-al region could have been obtained but was prevented by difficulties in the scoring of al-1 and al-2. However, the present modified values in the un-nic region combined with unmodified values in the leu-un, un-nic, and nic-al regions (see Table V) clearly indicate that recombination is normal in crosses homozygous for asc-3.

**Cytology.** The combined genetic data of crosses homozygous for asc-3 indicated normal recombination and segregation of homologs at the first meiotic division, followed by extensive nondisjunction at the second and post-meiotic divisions. In order to gain more insight into the nature and mechanism of this nondisjunction, and to determine the reason for the low fertility, crosses homozygous for asc-3 were analyzed cytologically. The asci that were produced showed normal pairing of homologs (Fig. 6c, d). This observation is in accord with the recombination data
Fig. 6  Chromosome development in crosses homozygous for asc-3. All preparations were stained with iron-haematoxylin. Very few asci were formed. Many cells which resemble croziers were observed (b) x2000; these cells contain a number of nuclei with nucleoli (a) x 2400; it appears that these nuclei are incapable of fusion. Pairing of homologous chromosomes appears normal at pachytene, so do the size and appearance of the nucleolus (c, d) x3400; at diakinesis, seven bivalents appear (e) x3400, and about equal amounts of chromatin segregate at the first division (f) x1300. The second division is highly abnormal: many second division figures were observed, with most chromatin attached to one spindle-pole body but not the other (arrowed) (g, h) x 1300; about equal amounts of chromatin may segregate in some asci (i) x1300; this division figure may also become similar to those shown in (g) and (h) since the spindle-pole bodies (in i) have not moved very far apart yet. Unequal amounts of chromatin have segregated into the ascospores (j, k) x1300.
presented. Seven bivalents condensed at diakinesis (Fig. 6e) and divided in a normal fashion distributing equal amounts of chromatin to the two poles (Fig. 6f). Subsequently, many asci were observed during the second division indicating that this stage takes an abnormally long time to complete (Fig. 6g, h, i). In addition, most division figures show one spindle-pole body (SPB) with a large amount of chromatin attached and the other with little or no chromatin attachment. Whether the defect is a property of the SPB or of the chromosomes cannot be determined but this differential attachment appears to be the cause of the extensive nondisjunction that has been observed genetically. Fig. 6j shows four out of eight ascospores in an ascus with no chromatin in two spores and a large amount in the two nuclei of the other two spores. Other spores contain very little chromatin with often different amounts of chromatin in sister ascospores, accounting for the many 1B:7W asci (e.g., Fig. 6k). The genetic and cytological data apparently supplement each other perfectly.

The first-acting defect of \textit{asc-3} takes place prior to ascus formation. Many cells resembling ascogenous hyphae have been observed (Fig. 6b). Fig. 6a shows one of these cells in some detail. The pre-meiotic nuclei can be readily identified by the presence of large nucleoli. Such a large number of nuclei is normally never obtained in the croziers. Thus, it appears that the defect results in blockage of most asci prior to or during karyogamy. This block in ascus formation is apparently the cause of the low fertility of these crosses. A more detailed analysis is needed, however, to determine the exact stage of the block. It may be tentatively concluded that the primary defect of \textit{asc-3} takes place
during crozier formation, possibly karyogamy, and that some cells can escape this developmental block to produce asci which nondisjoin extensively at the second and post-meiotic divisions.

asc-1 (P95)

Crosses homozygous for this recessive mutation generally result in about 40% ascospore abortion; however, up to about 70% abortion has been detected in some crosses. The fertility of crosses that were done on liquid medium appeared good. In some crosses made on solid medium, a near five-fold reduction in fertility was detected.

Recombination and Nondisjunction Frequencies. Random ascospore analysis of three crosses homozygous for asc-1 revealed reduced recombination and increased nondisjunction frequencies (Tables VIII and IX). RF was reduced in two out of three regions examined. The amount of reduction appeared variable in both the un-nic and nic-al regions. The frequency of PWT progeny was also quite variable and ranged from zero to 22.4%. No PWT cultures were detected among 239 progeny of a cross (Table VIII, row 1) that was made on liquid medium. In contrast, all crosses made on solid medium produced a variable number of PWT progeny. The majority of these PWT cultures were prototrophic for all LG I mutant markers. Therefore, these were apparently the result of nondisjunction during the first meiotic division.

The Nature of PWT Progeny. The nuclear composition of the eight PWT progeny recovered from cross 95-1 (Table VIII) was determined by the individual testing of conidial isolates from these cultures. All eight cultures were heterokaryotic. Five of them were heterokaryotic for the two original non-exchange chromosomes leu-3, a, arg-1, ad-3B and
Table VIII. Recombination and nondisjunction in three crosses homozygous for asc-1

<table>
<thead>
<tr>
<th>Cross*</th>
<th>Non-PWT Progeny</th>
<th>PWT Progeny</th>
<th>Genotypes of PWT's auxo+ other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Recombination frequency (%)</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Non-PWT Progeny</td>
<td>(no. recombinants in parentheses)</td>
<td>Progeny</td>
</tr>
<tr>
<td></td>
<td>leu-un</td>
<td>un-nic</td>
<td>nic-al</td>
</tr>
<tr>
<td>95A29 x 95a43†</td>
<td>239</td>
<td>15.9(38)</td>
<td>2.1(5)</td>
</tr>
<tr>
<td>95-1(Y₂ x X₁)‡</td>
<td>154</td>
<td>16.9(26)</td>
<td>9.8(15)</td>
</tr>
<tr>
<td>95-2(Y₅ x X₁₇)§</td>
<td>94</td>
<td>18.1(17)</td>
<td>10.6(10)</td>
</tr>
<tr>
<td>Wild type crosses§</td>
<td>11-17</td>
<td>15-20</td>
<td>30-35</td>
</tr>
</tbody>
</table>

* All strains used were ascospore isolates with genotypes as in Fig. 1.
† This cross was made on liquid medium; the ascospores from shot asci were analyzed (see Table X for ascus patterns from this cross).
‡ These crosses were made on solid medium, and random ascospores were analyzed.
§ RF values are normally slightly variable (see e.g., Catcheside, 1974).
Table IX. Nature of growth of ascospores from crosses homozygous for asc-1

<table>
<thead>
<tr>
<th>Cross*</th>
<th>Fraction of Black Ascospores Producing</th>
<th>Total No. Spores</th>
<th>Freq. of un/un⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonies</td>
<td>Germ Tube Only</td>
<td>No Germ Tube</td>
</tr>
<tr>
<td>95-1(Y₂ x X₁)</td>
<td>0.54</td>
<td>0.37</td>
<td>0.09</td>
</tr>
<tr>
<td>95-4(Y₅ x X₃)</td>
<td>0.48</td>
<td>0.32</td>
<td>0.20</td>
</tr>
<tr>
<td>95-6(Y₅ x X₁₅)</td>
<td>0.48</td>
<td>0.35</td>
<td>0.17</td>
</tr>
<tr>
<td>95-11(Y₄ x X₁)</td>
<td>0.45</td>
<td>0.34</td>
<td>0.21</td>
</tr>
<tr>
<td>95-12(Y₄ x X₁₇)</td>
<td>0.41</td>
<td>0.42</td>
<td>0.17</td>
</tr>
<tr>
<td>95-13(Y₇ x X₁₅)</td>
<td>0.47</td>
<td>0.38</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* All on solid crossing medium.
† Determined by plating of ascospores as described in Materials and Methods.
un-3, A, ad-3A, nic-2, al-2. These were presumably the result of non-
disjunction of non-exchange chromosomes during the first meiotic divi-
sion. 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 (see Fig. 3 and 5). These two PWT cultures may have resulted
from nondisjunction during the first or the second meiotic division.
If these were caused by a crossover in the relatively small un-ad region
followed by nondisjunction at the first meiotic division, one would have
expected the simultaneous recovery of many PWT cultures resulting from
the nondisjunction of non-exchange chromosomes or of chromosomes with an
exchange in another region (e.g., ad-al). In fact, only six PWT cultures
of this type were detected. Therefore, it is more plausible that the un
and the ad, nic, al cultures were produced by nondisjunction at the se-
cond meiotic division, especially since a crossover in the un-ad region
is a prerequisite to the detection of such nondisjunction (Fig. 3). The
leucine requiring PWT was heterokaryotic for the two crossover chromo-
somes 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 cross-
over chromosomes during the first meiotic division.

To What Extent Do Exchange-Chromosomes Nondisjoin During the First
Meiotic Division? The nature of the leucine requiring PWT isolate appeared
to indicate that nondisjunction of crossover chromosomes may take place at
the first meiotic division. To test the extent of such nondisjunction of
crossover chromosomes, 263 PWT isolates from ten crosses (95-1 through 95-
10) were obtained and scored for albino phenotype. The six albino PWT
cultures were apparently caused by a crossover event in the nic-al region followed by nondisjunction at the first meiotic division (see MATERIALS AND METHODS). The nic-al map distance of the subgroup of meioses in which nondisjunction of LG I took place during the first meiotic division was thus estimated at $2 \times \frac{6}{263} = 4.5 \mu$. In contrast, in three crosses examined, the nic-al recombinant frequencies among non-PWT progeny were 16.3%, 22.7% and 11.7% (see Table VIII). These data provided strong evidence supporting the idea that nondisjunction at the first meiotic division in this mutant involves primarily non-exchange chromosomes. However, these observations also suggest that some crossover chromosomes do nondisjoin at the first division.

Ascus Analysis. Many unordered asci from a cross homozygous for asc-1 have been analyzed (Table X). Most asci contained an even number of black ascospores suggesting a defect prior to the post-meiotic division. The analysis of ascospores from these asci revealed several aspects of disjunction in this mutant. First, the recombination frequency in the un-al region was similar for 8B:0W ascis (20%) and for all other asci combined (16.8%). Therefore, the reduction of RF is a general defect operative in all asci from this cross, and not just an expression of a subgroup with increased ascospore abortion.

Second, as pointed out previously, the defect in disjunction of chromosomes would have to take place prior to the post-meiotic division, i.e., during the first or second meiotic division. The absence of PWT progeny from this cross (Table VIII, row 1) and the presence of large numbers of 8B:0W and 6B:2W asci suggest regular segregation of at least LG I at the first meiotic division. One might argue that ascospore
Table X. Ascus analysis of a cross homozygous for the recessive meiotic mutation asc-1 (P95) (95A29 x 95a43)

<table>
<thead>
<tr>
<th>Types of Asci (black:white ascospores)</th>
<th>No. of Asci Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 : 0</td>
<td>41</td>
</tr>
<tr>
<td>6 : 2</td>
<td>19</td>
</tr>
<tr>
<td>4 : 4</td>
<td>22</td>
</tr>
<tr>
<td>2 : 6</td>
<td>18</td>
</tr>
<tr>
<td>0 : 8</td>
<td>19</td>
</tr>
<tr>
<td>other (all types)</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
</tr>
</tbody>
</table>
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 out of ten 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 the first meiotic division could be the cause of the observed ascospore abortion.

**Cytology.** To confirm and supplement the genetic data, three crosses were examined cytologically (95-11, 95-12 and 95-13). Crozier and ascus formation appeared regular (Fig. 7a); the first defect was visible during the zygo/pachytene stage when reduced pairing of homologous chromosomes is often evident (Fig. 7b, c). This observation is compatible with the reduced recombination values.

During this analysis, a number of metaphase I figures with up to 14 (= 2N) univalents were observed (Fig. 7d-f). In many cases, a close association between several of these univalents could be detected. For example, Fig. 7d shows a number of pairs of chromosomes and Fig. 7e shows a connection between three pairs of univalents. These latter connections may well result from the segregation of homologs whereas those chromosomes lacking this connection may be genuine univalents. It is plausible that the nature and extent of premature separation of these "pairs" of chromosomes determines the final frequency of nondisjunction.
Fig. 7 Chromosome development in crosses homozygous for asc-1. All preparations were stained with Feulgen and aceto-orcein. Crozier development is normal (a) x2700; the pairing of homogous chromosomes at pachytene is incomplete (b and c) x3400; subsequently, some univalents appear on the metaphase plate (d), and some chromosomes with bridges are seen at meta/anaphase I (e and f) x3400; the latter type may be dividing bivalents. The first meiotic division segregates about equal amounts of chromatin to the two poles (g) x1700, but some lagging occurs--see arrowed chromosomes (h) x1100. The second division is highly irregular: overlapping spindles (i), abnormal separation (j) x1400, and lagging of chromosomes (k) x1700, have all been observed frequently. An ascus at the prophase of the postmeiotic division shows regular placement of chromatin in asci but unequal amounts of chromatin in different nuclei (l); such unequal distribution is also observed at the ascospore stage (m) x900. Finally, many asci with eight ascospores are aborted; one of the few normal unabortased asci is shown (arrowed) as a comparison in (n) x200.
Further analysis of high and low nondisjunction crosses should shed some light on this subject.

Cytological preparations of crosses homozygous for asc-1 revealed many more metaphase I configurations than were observed in wild type crosses. This may be a reflection of an extended time interval spent in this stage. That division may be slowed down by the presence of univalents has been previously reported for rye (Prakken, 1943). The distribution of approximately equal amounts of chromatin to the two poles (e.g., Fig. 7g, h) seems to suggest that the univalents do not segregate purely at random. In addition, some lagging of chromosomes has been observed following the first division (Fig. 7h). These chromosomes will most likely join another nucleus later on, since only eight nuclei are ever observed at the eight-nucleus stage. Some PWT progeny may have originated in this manner.

The second meiotic division was almost always irregular: spindle overlap, lagging of chromosomes, apparent slow separation of dividing nuclei, and movement of segregating spindle bodies to the same pole, have been observed (Fig. 7i-k). In addition, unequal amounts of chromatin can be seen to segregate in many asci (Fig. 7l, m). These large-scale abnormalities of segregation agree well with genetic observations. The relative regularity of the post-meiotic division, suggested by the infrequent occurrence of asci with odd numbers of black ascospores (Table X) was confirmed by the equal amounts of chromatin-staining material in sister spores (e.g., Fig. 7m).

In the final analysis, the primary defect of this mutant appears to be a reduced pairing of homologous chromosomes with a resultant reduction
in recombination frequency in some regions. The consequence of this re-
duced pairing is the formation of univalents, the segregation of which
is somewhat irregular. The high amount of irregular segregation at the
second division appears to be the direct cause of much of the ascospore
abortion. This aberrant segregation may be the normal consequence of
the formation of univalents, or it may be due to a pleiotropic effect of
the mutant.

The Inviability of Many Black Ascospores from Crosses Homozygous
for asc-1 May be Due to Their Multiple Disomy. Nondisjunction can be
detected in Neurospora because of the viability of its aneuploid progeny.
In contrast, such progeny in most plants grow either very poorly or not
at all. It is possible that many aneuploid cultures are also quite in-
viable in Neurospora. To test this idea, ascospores from six crosses
were heat-shocked and plated out, and their germination and colony-forming
ability recorded. Only about 50% of the black ascospores produced colonies
(Table IX). Most other ascospores germinated but growth stopped very
quickly. A few germinated ascospores of the latter type resumed growth
at one or more points along the mycelium. The observation of such escape
suggests an explanation of inviability of so many black ascospores.

Crosses homozygous for asc-1 produce PWT progeny resulting from hyper-
ploid ascospores. It is plausible that the growth of many of these hyper-
ploid ascospores becomes inhibited upon germination. In some of these
young ascospore cultures, the process of haploidization may cause the loss
of extra chromosomes (Pittenger, 1954) and thus enable resumed growth.
mei-1

This recessive meiotic mutation has been analyzed using both genetic and cytological means (Smith, 1975; Lu and Galeazzi, 1979) and appears to be defective in a function necessary for the pairing of homologous chromosomes during the first prophase of meiosis. This is followed by the formation of univalents at metaphase I and abnormal segregation patterns, including 4-poled spindles, at subsequent divisions leading to about 90% ascospore abortion. Genetically, an almost complete absence of recombination is in accordance with the observed pairing defect, and the majority of black ascospores are disomic for most linkage groups.

The mei-1 mutant was primarily obtained to check allelism to any mutants isolated during this study, and to determine any potential interaction with some of these newly isolated mutations. During initial tests, it was found that crosses homozygous for mei-1 produced about 10% black ascospores when crossed on solid medium, as was observed by Smith (1975), but 30% black spores resulted when these same strains were crossed on liquid medium. From a cross (a^m(33), ad-3B; mei-1 x un-3, A, ad-3A, nic-2; mei-1) made on liquid medium, 22 out of 24 ascospore cultures were adenine-independent, indicating they were disomic for LG I. This value is as high or higher than was 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.

To determine the defect of such crosses made on liquid medium, the above-mentioned cross was analyzed cytologically. The observations on pairing (Fig. 8c, d) at metaphase I were identical to those obtained
Chromosome development in crosses homozygous for mei-1. (a, b, d, and g) were stained with Feulgen and aceto-orcein; the remaining preparations were stained with iron-haematoxylin. Pairing between homologs is apparently absent, although some loose associations may be observed (a, b) x3400; near metaphase I, 14 univalents are usually observed (c, d) x3400; sometimes, loose associations between homologous chromosomes appear to occur (c). About equal amounts of chromatin move to opposite poles at the first meiotic division (e, g) x3400. About 14 chromosomes individualize in each of the two dyad nuclei of prophase II (g) x2400; some chromosomes (arrowed) may lag. The second division is similar to that observed for mutant asc-6 (Fig. 5i): the chromatin takes a long time to separate (i, j, h) x2400, and usually unequal amounts of chromatin move to opposite poles (especially (j); spindle-pole bodies are arrowed).
previously (Lu and Galeazzi, 1979). However, observations on subsequent stages of meiotic development differed in several respects:

(1) Numerous asci were observed during the first interphase; these asci showed that about equal amounts of chromatin moved to opposite poles at the first meiotic division (Fig. 8e, f).

(2) Some asci corresponding to prophase II contained two nuclei, each with a chromosome number approaching a diploid nucleus (Fig. 8g).

(3) In accord with previous observations, the second division was characterized by a very slow separation of the two daughter nuclei (this was inferred from the high frequency of asci at this stage; Fig. 8h-j); however, spindle overlap was rarely observed and 4-poled spindles were absent.

(4) The post-meiotic division of four separate nuclei appeared regular.

In addition, several interesting aspects of these preparations are the apparent association of univalents during early metaphase (Fig. 8c), and the unequal distribution of chromatin to opposite poles in many second division figures (e.g., Fig. 8j).

It has been established that crosses homozygous for mei-1 result in 70% ascospore abortion when crossed on liquid medium, but 90% on solid medium. Cytological observations of crosses made on the two types of media were similar up to and including the formation of univalents; subsequently, preparations from the cross made on liquid medium contained more asci at interphase I and prophase II, but less asci with irregularities such as 4-poled spindles. Thus, it appears that the two types of crossing media differentially affect the segregation of univalents in
crosses homozygous for mei-1. Because of the absence of 4-poled spindles from asci produced on liquid medium, such irregular types of segregation probably do not, as proposed by Lu and Galeazzi, account for the observed frequency of disomy of this mutant. Instead, the present data suggest that univalents divide equationally during the first meiotic division. Even though a high frequency of PWT progeny might also be produced by the random movement of univalents, or by irregular spindle behavior or defective inclusion of nuclei in ascospores, these alternatives were virtually ruled out by the following observations. First, both spindle overlap and 4-poled spindles were rare or absent, and the stages subsequent to the second meiotic division were apparently normal. Thus, neither irregular spindle behavior nor defective ascospore inclusion could account for the extremely high frequency of PWT progeny. Second, contrary to observation, the random movement of univalents would cause unequal amounts of chromatin to segregate at the first meiotic division. In addition, the nuclei in the following prophase should contain a total of only 2N(= 14) chromosomes. In fact, the observed number was usually closer to 4N(= 28) chromosomes. By the process of elimination, it appears most probable that the univalents in crosses homozygous for mei-1 divide equationally. This would account for the observed segregation of roughly equal amounts of chromatin at the first division, and for the presence of a diploid or close to diploid number of chromosomes in each nucleus of the prophase II asci. In addition, the subsequent division would take a long time to complete since only chromatids are present.
Mapping

**asc-3.** A cross between mei-1 and asc-3, originally made to obtain double mutants, produced no recombinants among 62 ascospore isolates: 25 were asc-3, mei-1\(^+\), and 37 were asc-3\(^+\), mei-1. In order to perform a more detailed mapping, two flanking markers were introduced: trp-4, mapped 12/183 or about 6.5 mu to the right of asc-3; and pdx, mapped 3/92 or about 3.5 mu to the left of mei-1. Ascospores from the cross between asc-3, 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-3. The ratio of the two mutants roughly corresponds to the ratio of map distances previously determined for the regions pdx-mei-1 (3.5 mu) and asc-3-trp-4 (6.5 mu).

The pdx\(^+\), trp\(^+\) recombinants represent only about 5% of all progeny from this cross (half the map distance of the pdx-trp region). Since only recombinants in the pdx-trp region could contain a recombinant between mei-1 and asc-3, a total of 20 x 300 = 6000 progeny were tested for the latter type of recombinant (mei-1-asc-3). However, since only one out of two reciprocal crossover products would be detected, the total progeny tested would be \(\frac{1}{2} \times 6000 = 3000\). Therefore, no crossovers between mei-1 and asc-3 were observed among 3000 progeny. The recombination frequency between mei-1 and asc-3 is less than 0.1 mu.

**asc-1.** The mutant asc-1 was mapped close to asc-3, and therefore to mei-1, since only asc-1 (45) and asc-3 (51) progeny were detected among 96 isolates from a cross between the two mutants.
asc-6. Ascus analysis of a cross between asc-3 and asc-6 established the close proximity of asc-6 to one of the centromeres, since, in five out of seven ascii, the mutant and its wild type allele segregated during the first division. In subsequent crosses to centromere-linked markers, the mutant was mapped on LG II, about 3 mu from arg-5 (the slo (slow growth) mutation which was isolated with the meiotic mutation maps about 14 mu from arg-5 such that asc-6 lies between slo and arg-5). asc-6 cannot be definitely assigned to the right arm of LG II. However, two lines of evidence are in support of the location of asc-6 on the right arm of LG II to the right of arg-5: (a) second division segregation data put the asc-6 mutation approximately 10 mu from the centromere of this linkage group; and (b) arg-5 has previously been mapped about 5 mu to the right of the centromere of LG II (Radford, 1972). Therefore, the following arrangement of markers appears most likely: (centromere) - 5 mu - (arg-5) - 3 mu - (asc-6) - 11 mu - (slo).

Interaction of Meiotic Mutations

mei-1 and asc-6. Crosses homozygous for the double mutant mei-1; asc-6 produced about 70% spore abortion when made on liquid crossing medium. This is similar to values for either single mutant. The fertility of these crosses was good (i.e., many spores were formed) although somewhat reduced when compared to 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-6 x un-3, A, ad-3A, nic-2; mei-1; asc-6), showed that 38 out of 41 isolates (93%) were adenine-independent and therefore disomic for LG I. This value of PWT frequency is similar to mei-1 (80-90%), and
different from $\text{asc-6}$ (20-55%). Therefore, these data suggest an epistatic dominance of $\text{mei-1}$ over $\text{asc-6}$. This epistatic relationship was confirmed cytologically: in such crosses there was an absence of pairing as in $\text{mei-1}$, rather than reduced pairing as in $\text{asc-6}$.

$\text{asc-1}$ and $\text{asc-6}$. Crosses homozygous for the double mutant $\text{asc-1; asc-6}$ produced about 70% ascospore abortion ($70.3 \pm 6.7\%$ in 12 crosses examined). This value is similar to those obtained from either single mutant ($69 \pm 8.4\%$ in 10 crosses homozygous for $\text{asc-1}$ alone, and $62.3 \pm 9.4\%$ in 6 crosses homozygous for $\text{asc-6}$ alone). Thus, from these ascospore abortion data it appears that there is no epistatic interaction between these mutations. Genetic and cytological analyses of these crosses have not yet been performed. Until such time, no firm statement can be made on the interaction between these two mutations.

DISCUSSION

Characteristics of four recessive meiotic mutations of *Neurospora crassa* have been summarized in Table XI. The abortion of ascospores observed in crosses homozygous for each of the three newly isolated recessive mutations ($\text{asc-1, asc-3 and asc-6}$) was shown to be the consequence of abnormal disjunction of meiotic chromosomes. In two cases ($\text{asc-1}$ and $\text{asc-6}$), the abnormal disjunction was apparently caused by a defect in the pairing of homologs during the first meiotic prophase. In the third mutant ($\text{asc-3}$), a primary defect near karyogamy may have had a pleiotropic effect resulting in the nondisjunction observed during the second and post-meiotic divisions. Although the absence of pairing and the subsequent disjunction defect of $\text{mei-1}$ has been established previously.
Table XI. Characteristics of four recessive meiotic mutations in *Neurospora crassa* with a defect in the regular disjunction of chromosomes

<table>
<thead>
<tr>
<th>Allele</th>
<th>Approx. % Ascospore Abortion</th>
<th>Crosses made on liquid or solid medium</th>
<th>Fertility*</th>
<th>Recombination Frequency leu-un un-al</th>
<th>Paireing</th>
<th>Nondisjunction at MI MII PMD</th>
<th>Apparent Initial Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>asc-1</td>
<td>40-70</td>
<td>both</td>
<td>med → high</td>
<td>normal reduced reduced</td>
<td>yes</td>
<td>yes</td>
<td>?</td>
</tr>
<tr>
<td>asc-3</td>
<td>90-98</td>
<td>both</td>
<td>very low</td>
<td>normal normal normal</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>asc-6</td>
<td>70</td>
<td>both</td>
<td>low → med</td>
<td>reduced reduced  much reduced</td>
<td>yes</td>
<td>?</td>
<td>yes</td>
</tr>
<tr>
<td>mei-1</td>
<td>70</td>
<td>liquid high</td>
<td>--</td>
<td>absent† absent†</td>
<td>yes†</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>solid high</td>
<td>--</td>
<td>absent† absent†</td>
<td>yes†</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

*Total number of black and white ascospores produced; may be low (few ascospores), med. (intermediate amount of ascospores), or high.

† Data from Smith (1975), and Lu and Galeazzi (1979); recombination was determined on regions of another chromosome (Smith, 1975).
(Smith, 1975; Lu and Galeazzi, 1979), some new observations were made during this study which suggest a possible mechanism for the abnormal disjunction of univalents in *Neurospora*.

**Pairing- and Recombination-Defective Mutants**

Mutants with a reduced recombination frequency may have a defect in the pairing of homologs (i.e., a precondition to exchange), or in the exchange process itself.

The mutations *asc-1* and *asc-6* have cytologically detectable defects in the pairing of homologs during the zygotene and pachytene stages of the first meiotic prophase. In this respect, they resemble many asyndetic mutants in plants (reviewed in Baker *et al.*, 1976a) and the mutant *mei-1* in *Neurospora* (Smith, 1975; Lu and Galeazzi, 1979). The extent of the pairing defect of these three mutants in *Neurospora* (i.e., *asc-1*, *asc-6* and *mei-1*) was found to be perfectly correlated with the reduction in recombination frequency. Mutant *asc-1* was least affected while both pairing and recombination were almost completely absent in *mei-1*.

The cytological detection of a pairing defect does not necessarily mean that a defect in the pairing process *per se* operates. Instead, a defect in an immediate requirement for the process of exchange may secondarily cause some pairing abnormality. Mutants with a defect in the preconditions to exchange (such as pairing) and exchange processes have been distinguished on the basis of the uniformity of the reduction of exchange (Sandler *et al.*, 1968; Jones, 1974). Any mutant which reduces exchange in a uniform manner along the chromosome should be
considered defective in a process required for exchange itself. In contrast, non-uniformity of reduction would mean a defect in a precondition to exchange. Using these criteria, the wild type gene of \textit{asc-1} controls some function necessary for a precondition to exchange (presumably the establishment or maintenance of pairing). Such a function has not been firmly established for mutant \textit{asc-6}; however, the occurrence of most detectable pairing near the ends of the chromosomes may suggest a defect in a precondition function. The almost complete lack of pairing at all stages of meiotic development in crosses homozygous for \textit{mei-1} would not be expected if the exchange process was affected. Therefore, the wild type gene of \textit{mei-1} probably controls a function that is essential for the establishment of pairing.

\textbf{Disjunction of Chromosomes During the First Meiotic Division}

\textbf{Correlation between Lack of Exchange and Nondisjunction.} As a consequence of reduced pairing and exchange, many homologous chromosomes will not be held together by their chiasmata, and they will produce univalents instead of bivalents at metaphase I of meiosis. Bivalents normally control the regular segregation of its component homologous chromosomes. Therefore, if homologous chromosomes are not held together to form bivalents, segregation of the individual chromosomes (univalents) will be irregular. In this manner, mutants with a defect in pairing or exchange have been indirectly selected through the aberrant disjunction of the univalents it produces. In recombination, defective meiotic mutants of \textit{Drosophila}, only non-exchange chromosomes non-disjoin (Baker and Hall, 1976). Therefore, all bivalents which are
held together by one or more chiasma(ta), segregate in a normal fashion and irregular segregation is solely due to univalents produced by a lack of chiasmata. Similar analysis has not been performed in meiotic mutants of higher plants due to difficulties associated with the recovery of aneuploid products produced by nondisjunction. However, nondisjunction of some exchange chromosomes takes place in meiotic mutants of the nematode Caenorhabditis elegans (Hodgkin et al., 1979). Analysis of aneuploid progeny (PWT) produced by the mutants asc-1 and asc-6 of Neurospora showed that the majority of nondisjunction involves non-exchange chromosomes. Therefore, most nondisjunction is a consequence of the irregular segregation of the non-exchange univalents. However, a small but significant fraction of exchange chromosomes fail to disjoin during the first meiotic division. The low frequency of such events suggests that nondisjunction of exchange chromosomes is a secondary effect of the production of non-exchange univalents. For example, the long duration of metaphase I observed in these mutants (see also Prakken, 1943) could have caused the precocious terminalization of chiasmata and thus produced exchange univalents. Alternatively, lagging of chromosomes or spindle abnormalities induced by the abnormal nature of the chromosomes could cause nondisjunction of some bivalents. That the presence of univalents is not the only factor that can potentially cause nondisjunction in wild type strains is amply illustrated by high frequencies of spontaneous or chemically induced nondisjunction of exchange chromosomes in Neurospora (DeLange, unpublished; Griffiths and DeLange, 1977; Smith, 1974). 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. In fact, univalents appear to be scattered across the length of the spindle.

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., Catche-side, 1939; Sjodin, 1970); in many plant species, both types of segregation have been observed simultaneously (e.g., Prakken, 1943). In Neurospora, some evidence indicates that the equational division of centromeres during anaphase I is a common means of segregation of univalents.

First, the high frequency of 6B:2W asci obtained from a cross homozygous for asc-1 is compatible with aberrant segregation at the second meiotic division, or with the equational division of some univalents during the first division. Since the primary defect in pairing and in the production of bivalents has been established in subsequent crosses homozygous for asc-1, the latter explanation is preferable. In addition, non-disjunction of homologous chromosomes should have produced PWT progeny if LG I was involved, or 4B:4W asci with only one homologous LG I centromere (leu, a, arg, ad or un, A, ad, nic, al) if one or more other linkage groups were involved (see RESULTS). In fact, no PWT's were observed among the progeny of this cross, and both homologous LG I centromeres were obtained in seven out of ten 4B:4W asci. These observations would be consistent with the equational separation of the centromeres of some chromosomes other than LG I during the first meiotic division.
Second, in crosses homozygous for mei-1, 14 univalents can be counted in most metaphase I figures. If these univalents moved at random one would expect unequal amounts of chromatin to segregate to opposite poles at the first division. However, in all cases observed, approximately equal amounts of chromatin moved to each pole, consistent with equational separation of all 14 univalents. At the prophase of the next division, up to 14 chromosomes were observed in each dyad nucleus. In addition, the observation that about 90% of ascospore cultures were PWT, suggests that the nuclei in the viable ascospores were diploid or nearly so. These observations are clearly not compatible with random movement of univalents. By the process of elimination, the equational separation of univalents at the first meiotic division has likely taken place. Similar data have been obtained for asc-6. The lower frequency of nondisjunction (20-55%) is likely due to a higher frequency of bivalents at metaphase I.

Third, the equational division during the first meiotic division has been previously suggested in Neurospora by the occurrence of certain PWT-containing asci (Threlkeld and Stolz, 1970). Direct proof for equational division awaits further genetic and cytological 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. The failure to observe this event during this study is an indication that the separation of chromosomes at anaphase I proceeds very rapidly.

Disjunction of Chromosomes During the Second and Post-Meiotic Divisions

The equational division of univalents during the first meiotic division makes the subsequent division of these chromatids impossible. In some yeast and plant mutants, no second division follows the equational
separation, and either diploid spores or pollen are produced (Moens et al., 1976; Stringham, 1970; Smith, 1939) or the cells degenerate (Palmer, 1971). If nuclear division takes place, the chromatids will either move at random to opposite poles, possibly after a period of lag (Prakken, 1943), or they replicate before division. Even though the latter mechanism has never been reported, the behavior of mutant mei-1 suggests that an extra round of replication prior to the second division may occur in Neurospora. Alternatively, most chromosomes could move together to one of the two division poles. It has been noted that each dyad nucleus presumably has a diploid complement (or close to it) of chromatids. The unusually high number of asci with two dividing nuclei means that this division takes a long time to complete. If the chromatids move at random, then most resulting nuclei should miss at least one chromosome. Consequently, contrary to observation, most asci would contain only inviable ascospores. The high fertility and almost complete diploidy of most ascospores can be explained by an extra round of replication and regular centromere division, or by the preferential movement of most chromosomes to one division pole. Some cytological evidence is in favor of the latter hypothesis (see e.g., Fig. 8j). The occurrence of an extra round of replication could be tested by means of the Feulgen staining method (Iyengar et al., 1977).

In crosses with a mixture of univalents and bivalents at metaphase I, a combination of the regular segregation of the bivalents and random movement or centromere division of the univalents would produce a mixture of chromosomes and chromatids prior to the second division. In this case, a problem in the synchrony of division could be
expected. In rye, mutants with a mixture of univalents and bivalents had more aberrant second division figures than those with predominantly univalents (Prakken, 1943). Similarly, in Neurospora, more irregular types of chromosome movement (e.g., lagging, spindle overlap) during the second meiotic division have been observed in crosses with many bivalents and some univalents (e.g., asc-1), than in crosses with few or no bivalents (e.g., asc-6 and mei-1).

The post-meiotic divisions of these three mutants are usually regular. However, some chromosome loss or nondisjunction has been suggested by asci with odd numbers of viable black ascospores, and confirmed genetically by the recovery of an odd number of PWT progeny from six out of seven asci obtained in a cross homozygous for asc-6 (Table III).

Effect of Liquid and Solid Crossing Medium on Disjunction

Abnormal division figures have been observed in crosses homozygous for asc-1 and mei-1 on solid crossing medium. Examples are the movement of separating spindle pole bodies to the same side of the ascus in crosses homozygous for asc-1, or 4-poled spindles for mei-1. It has been proposed that the 4-poled spindles in crosses homozygous for mei-1 may account for the observed nondisjunction (Lu and Galeazzi, 1979). However, their absence from similar crosses on liquid medium provides firm evidence against such a postulate. That the differences in types of division figures is a consequence of different crossing media is suggested by the abortion of 90% of the ascospores on solid medium but only 70% on liquid medium. The higher spore abortion on solid medium can be readily explained by the abnormal types of divisions (e.g., 4-poled spindles); such abnormal
divisions might be expected to simultaneously reduce the frequency of viable aneuploids for each chromosome. A comparison of PWT frequencies from crosses homozygous for mei-1 on liquid medium (this chapter), with those on solid medium (Smith, 1975), is not incompatible with this prediction. These observations illustrate the importance of the combined genetic and cytological studies of several crosses. The use of different crossing conditions (e.g., solid vs. liquid crossing medium) may also provide valuable information for the final understanding of meiotic processes.

Are All Aneuploid (n + 1 through n + 6) Ascospores Viable in Neurospora?

In Neurospora, mutations which cause abnormal disjunction of chromosomes during meiosis have been detected by the presence of many hypoploid white ascospores, and they have been partially characterized by the study of hyperploid (PWT) isolates. In higher plants, most aneuploid products of meiosis are inviable. This makes the genetic characterization of mutants very difficult. Even though many aneuploid products can be obtained in Neurospora, it is not clear whether or not they are all viable. Therefore, ascospores from a cross homozygous for asc-1 were heat-shocked, plated and scored for their colony-forming ability. About 50% of the black ascospores did not produce colonies. Most of these germinated but then growth stopped abruptly. The observed sudden escape in some of the inhibited ascospore cultures may be explained if the inhibition were caused by aneuploidy, and the resumption in growth by the loss of one or more excess chromosomes (Pittenger, 1954). Germination frequencies (generally about 50%) of spores produced by crosses homozygous
for asc-6, asc-3 and mei-1 are also consistent with this interpretation.

Does Nondisjunction Affect All Chromosomes?

The study of the simultaneous nondisjunction of more than one chromosome may help in the characterization of these mutants. Three chromosomes (LG I, IV and V) were simultaneously monitored in crosses homozygous for asc-6. In two crosses examined, the three chromosomes were affected by nondisjunction at about equal frequency (Table IV). Similar observations have previously been made for mei-1 (Smith, 1975). However, it appeared at first that chromosomes in crosses homozygous for asc-6 did not nondisjoin independently: a prevalence of isolates disomic or haploid for all three chromosomes was observed. Even though this may be due to a dependence of disjunction of different chromosomes, the more probable explanation of this phenomenon is the selective death of ascospores with multiple disomic nuclei and survival of haploid and diploid (or nearly so) isolates. Such preferential survival has already been suggested in the previous section.

Interaction of Three Meiotic Mutations

Studies of interaction between the three mutations with a pairing defect suggested that each is defective in one aspect of the same process. In this case, the process affected is pairing, and double mutants appear to behave as the single mutant with the more extreme reduction in pairing. However, since data on the interaction between the mutants asc-1 and asc-6 are limited, it remains possible that these two mutations interact to virtually eliminate the pairing of homologs. This interpretation would be compatible with present observations,
since the amount of ascospore abortion of crosses homozygous for the double mutant asc-1;asc-6 is identical to that of crosses homozygous for mei-1 which lack pairing of homologs.

In summary, the combined genetic and cytological observations of the three meiotic mutants mei-1, asc-1 and asc-6 classify these mutants into a group with many common characteristics. In each case, some defect in pairing (or exchange) leads to the formation of univalents at metaphase I. The difference in frequency of univalents appears to partly control subsequent chromosome behavior during the following division. However, in each case, irregular segregation takes place during the first and second division, leading to the production of hypoploid products (recognized as white ascospores), and hyperploid products (those disomic for LG I are recognized as PWT progeny, which are used in the characterization of the mutant). The use of different crossing media apparently has an effect on chromosome movement in at least one of these mutants (mei-1). Therefore, the simultaneous study of mutants on both types of media may provide some insight into the process of disjunction during meiosis.

A Mutation Which Causes Nondisjunction During the Second Meiotic Division

The mutant asc-3 appears to be unique. The primary defect takes place before the formation of asci. Most cells are apparently blocked prior to karyogamy. Since pre-meiotic DNA synthesis in Neurospora takes place just prior to karyogamy (Iyengar et al., 1977), it is quite plausible that the wild type gene of asc-3 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-3 produced the same crossing phenotype. Thus, the few asci produced in crosses homozygous for asc-3 are the consequence of leakiness. Pairing of, and recombination between homologous chromosomes is normal. Similarly, no abnormalities have been detected in the disjunction of these chromosomes during the first meiotic division. However, an extremely high frequency of nondisjunction takes place during the second division, and there is also chromosome loss or nondisjunction at the post-meiotic division. The nondisjunction during the second division apparently involves attachment of most chromosomes to one, but not the other, spindle pole body (SPB). The extended duration of this division is probably caused by such abnormal attachment.

In some respects, this mutant resembles the mutants pal and cand of Drosophila (Baker and Hall, 1976). The mutant pal acts only in males. Chromosomes of homozygous pal males are preferentially lost during the first zygotic cleavage division and maybe during the meiotic divisions. Such loss also takes place in cand mutants, which act exclusively in females. In both cases, chromosomes are lost at one pole of the division. In these two mutants of Drosophila and the asc-3 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 Second Meiotic Division Stages

It has been suggested that the wild type genes for the mutants pal and cand 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., 1976a). Alternatively, the phenotype of these mutants might be produced by a defective spindle pole body. Similarly, in view of the stage of the first acting defect of asc-3, it is possible that its wild type gene product operates during the pre-meiotic S phase and modifies the centromere region of the newly synthesized DNA or produces a defective spindle pole body. Either defect would generally cause a developmental block; however, the few cells that escape this block would encounter problems during the second and subsequent divisions owing to the mutant product necessary for regular segregation. One could speculate that this particular 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 mutant, and of similar ones, e.g., mei-4 in Neurospora (Newmeyer and Galeazzi, 1978; Raju and Perkins, 1978). For example, mutants with similar primary defects, such as the pairing defective mutants already discussed, may have similar secondary defects in the segregation of chromosomes.

The Nature of the Defect at the Second Meiotic Division

If the postulated abnormal centromere regions would align at random, one would expect both chromatids of all seven chromosomes to move at random to either one or the other pole. Such segregation would produce an extremely high chance of abortion of resultant ascospores owing to hypoploidy. Both cytological and genetic observations appear to contradict these assumptions: (i) most chromosomes move to one pole,
few or none to the other, and (ii) 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 (Fig. 9). This type of preferential segregation has been observed in prokaryotic systems (Jacobs et al., 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.

Map Positions of asc Mutations

Mapping of the three newly isolated meiotic mutations has placed asc-1 and asc-3 extremely close to the previously isolated mutation mei-1. Mutation asc-6 was located on another linkage group (LG II). The three closely linked meiotic mutations (mei-1, asc-1 and asc-3) are mutually complementing (see Chapter 1) and appear to involve functions necessary only for the regular pairing and disjunction of chromosomes during meiosis. These mutations could be complementing alleles of the same gene. Alternatively, they may represent the first case of a gene cluster that is specifically active during meiosis (a gene cluster of functions required in DNA metabolism has been detected in Drosophila; some of these functions are also required during meiosis; Boyd et al., 1976a). The close proximity of asc-3, whose wild type gene operates before karyogamy, and mei-1 and asc-1, which exhibit pairing defects, may be an
Fig. 9 Possible explanation for abnormal disjunction in asc-3 mutant crosses. Upon separation of DNA strands to enable premeiotic 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-3 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).
Premeiotic S Phase

Nucleus of Parent 1  Parent 2
(only two of the seven chromosomes presented)

Double Helix

Metaphase I

Metaphase II (one dyad nucleus)

defective in asc-3
defective in asc-3
indication that the wild type alleles of mei-1 and asc-1 are also active before karyogamy and thus well before the actual pairing process where their effects are expressed.

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*. Two different types of mutants have been partly characterized and mapped. Future studies should extend to the isolation of temperature-sensitive mutants and mutants that interact with existing meiotic mutants. These may be used to determine the temporal period of activity of gene products and the nature of cell activity by the interaction of different components.
CHAPTER III

THE MUTATION SK(ad-3A) ALTERS THE DOMINANCE

OF ad-3A+ OVER ad-3A IN THE ASCUS OF NEUROSPORA
INTRODUCTION

Known causes of lethality of meiotic products include aneuploidy and mutation. For example, the deletion of any essential part of the genome from the pollen of higher plants or ascospores of fungi results in their abortion. In contrast, deficiencies of nuclei in the meiocytes of animals does not generally result in lethality of the egg or sperm cells. There are three known types of lethality of meiotic products which are not associated with aneuploidy but, instead, involve specific gene mutations. In each case, meiotic products which carry a specific allele are inviable.

Alleles of certain genes are recovered in a regular fashion when crossed with the same allele, but they are less frequently or not recovered when crossed with another allele. This phenomenon, called segregation distortion, can be the result of the lethality of meiotic products containing the sensitive genes (for review see Zimmering et al., 1970; Hartl and Hiraizumi, 1976). Examples are SD in Drosophila, which causes an arrest in maturation of Sd+ spermatids (Hartl and Hiraizumi, 1976) and SK in Neurospora, which causes abortion of SK containing ascospores (Perkins and Barry, 1977; Turner and Perkins, 1976).

Alleles from a second kind of gene cause the abortion of all meiotic products when crossed with the same allele. This kind of mutant effect may be expressed in an autonomous or recessive manner, i.e., in crosses to the wild type allele, meiotic products containing
the mutant allele may abort (autonomous) or they may be viable (recessive).

These types of mutants are most easily detected in fungi, especially ascomycetes, since all products of a meiotic division can be observed in a single ascus. In addition, viable and inviable ascospores can be readily distinguished by their different color (and often their size). Ascospore lethals are usually colorless, while normal viable ascospores are generally colored. In this manner, the autonomously expressed ascospore lethals asco (Stadler, 1956), tan (Nakamura, 1961), cys-3 (Murray, 1965) and ws (Phillips and Srb, 1967) were detected in Neurospora. This class of mutants superficially resembles viable ascospore color mutants in Sordaria (Chen, 1965; Olive, 1965), Podospora (Esser, 1974) and Ascobolus (Bistis, 1956; Lissouba et al., 1962). However, the two types of mutants probably represent quite different defects.

Examples of mutations which express their effect in a recessive manner are ad-3A and ad-3B in Neurospora (Griffiths, 1970) and ms_13 and ms_14 in tomato (Rick and Butler, 1956).

This chapter reports a case in Neurospora crassa where one mutant (ad-3A) can be expressed as either a recessive or an autonomous ascospore lethal, depending on whether it is crossed with wild type (ad-3A^+) or a newly induced mutation called SK(ad-3A). This is, to the author's knowledge, the first case of such a relationship.

5Some exceptions are the ascospore lethals le-1 and le-2 in Neurospora (Murray and Srb, 1961; Garnjobst and Tatum, 1967). Ascospores containing these mutants are black.
Moreover, the system is also unique in that the affected enzyme coded for by the \textit{ad-3A} locus is known and characteristics of it can be studied on a biochemical level (Fisher, 1969b). Therefore, the study of this mutation may give an insight into processes involving gametic lethality and the interaction between genomes in the ascus.

\textbf{MATERIALS AND METHODS}

\textbf{Strains}

The following mutant alleles were used during this study: \textit{leu-3} (R156), \textit{un-3} (55701-t), \textit{arg-1} (36703), \textit{nic-2} (43002), \textit{al-2} (74-Y-112-M38), \textit{tol} (N83), \textit{ad-3A} (2-17-19, 2-17-124, 2-17-186, 2-17-232, 2-17-233, 2-17-814, 2-17-825, 2-31-2, 2-32-10, 2-33-3, 2-33-4, 2-33-22, 2-33-30, 2-33-34, 5-5-4, 5-5-23, 5-5-47, 5-5-52, 5-5-74), \textit{ad-3B} (2-17-114, 2-17-76, 2-17-82, 2-17-85, 2-17-99, 2-17-128). The last five alleles of \textit{ad-3B} complement allele 2-17-114, and none of the \textit{ad-3A} alleles complement each other. The approximate map distances (Radford, 1972) of mutations on LG I are shown in Fig. 1 (see also Chapter I). Discrepancies between these values and those obtained in this study may be partly due to variability caused by genetic background.

The procedure used in the isolation and detection of mutant strain P917 has been described in Chapter I. This strain is a pseudo-wild type (PWT) culture obtained from a screen for recessive meiotic mutants. It is composed of two nuclear components of genotypes \textit{leu-3}, \textit{a}, \textit{arg-1}, \textit{ad-3B} and \textit{un-3}, \textit{A}, \textit{ad-3A}, \textit{nic-2}, \textit{al-2}; each component carries the \textit{tol} mutation which allows normal growth of (\textit{A} + \textit{a}) heterokaryons (Newmeyer, 1970; DeLange and Griffiths, 1975), and identical (but
Fig. 1  The two nuclear components of strain P917.
Heterokaryon

Component 1

leu-3 + a arg-1 + ad-3B + +

Component 2

+ un-3 A + ad-3A + nic-2 al-2

Approximate map distance
(Radford, 1972)

10 0.1 10 9 0.3 4 28
unknown) het genotype necessary for vigorous growth of the heterokaryon (Garnjobst and Wilson, 1956). Mutant P917 was detected by its production of about 60% white aborted ascospores.

The two ascospore isolates 917A36 and 917a38 were obtained from crosses between strains P917 and the wild type strains OR-A and OR-a, respectively. Their genotypes are leu-3, a, arg-1, ad-3B; tol; Cde (917A36) and un-3, A, ad-3A, nic-2, al-2; tol; Cde (917a38), where C, d, and e are alleles of three heterokaryon compatibility loci. The presence of the tol mutation and Cde genotype in both strains allows the formation of a heterokaryon between these strains. The identity of the two adenine requiring mutations ad-3A and ad-3B was determined, where necessary, by heterokaryon tests with ad-3A and ad-3B tester strains (see Delange and Griffiths, 1975).

Procedures

Crosses were performed by the simultaneous inoculation of two strains of opposite mating type (A and a) into 18 x 150 mm test tubes containing 5 ml liquid crossing medium and a strip of filter paper (Newcombe and Griffiths, 1972). All crosses were incubated at 25°C.

Ascospore analysis was usually performed by the isolation of individual ascospores and testing of the resulting cultures. To detect ad+ recombinants among the progeny from a cross between leu-3, a, arg-1, ad-3B and A, ad-3A, ascospores were plated on solid medium supplemented with leucine and arginine. The resulting colonies were transferred from the plates to slants of vegetative medium supplemented with leucine and arginine, and tested for their leucine and arginine requirements. Some wild type (PWT) cultures were presumably the result of nondisjunction.
of LG I. Only the *leu*, *arg*, *ad* recombinants were further used.

The *ad-3B* mutation in strain 917A36 has been reverted in one experiment. A conidial suspension of strain 917A36 was irradiated with UV at $5 \times 10^3$ ergs/cm$^2$ for 30, 60, or 90 seconds, and the irradiated conidia were plated on medium supplemented with leucine and arginine. The *ad* revertant colonies were crossed with an *ad-3A* mutant strain, and the resulting *leu*, *arg*, *ad* ascospore isolates were used to test for mutant (ascospore abortion) phenotype.

The observation of linear asci, and other routine manipulations have been described previously (Davis and deSerres, 1970; Chapter I).

**RESULTS**

Strain P917 is a self-fertile (*A + a*) heterokaryon consisting of two nuclear types, each having several complementing mutations on LG I (see Fig. 1). Upon selfing (a cross between the *a* and *A* nuclei of this heterokaryon), this strain produced about 60% aborted white ascospores.

**Is the Ascospore Abortion of P917 Caused by a Dominant or a Recessive Factor?**

To determine whether a dominant or recessive factor caused ascospore abortion in P917, it was crossed with wild type strains OR-*A* and OR-*a*. The absence of aborted spores from crosses with both wild type strains suggested that abortion was caused by a recessive rather than a dominant factor. However, the analysis of isolates from these crosses was, paradoxically, more consistent with the presence of a dominant mutation on LG I (see Table I). In backcrosses with P917, all
Table I. Analysis of isolates* from crosses between P917 to OR-A and OR-a wild type strains

<table>
<thead>
<tr>
<th>Cross</th>
<th>Genotype LG I</th>
<th>Number of progeny</th>
<th>% ascospore abortion when crossed with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P917</td>
<td>917A36†</td>
</tr>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P917</td>
<td>±, a</td>
<td>20</td>
<td>0-10</td>
</tr>
<tr>
<td></td>
<td>x un, ad, nic, al A</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>OR-a</td>
<td>un, ad, al A</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>ad, nic, al a</td>
<td>1</td>
<td>--</td>
</tr>
</tbody>
</table>

| (b)   |                        |                   |                                        |                                        |
| P917  | ±, A                   | 11                | 0-10                                   | 0-10                                  |
|       | ±, A                   | 7                 | 90                                     | 90                                     |
| OR-A  | arg, ad A              | 3                 | --                                     | --                                    |
|       | leu, a                 | 4                 | 0-10                                   | --                                    |
|       | leu, arg a             | 1                 | 0-10                                   | --                                    |
|       | leu, arg, ad a         | 12                | 60                                     | 60                                     |

* Recombinants between ad and al were not tested.
† 917A36 is an isolate from cross (b) of genotype leu, arg, ad, a.
‡ 917a38 is an isolate from cross (a) of genotype un, ad, nic, al, A.
20 wild type (+) a isolates from the cross between P917 and OR-a produced only black ascospores, and all 18 un, A, ad, nic, al isolates produced about 60% ascospore abortion. The abortion phenotype apparently segregated with the un, A, ad, nic, al chromosome (LG I). Similarly, in the cross between P917 and OR-A, the abortion phenotype segregated with the leu, a, arg, ad chromosome (LG I). The abortion of ascospores from these crosses could not have been due to the selfing of P917 since crosses with either P917 or the ascospore isolate 917a38 produced the same amount of ascospore abortion. The 90% ascospore abortion observed in 7 wild type (+) A isolates was later attributed to a separate recessive point mutation (asc-7; see Chapter I). The abortion factor on the leu, a, arg, ad chromosome could be located to a small region spanning the centromere of LG I, since leu, a and leu, a, arg crossover products did not produce any aborted ascospores when crossed with P917 (the centromere of LG I is located between arg-1 and ad-3). Since the mutation causing about 60% ascospore abortion is located in a well-marked region of LG I, it is unlikely that the same mutation is present in both the un, A, ad, nic, al and leu, a, arg, ad components of strain P917. These observations are consistent with a mutation located near the centromere of LG I, which either causes ascospore abortion in dominant fashion (in P917) or has no effect (in crosses between P917 and OR-A or OR-a).

Nature of Defect

To further determine the nature of the defect leading to ascospore abortion, a cross between the two ascospore isolates 917A36 (leu,
a, arg, ad) and 917a38 (un, A, ad, nic, al) was analyzed. About 60% of ascospores produced by this cross were aborted. Table II shows that recombination and nondisjunction (PWT) frequencies were normal. However, only one of the two homologous parental chromosomes (leu, a, arg, ad) was recovered from this cross. In addition, reciprocal crossover products were only detected in the arg-nic region. Consequently, a small region or gene in the arg-nic region on the un, A, ad, nic, al chromosome cannot be recovered when crossed with strain 917A36 (the leu, a, arg, ad chromosome). These data suggested the involvement of three closely linked genes which may be alleles. A gene on the leu, a, arg, ad chromosome apparently caused the death of ascospores containing a second gene which was located on the un, A, ad, nic, al chromosome. Both genes were located near the centromere of LG I. A third gene, presumably also on LG I, was not affected by the killing of the first gene, nor was it capable of killing the second gene.

Identity of Gene Sensitive to Killing Action

To locate the sensitive gene more precisely within the arg-nic region, all crossover products in this region were tested for the presence of ad-3A and ad-3B mutations. All these products were ad-3B rather than ad-3A. Apparently, the sensitive gene was located near or at the ad-3A locus.

6 This cross was used rather than a P917 selfing, to ensure that only two nuclear components of known genotype were involved. P917 may contain a small proportion of contaminating nuclei, e.g., produced by somatic crossing-over (Pittenger and Coyle, 1963).
Table II. Genotype of 79 isolates from the cross between strains 917A36 and 917a38 (for LG I markers, see Fig. 1) which produced about 60% aborted ascospores

<table>
<thead>
<tr>
<th>LG I Markers</th>
<th>Genotype</th>
<th>Number Ascospore Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leu, arg, ad</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>un, ad, nic, al</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Crossover:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leu-un</td>
<td>arg, ad</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>leu, un, ad, nic, al</td>
<td>0</td>
</tr>
<tr>
<td>arg-nic</td>
<td>un, ad</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>leu, arg, ad, nic, al</td>
<td>4</td>
</tr>
<tr>
<td>nic-al</td>
<td>leu, arg, ad, al</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>un, ad, nic</td>
<td>0</td>
</tr>
<tr>
<td>Double Crossover:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leu-un/nic-al</td>
<td>arg, ad, al</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>leu, un, ad, nic</td>
<td>0</td>
</tr>
<tr>
<td>arg-nic/nic-al</td>
<td>un, ad, al</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>leu, arg, ad, nic</td>
<td>1</td>
</tr>
</tbody>
</table>

RF in Wild Type Crosses

RF (leu-un) = 13/79 (16%)  11-17
RF (un-arg) = 0/79 (0%)    15-20
RF (arg-nic) = 12/79 (15%) 30-35
RF (nic-al) = 32/79 (40%)  

Nondisjunction Frequency = 0/79 (0%)
To test the possibility that the ad-3A mutation itself could not be recovered in these crosses, 19 independently derived ad-3A alleles were crossed with strain 917A36. As a control, five complementing ad-3B mutants and seven wild type strains were also crossed with 917A36. Crosses with each of the 19 ad-3A alleles resulted in 50-60% ascospore abortion; in contrast, abortion was absent in the remaining crosses. Thus it appeared that only adenine requiring ad-3A mutations could not be recovered when crossed to the "killer" leu, a, arg, ad chromosome.

If all ascospores containing ad-3A were inviable when crossed with strain 917A36, which contains the leu, a, arg, ad chromosome, most asci produced from this cross should contain four black and four white ascospores (4B:4W). In fact, 54 out of 56 asci were of this type; the remaining two were 2B:6W. Since ad-3A is closely linked to the centromere, a low frequency of second division segregation of white and black ascospores would be expected. Again, only 2 out of 54 asci had a second division pattern of segregation of black and white ascospores.

It was concluded that a mutation on the leu, a, arg, ad chromosome caused ad-3A-containing ascospores to abort. This mutation will be referred to as spore killer of ad-3A, or SK(ad-3A).

Location of the Newly Induced Spore Killer SK(ad-3A) Mutation

In order to locate SK(ad-3A) on LG I, 253 isolates from the cross between 917A36 (leu, a, arg, ad-3B, SK(ad-3A)) and a wild type strain (FGSC 1228, which is ad-3A+ and therefore resistant to SK(ad-3A) action) were tested for the killer character. No recombinants between SK(ad-3A) and ad-3B were recovered. Thus, SK(ad-3A) was linked very
closely to ad-3B.

To show that ad-3B was not required for the killing of ad-3A-containing ascospores, the ad-3B mutation in strain 917A36 was reverted. The killing action of leu, a, arg, ad+ revertants clearly demonstrated that SK(ad-3A) was still present and acted independently of the ad-3B mutation.

A more precise localization of SK(ad-3A) with respect to the ad-3A and ad-3B loci was obtained by isolating ad+ recombinant progeny from crosses between 917A36 (leu, a, arg, ad-3B, SK(ad-3A)) and two strains containing alleles 2-17-814 or 2-17-825 of ad-3A. Four crossover products of genotype leu, a, arg, ad+ were obtained (Fig. 2). All four recombinants contained the SK(ad-3A) mutation. Therefore, since SK(ad-3A) is very closely linked to ad-3B (0/253 recombinants), but nearer ad-3A than ad-3B, it was concluded that SK(ad-3A) and ad-3A are very tightly linked and may be alleles of the same gene.

Other Characteristics of ad-3A and SK(ad-3A) Mutations

The only known effect of the SK(ad-3A) mutation is its killing action on ad-3A-containing ascospores. Thus, crosses homozygous for the SK(ad-3A) mutation produce only black ascospores. In addition, SK(ad-3A) cultures grow at wild type rates and do not require adenine for growth.

To determine whether or not ad-3A-containing conidia could be obtained from vegetative heterokaryons between ad-3A and SK(ad-3A), several heterokaryons between strain 917A36 (leu, a, arg, SK(ad-3A), ad-3B) and 2-17-825a (a, ad-3A) were allowed to grow in 50 cm race tubes.
Fig. 2  Selection of $\text{ad}^+$ recombinants to localize the $\text{SK(ad-3A)}$ mutation with respect to the $\text{ad-3A}$ and $\text{ad-3B}$ loci.
centromere

leu-3  a  arg-1  +  ad-3B

+  A  +  ad-3A  +

0.3 mu
These heterokaryons grew at a rate comparable to several heterokaryons between ad-3A and SK(ad-3A). Conidia from the beginning and the end of one race tube were isolated and their genotypes were tested. The ad and leu, arg, ad genotypes were recovered with approximately equal frequency (Table III). Thus, the SK(ad-3A) mutation does not appear to affect the viability of ad-3A-containing conidia.

DISCUSSION

The ad-3A mutation in Neurospora crassa behaves as a recessive ascospore lethal, i.e., crosses homozygous for ad-3A produce mainly inviable unpigmented ascospores, but most ascospores from a cross between ad-3A and its wild type allele ad-3A⁺ are pigmented and viable. This chapter reports the isolation of a mutation which, when paired in a cross with an ad-3A mutant, causes the abortion of ad-3A-containing ascospores. The new mutation was called spore killer of ad-3A, or SK(ad-3A), and was found to be located at or very close to the ad-3A locus. Both this close proximity of SK(ad-3A) to the ad-3A locus and its specific effect on the viability of only ad-3A-containing ascospores strongly suggest that this new mutation is an allele, control or structural, of the ad-3A locus. Thus, depending on which allele ad-3A is crossed with, it may act as a recessive or an autonomous ascospore lethal. While many cases of both types of lethality have been previously described, both types have never before been associated with the same mutation.

The lethality of the ad-3A mutations superficially resembles some cases of segregation distortion which involve the lethality of meiotic
### Table III. Analysis of conidial isolates from a heterokaryon between strain 917A36 (*leu*-3, *a*, *arg*-1 SK(*ad*-3A), *ad*-3B) and 2-17-825a.(*a*, *ad*-3A)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Beginning of Race Tube</th>
<th>End of Race Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK *</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td><em>leu</em>, <em>arg</em>, <em>ad</em></td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td><em>ad</em></td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

*Wild type due to complementation of the two nuclear types.*
products. For example, the lethality of $\text{Sd}^+$ in *Drosophila* (Hartl and Hiraizumi, 1976), $\text{SK}^5$ in *Neurospora* (Turner and Perkins, 1976), and "1" in *Ascobolus* (Makarewicz, 1966), depends on the other allele of these loci and on the genetic background in the meiocytes. Since meiocytes that are homozygous for these alleles produce only viable products (ascospores or sperm), lethality appears to be caused by the interaction of two different alleles at a particular locus. In contrast, meiocytes that are homozygous for $\text{ad-3A}$ produce mainly inviable ascospores. Therefore, the lethality of $\text{ad-3A}$-containing ascospores is apparently the result of a deficiency in these ascospores.

Assuming that $\text{SK(ad-3A)}$ is an allele of the $\text{ad-3A}$ locus, lethality of $\text{ad-3A}$-containing ascospores would be caused by a failure of the altered $\text{SK(ad-3A)}$ gene or gene product to complement the deficiency in these ascospores. Since complementation is normal in vegetative heterokaryons, the reduced ability to complement the deficiency is restricted to the ascus. Several mechanisms for such reduced complementing ability of the $\text{SK(ad-3A)}$ gene can be visualized.

The mutation $\text{SK(ad-3A)}$ could be within the structural $\text{ad-3A}$ gene, and produce an altered polypeptide with a differentially low activity in the ascus and ascospores. Such reduced activity might affect the maturation of $\text{ad-3A}$-containing ascospores if a specific threshold level of activity would be required prior to ascospore enclosure. This threshold level would not be easily reached in the case of the relatively inactive $\text{SK(ad-3A)}$ gene product. A similar model has been proposed to explain some irregular features of segregation distortion in *Drosophila* (Miklos and Smith-White, 1971).
The lost ability of SK(ad-3A) to complement ad-3A in the ascus could also be caused by changes in the control of enzyme synthesis, its modification, stabilization, or an altered property of transfer through the cytoplasm in the ascus. The mutation cys-3 (Murray, 1965) in Neurospora may be an example of a defect in transfer in asci. It lacks a permease, and is the only cysteine-requiring mutation which produces mainly light inviable ascospores.

The SK(ad-3A) mutation represents a unique situation which may enable the study of different processes in the development of ascospore enclosure and maturation. In more general terms, this situation resembles the critical step of determination in the development of two types of cells (here ascospores) from a single type. Therefore, the study of this process may contribute to the understanding of the process of differentiation of cell types during development in multicellular organisms. Such analysis in this system is facilitated by the knowledge of several components. First, ascospore abortion can not be due to a lack of adenine in the ascospores because abortion has not been associated with other adenine requiring mutants (e.g., Ishikawa, 1962). Abortion may be indirectly caused by the ad-3A mutation. For example, the accumulation of an intermediate (AIR) in the purine synthetic pathway in ad-3A mutants may be the direct cause of abortion (Fisher, 1969a). Mutants in the same enzyme in Schizosaccharomyces show a correlation between the accumulation of the polymer of AIR, the accumulation of red pigment, and a slight decrease in growth rate. Both the red pigmentation and reduced growth rate can be eliminated by a secondary mutation located in the purine pathway before the
formation of the intermediate AIR (Gutz et al., 1974). These findings suggest that similar secondary mutations should be able to suppress the ascospore lethality, if the accumulation of AIR polymers is the direct cause of the lethality. Second, the enzyme involved in the primary defect has been partially purified and characterized (Fisher, 1969b). Therefore, any altered properties of the enzyme in the SK(ad-3A) mutant could be determined. Finally, genetic means of analysis are available. For example, more detailed mapping of the SK(ad-3A) mutation may locate it within the control or structural part of the ad-3A locus. In addition, mutations which interact with SK(ad-3A) to modify the abortion phenotype should be readily obtainable.
CHAPTER IV

GENERAL DISCUSSION
The previous chapters have described the development and successful use of a system in *Neurospora crassa* which facilitates the isolation of recessive mutants with a defect in ascus or viable ascospore formation. Subsequent cytological and genetic observations have shown that some of these mutants have their defect during meiosis. These meiotic mutants are of particular interest to the study of the genetic control of meiosis, and may ultimately help in gaining a complete understanding of the molecular processes that control meiosis.

**Genetic Control of the Sexual Cycle (Including Meiosis) in *Neurospora crassa***

The sexual cycle in *Neurospora* involves the development of perithecia, meiosis, and the formation and maturation of ascospores. Mutants with defects in each of these events have been detected during this study. These mutants have already contributed to our understanding of the genetic control of the sexual cycle in *Neurospora*. Thus, it was shown that the development of perithecia is primarily controlled by genes of the maternal parent. In addition, the study of mutants with an apparent defect in the maturation of ascospores suggests that the reaching of a threshold amount of a particular substance within the ascus (*asc-5*; Chapter I), or within each nucleus (*SK(ad-3A)*; Chapter III) was essential for the maturation of ascospores. Finally, the study of two types of mutants has provided some insight into the control of meiotic divisions in *Neurospora*. Thus, the apparent primary
defect in pairing and exchange in mutants asc-1, asc-6, and mei-1 appears to be followed by the equational centromere division of many or all univalents produced during the first prophase. A second type of mutant (asc-3) with a partial block before karyogamy, has defective attachment of chromosomes to one of the two spindle pole bodies of the second meiotic division. These observations may mean that a product produced before karyogamy (possibly during the pre-meiotic S phase) later facilitates the attachment of one set of chromatids to one of the spindle pole bodies (some possible models have been described in the discussion of Chapter II).

Even though, at this stage, the information obtained from these mutants is speculative, the use of such mutants appears quite promising in Neurospora. Future research should involve the isolation of temperature-sensitive mutants, and of mutants that interact to change the phenotype of existing mutants. In addition, the identification of the molecular nature of the defect of mutants with cytologically and genetically defined defects may become possible through the use of electron microscopy or biochemistry (as was previously mentioned in the Introduction).

**Isolation of Temperature-Sensitive Mutants**

Temperature-sensitive mutants may be instrumental in determining the temporal period during which their normal product is active, and in identifying that product. Such meiotic mutants have already been obtained in Drosophila (Grell, 1978) and yeast (Roth, 1976; Esposito and Esposito, 1974). In the absence of a direct selection method for these mutants, large numbers of PWT cultures should be
screened for temperature-sensitive defects. However, the isolation of many PWT cultures is still quite time-consuming because of the low PWT frequency of the cross used to isolate these cultures.

The present selection system may be improved in several different ways, all involving crosses with increased PWT frequencies. The nature of the selection system for recessive meiotic mutations requires that the PWT cultures should be primarily disomic for LG I only. Because of the high frequency of multiple disomies obtained from crosses homozygous for asc-3, asc-6 or mei-1, PWT cultures from these crosses are not useful. However, some crosses homozygous for asc-1 with an intermediate PWT frequency (about 5%) might be of use if the nondisjunction frequency of chromosomes other than LG I is also low. Since crosses homozygous for this mutation usually produce about 40% ascospore abortion, it may not be possible to isolate certain types of mutations similar to asc-1 and asc-6 (the double mutant between asc-1 and asc-6 is indistinguishable from asc-1 and asc-6 separately). To overcome this difficulty, several other systems may be used.

First, PWT progeny may be obtained from a cross heterozygous for the dominant meiotic mutant Mei-2 (Smith, 1975). Since crosses involving Mei-2 produce about 40% ascospore abortion, only PWT progeny containing Mei-2 should be selected and tested. This may be achieved by selecting against an auxotrophic mutation closely linked to the Mei-2 locus. The success of this system depends on the frequency of PWT progeny that can be obtained.

Second, a mutant with a temperature-sensitive defect leading to PWT formation would be extremely useful. PWT cultures could be obtained
from crosses at one temperature and tested for mutants at the other

Third, the frequency of PWT progeny can be increased markedly
with the use of the chemical p-fluorophenylalanine (Griffiths and

**Interacting Mutations**

It may be anticipated that mutations which interact with exis­
ting meiotic mutations will become partially instrumental in the un­
derstanding of meiotic development and its control. In the past,
interaction-type mutants have indeed been quite useful. A relevant
example is the study of recombination in *E. coli*. First, suppressors
*sbc A* and *sbc B* of the recombination-defective mutants *rec B* and *rec C*
have inactive exonuclease VIII and exonuclease I, respectively
(Barbour and Clark, 1970; Kushner et al., 1972). Second, four mutant
genes result in recombination deficiency in the reverted *rec B rec C*
*sbc B* strain (Horii and Clark, 1973). These interaction mutants have
provided some insight into the alternative means (or pathways) of re­
combination in *E. coli*. In this manner, three pathways have been de­
tected (Clark, 1974). Although many other examples of interacting
mutants have been instrumental in the understanding of cellular pro­
cesses, the example of recombination control in *E. coli* is most
closely relevant to the control of recombination and meiosis in eu­
karyotic organisms. Therefore, it should be anticipated that similar
studies in several eukaryotic organisms (e.g., *Neurospora*) may enable
the successful dissection of recombination and meiotic processes.
Second-site reversions may be dominant or recessive. It will be virtually impossible to obtain recessive suppressor mutations of sterility mutants in *Neurospora* (e.g., asc-2, asc-4, mei-3, uvs-3, uvs-5, uvs-6) because PWT cultures needed to screen for such mutations cannot be obtained from crosses homozygous for sterility mutations. However, the isolation of revertants would be possible in conditional (e.g., temperature-sensitive) mutants: PWT cultures could be obtained at one temperature and the screening would be performed at the restrictive temperature.

The isolation of revertants of mutations with a defect in pairing or exchange, and therefore chromosome disjunction, is potentially possible, since PWT frequencies in such mutants are usually high. The isolation of dominant revertants would be even simpler because mutagenized conidia, instead of PWT cultures, can be screened. In addition, variants obtained from nature or in existing laboratory stocks may also modify the phenotype of meiotic mutations. The high frequency of black ascospores obtained in some crosses homozygous for asc-3 (P393) may possibly be an example of this.

Finally, mutations that interact with the spore killer mutation *SK(ad-3A)* should be readily obtained. Since the mutation *SK(ad-3A)* apparently causes a defect in complementation, it is quite plausible that certain other components interact to achieve such complementation. In effect, alteration of one or more of these interacting components could act as a suppressor of *SK*, and thus restore the ability of *SK* to complement *ad-3A* mutations. The study of such interaction mutations may provide insight in the developmental steps involved.
In this case, detection of suppression may be a fast procedure since thousands of asci could be quickly scored for the presence or absence of mostly black ascospores.

Potential of Future Studies on the Molecular or Micro-Structure Level

The ultimate understanding of meiotic development necessarily involves biochemical characterization. Because of the asynchrony of development of asci and their attachment to vegetative cells, such analysis is presently not possible in *Neurospora*. However, some genetic manipulation and development of techniques that allow the separation of asci from surrounding cells should largely overcome this problem. The partial synchronization of meiotic development has been achieved by temperature shock in *Coprinus lagopus* (Lu, 1974; Lu and Jeng, 1975), or by a temporary inhibition of DNA synthesis in *Schizophyllum commune* (Carmi et al., 1978). The same may possibly be achieved by certain mutations, e.g., Ban in *Neurospora*, which causes alternate waves of meiotic and mitotic cycles (Raju and Newmeyer, 1977).
BIBLIOGRAPHY


APPENDIX

LIST OF ABBREVIATIONS

asc  Recessive mutation resulting in the abortion of asci and/or ascospores.
CO   Crossover.
HK   Heterokaryon or heterokaryotic.
HN₂  Nitrogen mustard.
LG   Linkage group.
MI   First meiotic division.
MII  Second meiotic division.
mei  Meiotic mutation.
MMS  Methyl methane sulphonate.
MNNG N-methyl N-nitro N-nitrosoguanidine.
PWT  Pseudo-wild type.
RF   Recombinant frequency.
SK   Spore killer mutation.
SPB  Spindle pole body.
UV   Ultraviolet.
PUBLICATIONS


A.M.DeLange, 1980. The mutation Sk(ad-3A) alters the dominance of ad-3A+ over ad-3A in the ascus of *Neurospora*. (Submitted).