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Evidence for Separate Genetic Control of Crossing Over and Chiasma Maintenance in Maize

Marjorie P. Maguire

Zoology Department, University of Texas, Austin, Texas 78712, U.S.A.

Abstract. The meiotic cytological behavior of chromosomes in maize microsporocytes homozygous for the recessive mutant *desynaptic* was studied at various stages. It was found that following apparently normal pachytene synapsis there appears to be sporadic precocious desynapsis. By diakinesis bivalents heterozygous for a distal knob have often separated to pairs of univalents, each with a knob-carrying and a knobless chromatid. From the frequency of such events it is inferred that the crossover process is probably not affected by the mutant and that the genetic defect affects instead a distinct function concerned with chiasma maintenance following crossing over. Since precocious separation of dyads to monads at prophase II was also found in the *desynaptic* material, it is suggested that normal chiasma maintenance until anaphase I and normal dyad integrity maintenance between anaphase I and anaphase II may depend upon the same mechanism; it is also suggested that this may involve a special tendency for cohesiveness of sister chromatids during meiosis, beyond that which is ordinarily found at mitosis.

Introduction

Two major contributions of meiosis in most organisms to the adaptive value of sexual reproduction are usually attributed to the process of crossing over. These are: (1) provision for recombination of genes located together (linked) on the same chromosome, and (2) provision for the orderly distribution of homologues to opposite poles at anaphase I. Accumulated evidence seems compelling that crossovers are generally accompanied by chiasma formation and that (at least) one persistent chiasma per bivalent will normally serve the function of assuring orderly homologue disjunction (for review see Whitehouse, 1973). Frequently overlooked are unsolved problems of the nature of chiasma anatomy, for, a simple recombination of homologous non-sister chromatids would probably not be expected to provide the basis for a bond between homologues which

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could hold together through the processes of chromosome condensation, congression and coorientation typical of later meiotic prophase and metaphase I in most organisms. Reports of lasting remnants into diplotene and later stages of short stretches of synaptonemal complex (SC) at possible chiasma sites and elsewhere (Moens, 1969a; Solari, 1970; Sotelo et al., 1973) seem so far to offer scant hope for provision of satisfactory candidates for the missing bond. The long recognized view (Darlington, 1932) which is widely accepted on the basis of many light microscope observations, is that sister chromatids tend to cohere all along their length at later prophase I and metaphase I except in regions where terminalization of chiasmata has brought non-sisters together. Such cohesiveness of sister chromatids appears to be the stuff of chiasma maintenance, but a physical basis for it is unknown. Suggested possibilities for a physical basis for sister chromatid cohesiveness have been summarized (Maguire, 1974) and include: (1) Sister chromatids may be relationally coiled. Such coiling would presumably have to exist prior to synapsis and would present grave difficulties to the deployment of the SC as it is commonly conceived to occur (Westergaard and von Wettstein, 1972). Also, extensive intertwining of sister chromatids is not generally evident in diakinesis bivalents where the four chromatids can be traced. (2) A binding substance may be installed between sister chromatids at the appropriate stage. To my knowledge no direct observational evidence of such a substance has yet been reported although technology adequate for its demonstration may not have been applied. Perhaps the possibility that the lateral element of the SC may somehow represent a potential precursor should be explored. (3) Unreplicated segments of DNA may still exist at this stage so that sister chromatids are, as a result, joined at a series of points. Hotta and Stern (1976) have reported that in *Lilium* small portions of DNA still unreplicated at early zygotene do not in fact become replicated until after pachytene. If sister chromatids remain segmentally unreplicated until very late and are segmentally joined for this reason, the junctions are expected to be subtle; in fact, visualization of four chromatids throughout bivalents at diplotene and diakinesis at light microscope level commonly seems clear. Although both light and electron microscope resolution sometimes reveal fine connections within bivalents, similar connections are also seen between bivalents, and their nature is not understood.

In view of the unsatisfactory current status of understanding of chiasma maintenance mechanism, it seems reasonable to resort to a search for less direct evidence of its existence. It can be argued that perhaps after all, the stresses presumed to be imposed upon bivalents during diplotene, diakinesis and metaphase I are not so severe as to separate homologues with crossovers, and perhaps the appearance that chiasmata offer considerable resistance to separation of homologues at early anaphase I is somehow artifactual. But if a specific function to provide for chiasma maintenance normally exists independently of functions concerned with crossing over, then genetic lesions (mutations of this function) might reasonably be expected to occur. A number of mutants generally termed *desynaptic* have been described in various organisms (Baker et al., 1976); these characteristically show normal or near normal synapsis where pachytene is amenable to study, but irregular, abnormal separation of bivalents

to univalents characteristically occurs throughout diplotene, diakinsis and meta phase I, and this is followed by irregular chromosome distribution and variable sterility. Studies of recombination frequency in the progeny of organisms displaying these mutant phenotypes are complicated by possible, but not readily measurable, dependence of normal chromosome distribution and resulting viable gamete production on relatively normal occurrence of crossing over (Moens, 1969b). Hence it has not been feasible to determine whether these mutants represent an intrinsic defect of chiasma maintenance. In this report a *desynaptic* mutant of maize is described in which crossover and non-crossover products for a specific chromosome region can be directly observed cytologically in diakinesis univalents. Evidence is presented that chiasma maintenance *following crossing over* fails in microporocytes homozygous for this mutant. This *desynaptic* mutant is therefore thought to represent a true lesion of chiasma maintenance function, and thus to provide evidence for the existence of such a function distinct from crossing over functions.

Materials and Methods

The recessive meiotic mutant *desynaptic* was first described by Nelson and Clary (1952) and to my knowledge has not been studied further until this work. Nelson and Clary reported that in microsporocytes of plants homozygous for this mutant pachytene stage was normal but that bivalents separated to univalents with variable frequency during diplotene and diakinesis; many of the persisting bivalents were rod-shaped. At anaphase I univalents either oriented amphitelically, late, so that their chromatids were distributed to opposite poles, or univalents were variously scattered without division and could be included in a daughter nucleus intact for an equational second division. Variable pollen sterility resulted from the irregular meiotic chromosome distribution. It was not known whether the precocious separation of bivalents was due to failure of crossing over to occur, so as to initiate chiasma formation, or to early loss of chiasmate association following crossing over. It was inferred that megasporocyte meiosis was unaffected by this mutant since plants homozygous for it showed no female sterility, and trisomes failed to appear in the progeny. It is interesting to note that this mutant seems to affect only male meiosis in maize where, unlike *Drosophila melanogaster*, synapsis and crossing over occur normally in both male and female meiosis. Reasons for the differential effect with respect to sex are unknown and must be fundamentally more complex than those proposed for differential effects of meiotic mutants of Drosophila in the two sexes (Baker et al., 1976).

The mutant described above was designated *desynaptic* (*dy*) by Nelson and Clary. Its chromosome location remains unknown. A stock carrying it is maintained by the Maize Genetics Coop, from whom heterozygous and homozygous seeds were obtained for the present study.

Microsporocyte samples were collected from *Dy dy* and *dy dy* plants, fixed in ethanol-acetic 3:1 mixture and stored in a freezer until observed in acetocarmine squash preparations by light microscopy. Similar samples were fixed in glutaraldehyde and osmium tetroxide, stained with uranyl acetate, embedded in plastic and thin-sectioned for electron microscope observation of pachytene cells. Preparation techniques used for material for electron microscope observations are described in detail by Riess and Biesele (1972).

Results and Discussion

Pachytene cells from *dy dy* plants appeared normal with light microscope resolution at early to mid-stage (Fig. 1a), but sporadically showed varying degrees

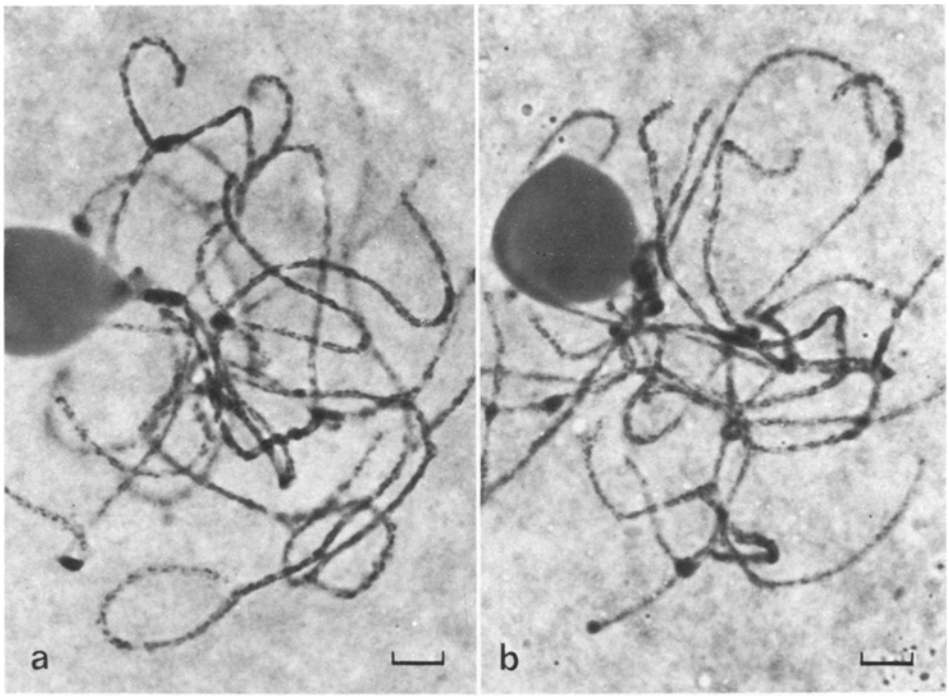

Fig. 1a and b. Photomicrographs of microsporocytes at mid-pachytene. **a** Cell from *dy dy* plant. **b** Cell from *Dy dy* plant. Note normal synapsis in both. Magnification bars represent 5 μm

of homologue separation at later pachytene, from more advanced portions of the tassel (Fig. 2). In some cases homologue separation was apparent but slight, and relational coiling of homologues was evident (Fig. 2b, c). Similar desynaptic configurations are seen at pachytene after ethanol treatment of genetically normal maize microsporocytes (Maguire, 1976). With electron microscope observation of thin-sectioned *dy dy* pachytene cells many apparently normal stretches of SC were found (Fig. 3a), and no convincing evidence of abnormal SC structure was seen. It is inferred that in *dy dy* microsporocytes the SC is normally formed but probably tends to be lost early in erratic fashion. Pachytene cells from *Dy dy* plants appeared normal (Fig. 1b, 3b).

During diplotene in *dy dy* microsporocytes bivalents showed irregular chromosome condensation, compared to the same stage in *Dy dy* microsporocytes, and sporadic loss of association of homologues (Figs. 4, 5). At diakinesis variable numbers of univalents and rod bivalents were found, and the univalents were distributed at anaphase I as described by Nelson and Clary. At prophase II precocious dissociation of dyads to monads was also observed (Fig. 6). The latter observation suggests a possible relationship between normal chiasma maintenance until anaphase I and maintenance of normal dyad integrity between anaphase I and anaphase II; it is conceivable that both functions normally rely upon the same basic mechanism and that the *dy* mutant represents a lesion of this mechanism. A consistent speculation is that the mechanism in both

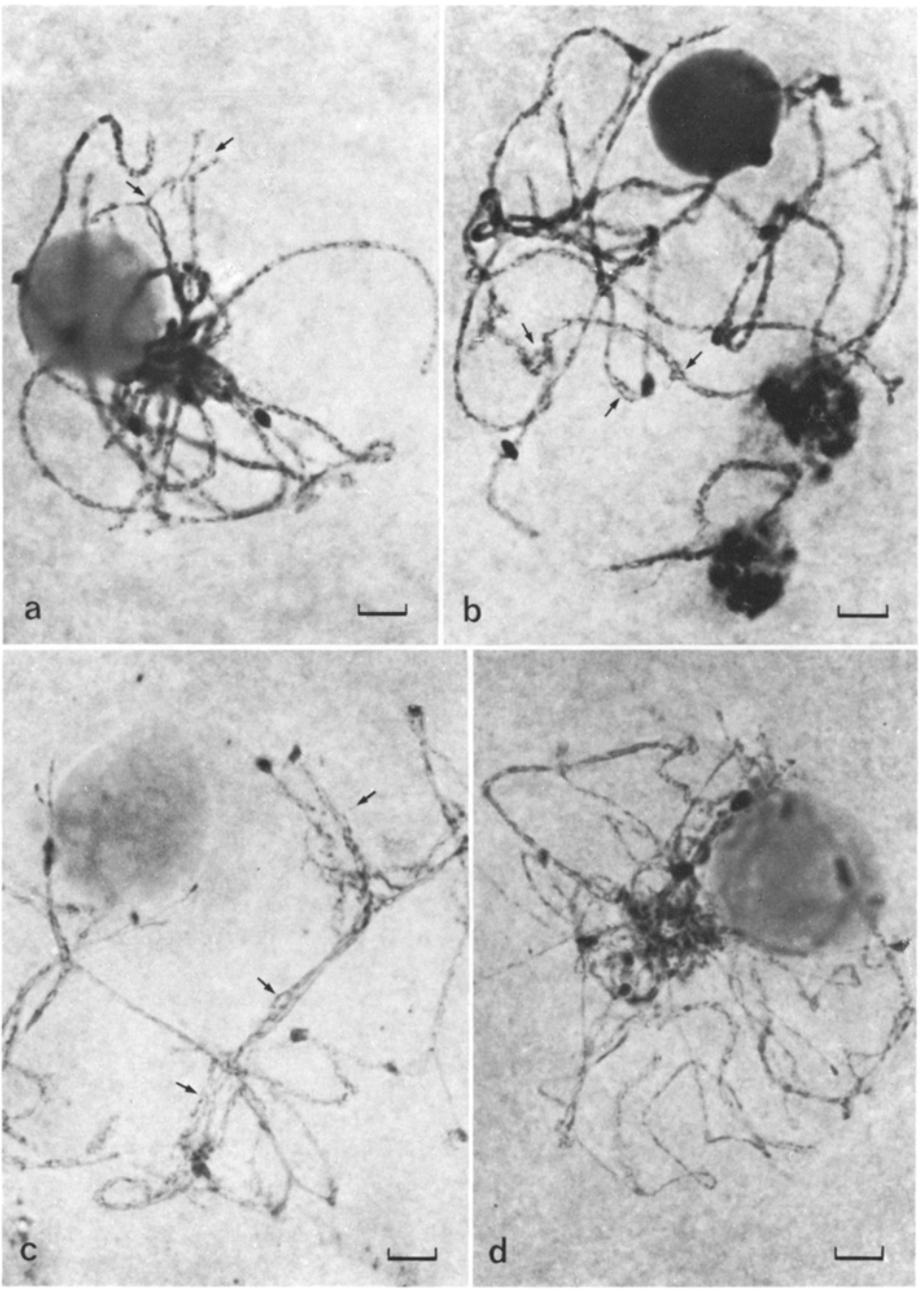

Fig. 2a–d. Microsporocytes from *dy dy* plant at later pachytene. **a** Short segments with synaptic failure. **b** More extensive segments of loose homologue pairing within bivalents. **c** Generalized loose homologue association within bivalents, relational coiling of homologues apparent in some regions. **d** Loose homologue association in some regions. Arrows mark regions of particular interest. Magnification bars represent 5 μm

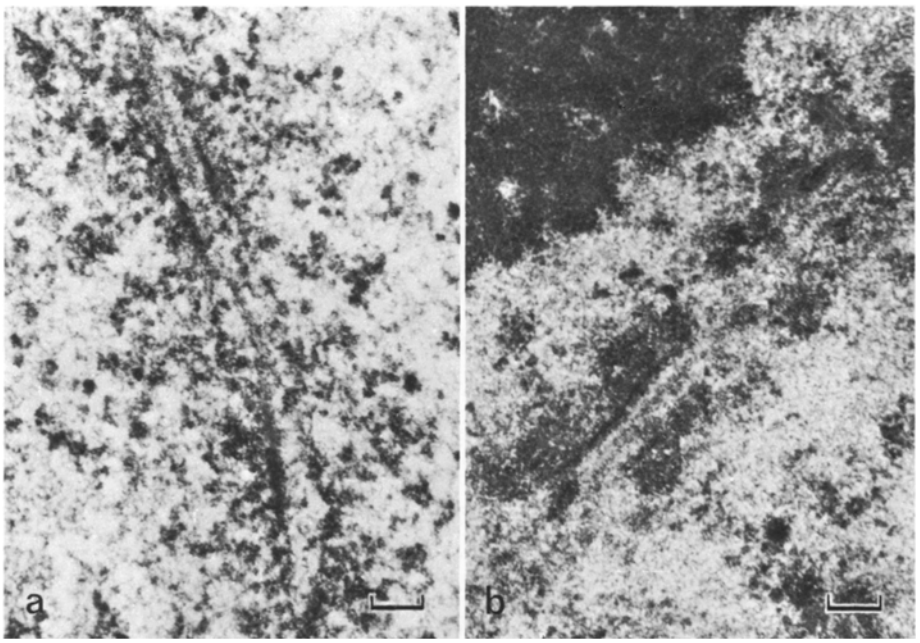

Fig. 3a and b. Electron micrographs of sections of microsporocytes at pachytene showing apparently normal SC structure. **a** From *dy dy* plant. **b** From *Dy dy* plant. The bivalent segment in **b** happens to be from a region of more condensed chromatin than that in **a**. Magnification bars represent 1560 Å

cases depends upon sister chromatid cohesiveness. It is almost axiomatic that the first division is virtually universally disjunctive in the centromere region in normal material and that the two chromatids of each dyad remain associated in the centromere region from anaphase I until anaphase II, although the four arms commonly appear widely separated distally. Crossing over is often strongly inhibited in regions adjacent to centromeres, and a commonly observed dyad type could result from one crossover per chromosome arm and would contain non-sister chromatids for regions distal to each such crossover. However, in dyads at prophase II in maize the two chromatids usually intersect for a very short segment only (presumably containing the centromere); although differential condensation during development or differential stretching during squash preparation could conceivably contribute to this appearance, it seems unlikely that the boundaries of the cohesive regions usually actually mark the positions of crossovers. Rather, it is probable that cohesiveness tends to be concentrated in more proximal zones (proximalization of association?). Although it has been suggested that centromeres normally remain unreplicated until the second meiotic division and that dyad integrity maintenance results from this, the commonly observed ability of univalents to separate equationally at anaphase I, as well as direct cytological observations of centromere doubleness during the first meiotic division argue strongly against this view (Kezer and Macgregor, 1971); Müller, 1972).

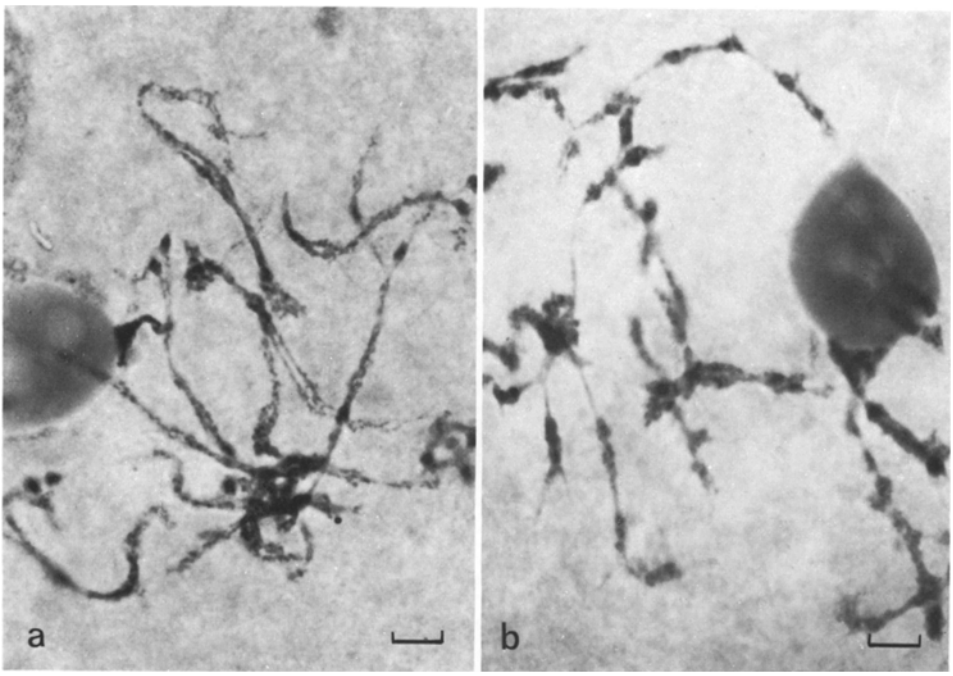

Fig. 4a and b. Microsporocytes from *dy dy* plant at late pachytene to diplotene. **a** Late pachytene or early diplotene (degree of homologue separation more typical of normal early diplotene but chromosome length more typical of late pachytene). **b** Late diplotene with irregular chromosome condensation and failure of chiasmate association. Magnification bars represent 5 μm

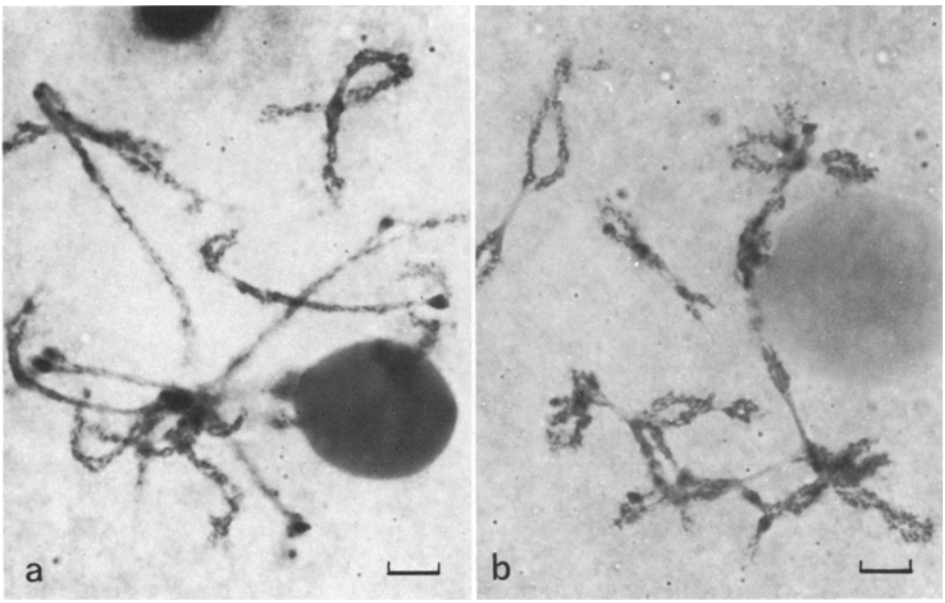

Fig. 5a and b. Microsporocytes from *Dy dy* plant at diplotene. **a** Early diplotene. **b** Late diplotene, bivalents with intact chiasmata. Magnification bars represent 5 μm

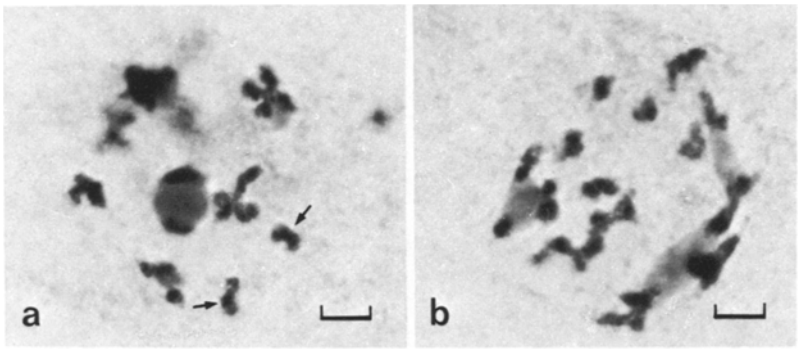

Fig. 6a and b. Microsporocytes from *dy dy* plant at prophase II. **a** One dyad separated to monads (marked by arrows). **b** A number of dyads separated to monads or apparently in the process of separating. Magnification bars represent 5 μ

A special property of maize chromosomes, in addition to their general excellence of meiotic cytology, permits a study of the presence of crossover chromatids in univalent chromosomes at diakinesis. This property is: the apparently optional presence of a variety of (mostly intercalary) heterochromatic regions, called "knobs" in mize. These knobs do not seem to make vital contributions to the metabolism of maize, for stocks vary widely in the repertoire of knobs which they carry, some being virtually knobless; within stocks it does not seem to be readily possible to distinguish phenotypically among plants which are homozygous positive, homozygous negative or heterozygous for a specific knob. Knobs are cytologically observable at pachytene, diplotene and diakinesis, and at prophase in at least some mitotic divisions as well.

In the present study plants of the *dy* carrying Maize Genetics Coop stock utilized were found to be generally homozygous for a number of large knobs but some *dy dy* and *Dy dy* plants were heterozygous for one large knob. The presence of homozygosity or heterozygosity is most easily determined at pachytene or early diplotene in the occasional cell where there is synaptic failure in the knob region (Fig. 7a, b). In these plants the region of knob heterozygosity was identified as the long arm of chromosome 4, on the basis of pachytene chromosome arm ratio, total length and knob position. The position of the knob on the genetic map of the long arm of chromosome 4 is not precisely known, but it is cytologically distal; the known genetic map of this arm comprises more than 44 units, while the true genetic map may well be substantially longer (Neuffer et al., 1968). It is reasonable to expect that a crossover will normally occur between the centromere and the heterozygous knob in most microsporocytes. If such crossing over is followed by failure of chiasmate association, with the resulting formation of univalents (or of a rod-bivalent with the persistent chiasma association present in the short arm), equational separation of knob-bearing chromatids can be easily recognized in appropriately stained cells at diakinesis. Miller (1963) utilized a search for such equational knob-bearing chromatid separation into diakinesis univalents of *asynaptic* maize plants to find that in the presence of that mutant, chromosomes destined to become

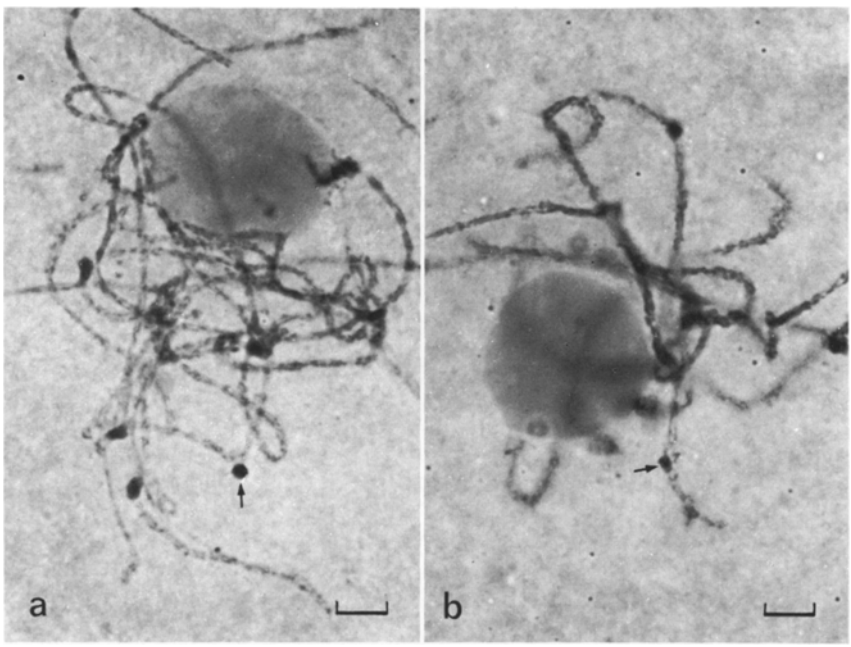

Fig. 7a and b. Microsporocytes showing heterozygosity for a large knob. **a** A *dy dy* cell at mid-pachytene. **b** A *Dy dy* cell at the late pachytene to early diplotene stage (incipient separation of homologues is evident, but note that chromosomes are distinctly shorter than those in *dy dy* cells showing incipient homologue separation in Fig. 2). The heterozygous knob is marked in each cell by an arrow. Magnification bars represent 5 μm

univalents at diakinesis probably have not engaged in crossing over; all univalents observed of *asynaptic* microsporocytes were found to show disjunctional separation of heterozygous knobs at diakinesis.

In contrast to the behavior in *asynaptic* microsporocytes, in the present study, most (31/32) *dy dy* diakinesis microsporocytes (appropriately stained) with dissociation of homologues in the arm with knob heterozygosity, showed equational assortment of knob-bearing chromatids (Fig. 8a); only one (1/32) of these showed disjunctional assortment of knob-bearing chromatids (Fig. 8b), reflecting lack of crossing over between the centromere and the heterozygous knob. This is considered strong evidence that with *dy* phenotypic expression crossing over occurs frequently in chromosome regions which become univalent by diakinesis, i.e. exhibit failure of chiasmate association. In a similar study of diakinesis in *Dy dy* microsporocytes most cells (121/124) showed a persistent chiasma in the vicinity of the heterozygous knob, while three (3/124) showed disjunctional separation of knob-bearing chromatids and absence of a chiasma in the arm heterozygous for the knob. In the two cases, inferred crossover frequencies are equal and consistent with normal expectation of crossover frequency in a chromosome region of the length in question. It therefore seems probable that crossover frequency in the region between the centromere and the heterozygous knob was virtually unaffected by the *dy* mutant phenotypic

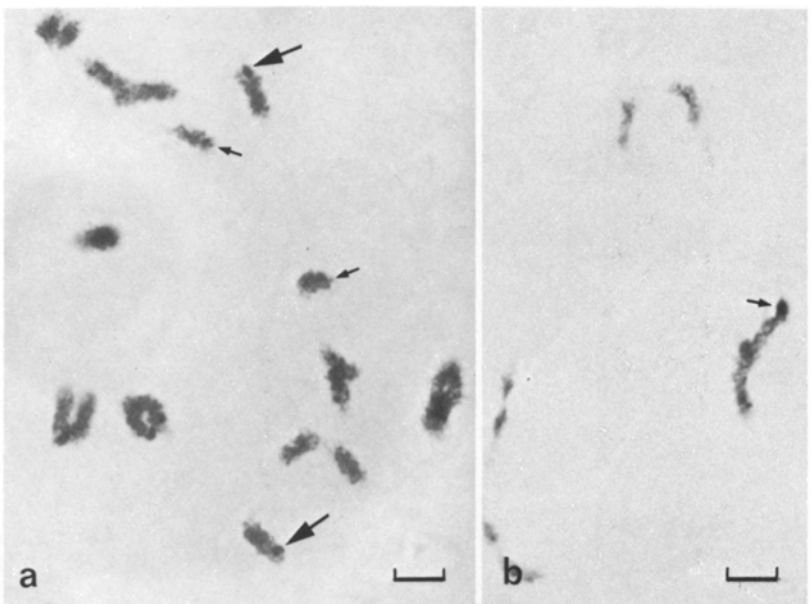

Fig. 8a and b. Microsporocytes from *dy dy* plant at diakinesis. **a** Two bivalents completely separated to univalents. Univalent members of homologous pairs are marked by similar arrows. One of these pairs shows equational separation of the heterozygous knob (arrows) such that each univalent contains a knob-carrying and a knobless chromatid (indicating that crossing over has occurred between the knob and the centromere but that chiasmate association has failed). **b** Part of another, somewhat earlier diakinesis cell from the same plant showing the heterozygous knob carrying bivalent in rod configuration with a persistent chiasma at the end opposite the knob. There is evident disjunctional separation of the knob-carrying chromatids from the knobless chromatids (evidence that crossing over probably did not occur between the knob and the centromere). This is the only case of such disjunctional separation seen. An arrow marks the knob. Magnification bars represent 5 μ

expression and that instead, lesion of chiasma maintenance function following crossing over is symptomatic of *dy* mutant expression. Genetic control of chiasma maintenance therefore seems independent of and separable from crossover control.

Since precocious loss of synapsis appears to be an early expression of the *dy* phenotype, it is tempting to speculate that provision for chiasma maintenance, perhaps the establishment of sister chromatid cohesiveness, may be a late function of the synaptonemal complex. Exploration of this possibility is underway.

It should be pointed out that sister chromatids at mitosis commonly appear to be cohesive all along their length until sister centromeres separate at the initiation of anaphase movement; at this time sister chromatids often appear to fall apart at once all along their length. Mitotic chiasmata which persist until anaphase have been reported to accompany mitotic crossing over (Therman and Kuhn, 1976). However, in these mitotic counterparts of meiotic bivalent configurations, sister centromeres usually appear to orient amphitelically, and

mitotic chromosome distribution is therefore normal. Additional constraints of chromosome behavior are obviously required at meiosis.

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