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Cytological Studies of Ameiotic and Normal Maize with Reference to Premeiotic Pairing

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Abstract. This study reports cytological observations in maize plants homozygous for the recessive *am* allele which suppresses meiosis in both male and female meiocytes. The ultimate premeiotic mitosis in anthers from ameiotic plants is normal, but the resulting nuclei do not undergo meiosis. Instead, a synchronized mitosis occurs after which the cells degenerate. No evidence was found for a non-random association of homologous chromosomes in the premeiotic or ameiotic mitoses of homozygous ameiotic plants or in the premeiotic mitosis of normal sibs. These observations are in agreement with the classical view that synapsis of homologous chromosomes does not occur until zygotene.

Introduction

Association of homologous chromosomes in somatic cells has been well documented, but a consistent relation between somatic and meiotic pairing has not been conclusively demonstrated. According to Smith (1942), "the meiotic pairing consummated at pachytene is initiated at the latest by the telophase of the last premeiotic division". Cytological observations of premeiotic pairing have been reported in a number of organisms: *Triticum* (Feldman, Mello-Sampayo and Sears, 1966), *Zea mays* (Maguire, 1967), *Haplopappus* and *Rhoeo* (Brown and Stack, 1968), and *Plantago* (Stack and Brown, 1969).

It has been assumed that the premeiotic associations prepare and orient the chromosomes for more intimate synapsis in meiosis. Brown and Stack (1968) have suggested that premeiotic pairing begins during early bud formation and that, gradually and cumulatively, it increases to a high level by the ultimate premeiotic division. On this basis, the infrequent occurrence of interlocked bivalents is explicable since there is no need to postulate long range pairing forces in the early meiotic prophase when the homologues are already somatically paired.

However attractive is the concept of premeiotic association, it need not be, and probably is not, of universal occurrence. In *Lilium longiflorum* "Croft", Walters (1970) found no evidence of association or alignment of chromosomes in premeiotic cells. The singleness of the

chromosomes persisted into leptotene. Moreover, there is no opportunity for premeiotic association of homologous chromosomes in forms such as the fungi where a zygotic meiosis occurs immediately following syngamy of two previously separated haploid nuclei. It is clear, therefore, that such an association is not a prerequisite either for meiosis or for exchange pairing.

During cytological studies of the ameiotic mutant in maize, premeiotic mitoses were analyzed in normal *Am Am* and *Am am* plants as well as in the recessive *am am* sibs. These observations provide evidence on chromosome distribution during the premeiotic mitosis in *Zea mays*, but the factors responsible for the transition from somatic mitosis to meiosis remain unresolved.

Materials and Methods

The ameiotic mutant was first observed by Rhoades (1956). Ameiotic plants differ from their sibs in being completely male sterile and almost completely female sterile. In segregating progenies a 3:1 ratio of normal to ameiotic plants indicated that a single Mendelian factor was involved. The ameiotic locus has been located on the short arm of chromosome 5 (Palmer, 1970). Ameiotic plants are phenotypically indistinguishable from normal sibs until tassel emergence since the gene has little if any effect on vegetative growth and development.

Microsporocyte samples were fixed in 3:1 (V/V) ethanol : glacial acetic acid, stored at $-20°$C and examined in aceto-carmine squash preparations. A portion of the tassel was left in the plant to be used in pollinations. Since certain meiotic mutants alter the behavior of chromosomes even when one normal allele is present (Hinton, 1966; Nel, 1971), it was necessary to distinguish the *Am am* and *Am Am* genotypes. The plants were self-pollinated and the progeny examined the following year. If no ameiotic individuals were found, the tested plant was assumed to be *Am Am*, while segregation of ameiotic plants indicated an *Am am* genotype.

The study of the course of events in ameiotic and fertile anthers requires a parameter by which comparable stages can be recognized. Erickson (1948) reported that anther length in *Lilium longiflorum* was correlated with developmental stage, *i.e.* a given anther length was diagnostic for a specific meiotic stage. If a similar correlation could be established in maize, it would permit a meaningful comparison between ameiotic and normal sporocytes at comparable times in development. Anthers of different lengths were collected from normal and ameiotic tassels and the developmental stage determined for each anther size. In normal maize we found a strong correlation between anther length and meiotic stage (Table 1). The importance of using anthers from sib plants in comparing fertile and ameiotic cannot be overemphasized since great variation in anther size at a specific meiotic stage is found in different races of maize.

One of the three anthers of a flower was placed on a line subdivided into tenths of millimeters and viewed with a dissecting microscope. After the length was recorded, the cytological stage was determined. The remaining two anthers were removed from the flower and stored in 70% ethanol.

Sections of anthers were prepared in order to count numbers of cells at various stages in anthers from the two phenotypes. Anthers stored in 70% ethanol were carried through the tertiary butyl alcohol dehydration series (Johansen, 1940) and embedded in filtered Tissuemat. Longitudinal sections 10 and 20 μ in thickness

were cut with a rotary microtome and stained with iron-alum hematoxylin (Brooks *et al.*, 1963), a modification of Heidenhain's iron hematoxylin (Johansen, 1940).

Microsporogenesis, megasporogenesis, and tapetal cell divisions were studied in normal and ameiotic sibs by Sinha (1960). Since the only cell divisions occurring in the sporogenous cells of ameiotic anthers were mitotic, Sinha concluded that meiosis was replaced by a mitotic-like division. No consideration was given to the possibility that all cell division might cease in the sporogenous cells of ameiotic anthers after they had undergone the last premeiotic division, *i.e.*, not only did meiosis fail to occur in the potential pollen mother cells (PMC's) but it was not replaced by a mitotic division. In order to distinguish between elimination and substitution, it was necessary to study the pattern of cell division in the anthers of normal and ameiotic plants at various stages of development. Thus, both sectioned and squashed preparations were examined.

Results and Discussion

The data in Table 1 are derived from observations on squashed anthers of various lengths. No striking difference was found in cells from fertile and ameiotic anthers ranging in length from 0.8 to 1.7 mm. Both kinds of anthers contained sporogenous cells only; none included cells in meiotic prophase. Most of the nuclei were in interphase but an occasional cell was found in division. Premeiotic mitoses were observed in anthers ranging in length from 1.2–1.8 mm. In anthers 1.8–2.1 mm long, some cells of fertile anthers were in premeiotic interphase while others had entered meiosis. Anthers measuring 2.2–2.9 mm contained cells in leptonema, zygonema and pachynema. Unlike the anthers from normal plants, the 1.8–2.1 mm anthers from ameiotic sibs showed no indication of the onset of meiosis. Cells undergoing mitosis were frequently observed, the mitotic index being considerably higher than in the smaller anthers. In anthers longer than 2.2 mm from ameiotic sibs, there was little or no mitotic activity; all nuclei were beginning to degenerate. In contrast to the situation in ameiotic anthers, successively longer anthers from fertile florets contained correspondingly more advanced meiotic stages with quartets occurring in anthers 3.7–4.1 mm in length.

The data presented in Table 1 allow the correlation of developmental stage with anther length but provide no information on the number of cells per locule.

In order to determine whether a mitotic division replaces meiosis in ameiotic anthers, longitudinal sections were prepared of normal and ameiotic anthers of various lengths and the number of sporogenous cells correlated with developmental stage. These data, given in Tables 2 and 3, indicated that the number of cells per locule in 2.2 mm ameiotic anthers, which have completed the mitotic division observed in 1.9 mm anthers, is twice that found in normal anthers of the same length and presumably therefore of comparable developmental stage. Photomicro-

Table 1. *Anther length and the cytological stage in normal and ameiotic sib plants*

Anther length (mm)	Normal	Ameiotic
0.8–0.9	interphase	interphase
1.0–1.1	interphase	interphase
1.2–1.3	interphase, mitosis	interphase, mitosis
1.4–1.5	interphase, mitosis	interphase, mitosis
1.6–1.7	interphase, mitosis	interphase, mitosis
1.8–1.9	interphase, mitosis, leptonema, zygonema[a]	interphase, ameiotic mitosis
2.0–2.1	interphase, leptonema, zygonema	interphase, ameiotic mitosis
2.2–2.3	leptonema, zygonema	beginning of degeneration
2.4–2.5	leptonema, zygonema	
2.6–2.7	leptonema, zygonema	
2.8–2.9	leptonema, zygonema, pachynema	
3.0–3.1	zygonema, pachynema, M-I	
3.2–3.3	zygonema, pachynema, M-I	
3.4–3.5	pachynema, M-I, diplonema, diakinesis, A-I	
3.6–3.7	M-II, A-II, quartets	
3.8–3.9	A-II, quartets	
4.0–4.1	quartets	complete degeneration

[a] Cells in synizesis are classified as being in the zygonema stage.

graphs of sections of normal and ameiotic anthers at a premeiotic stage and at a later stage are shown in Figs. 1 and 2. While the number of cells in 1.0 mm anthers from ameiotic and normal plants was about the same, it is evident that ameiotic anthers of 2.2 mm length contain more cells than those from the normal sibs (compare Figs. 1b and 2d).

The following conclusions can be drawn regarding the effect of the *am* gene on the course of meiosis. Counts of cell number in longitudinal sections of fertile and sterile anthers of similar length reveal that the number of sporogenous cells formed by the end of the premeiotic

Table 2. *Average number of cells observed in a microscopic field at 430×. Values in parentheses represent number of determinations. In several examples two anthers from the same flower were used for sectioning, one at 10μ, the other at 20 μ (e.g. 3–11). Cells undergoing mitosis were counted twice.*

Anther length (mm)	Normal			Ameiotic		
	Plant no.	Section thickness		Plant no.	Section thickness	
		10 μ	20 μ		10 μ	20 μ
1.0	3–1	31 (6)	—	—	—	—
1.0	16–9	31 (6)	—	15–7	30 (6)	—
1.1	—	—	—	15–8	30 (6)	—
1.9	3–11	23 (4)	23 (14)	6–3	—	45 (7)
1.9	—	—	—	6–5	47 (4)	48 (7)
2.0	3–14	—	24 (6)	12–10	46 (4)	49 (4)
2.0	3–17	24 (7)	24 (9)	—	—	—
2.0	3–19	24 (7)	25 (10)	—	—	—
2.2	—	—	—	6–1	48 (4)	49 (6)

Table 3. *Total number of cells observed in longitudinal sections of anthers. Values in parentheses represent number of determinations. In several examples two anthers from the same flower were used for sectioning, one at 10 μ, the other at 20 μ (e.g. 3–11). Cells undergoing mitosis were counted twice.*

Anther length (mm)	Normal			Ameiotic		
	Plant no.	Section thickness		Plant no.	Section thickness	
		10 μ	20 μ		10 μ	20 μ
1.0	3–1	52 (3)	—	—	—	—
1.0	16–9	57 (3)	—	15–7	49 (3)	—
1.1	—	—	—	15–8	57 (3)	—
1.9	3–11	85 (2)	91 (3)	6–3	—	194 (1)
1.9	—	—	—	6–5	197 (2)	204 (3)
2.0	3–14	—	88 (2)	12–10	—	194 (1)
2.0	3–17	—	—	—	—	—
2.0	3–19	101 (2)	110 (2)	—	—	—
2.2	—	—	—	6–1		196 (3)

divisions is approximately the same in the two types—*i.e.*, the *am* gene has no effect on the premeiotic divisions insofar as the number and character of the mitoses are concerned. However, a profound difference arises in anthers approximately 1.9–2.0 mm long. In normal anthers, the archesporial cells have entered the extended meiotic prophase while

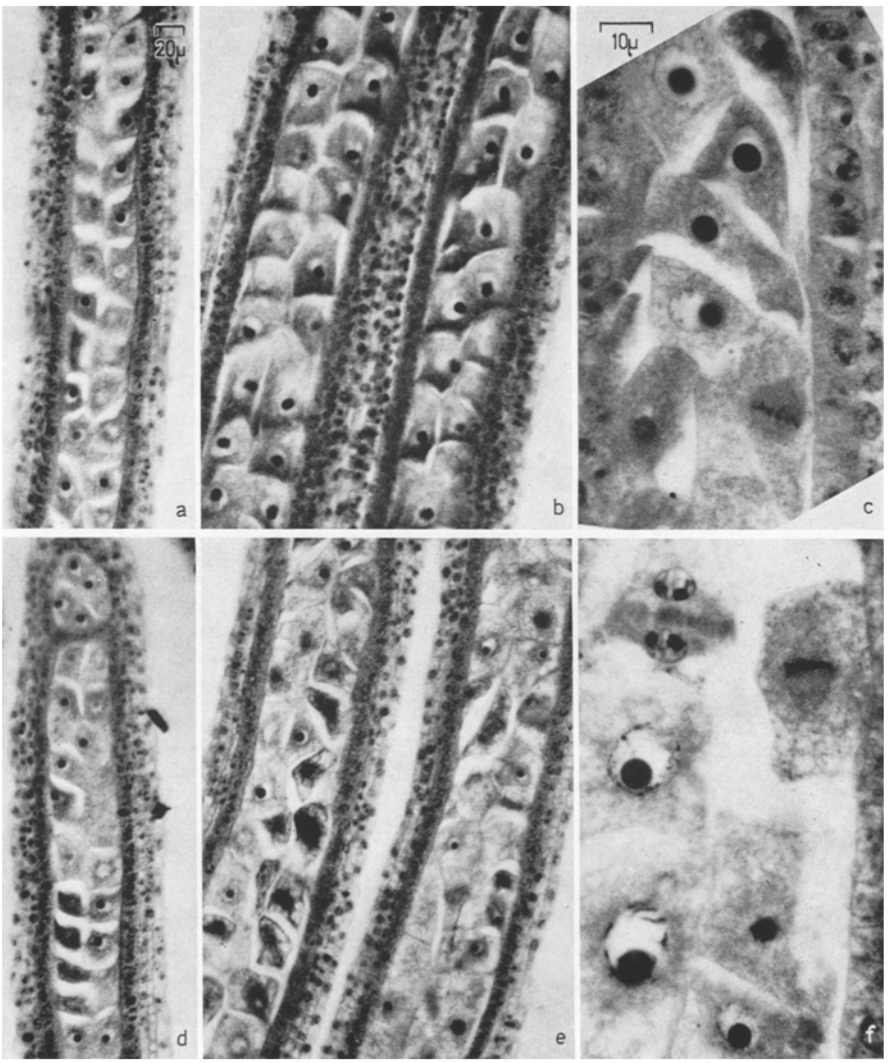

Fig. 1a–f. Longitudinal sections of anthers of the indicated lengths from normal plants (a–c) and from ameiotic plants (d–f). Sections are 10 μ in thickness. a 1.0 mm. The cells are in interphase. b 2.0 mm. The cells are in leptonema-zygonema and are synchronized. c 1.0 mm. Mitotic divisions observed in normal anthers of 1.0–2.0 mm length are sporadic and unsynchronized. d 1.1 mm. Cells are in interphase. Cell number is similar to a. e 1.9 mm. As the cells complete the ameiotic mitosis, plasmolysis and degeneration are starting to occur. f 1.9 mm. Synchronization of the sporogenous cells undergoing the ameiotic mitosis is evident. a, b, d, e × 190; c, f × 680

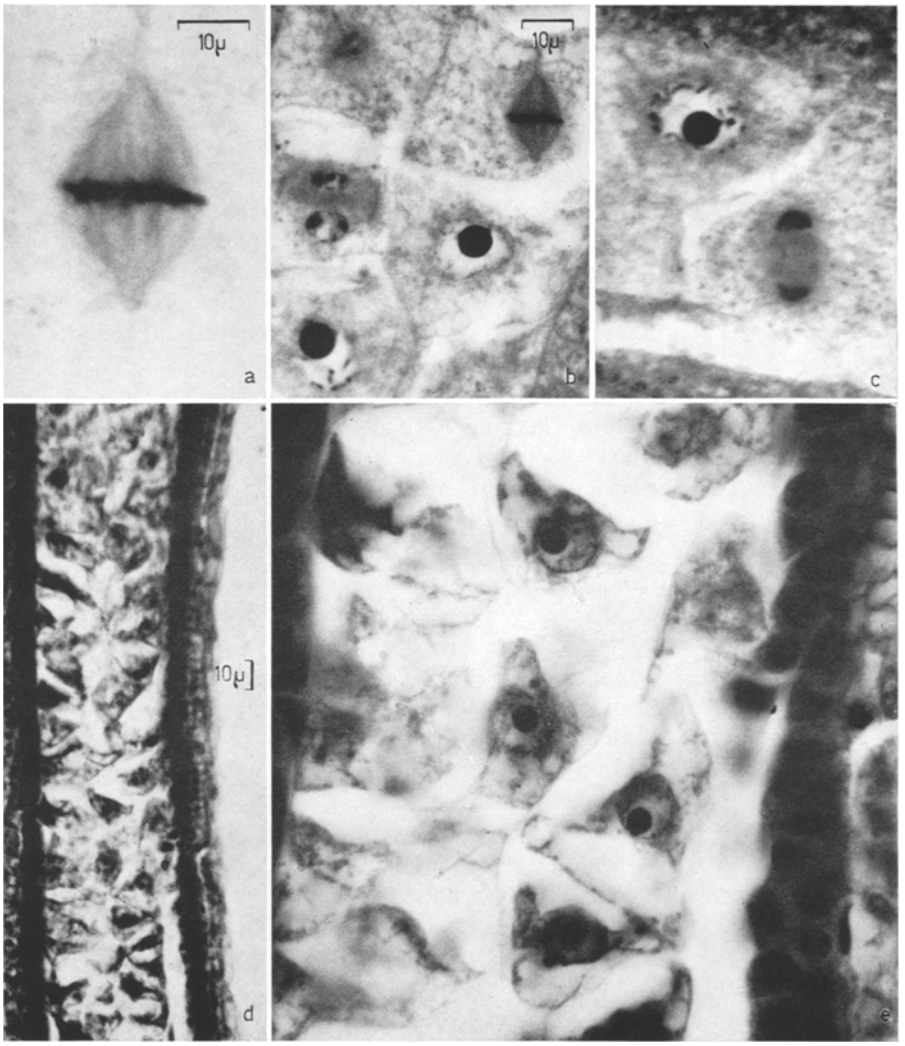

Fig. 2a–e. Longitudinal sections of anthers of the indicated lengths from ameiotic plants. a–c 10 μ thickness, d and e 20 μ. a 1.9 mm Metaphase of the ameiotic mitosis illustrating spindle fibers. b 1.9 mm. Illustrates the synchronization of the ameiotic mitotic division and shows a lower magnification of a. c 1.9 mm. Ameiotic mitosis. d 2.2 mm. After the ameiotic mitosis is completed cell degeneration occurs. e 2.2 mm. A higher magnification of the lower $^1/_3$ of d. a × 935; b, c, e × 680; d × 180

in ameiotic anthers cells of comparable age are in a somatic mitosis. We shall call this the ameiotic mitosis since it is a substitute for the first meiotic division. The ameiotic mitosis, with rare exceptions, proceeds

rapidly and is completed in 2.2 mm anthers, while in fertile anthers of this length all cells are still in early meiotic prophase. Significantly, the number of cells in 2.2 mm anthers, which have undergone the ameiotic mitosis, is approximately twice the number of pollen mother cells in fertile anthers which are in leptonema-zygonema. It is the doubled cell number at this developmental stage which offers the most cogent evidence that a mitotic division replaces meiosis. The occurrence of a second ameiotic division (corresponding to the second meiotic division) is doubtful because degeneration of the archesporial cells is evident in anthers 2.2 mm and longer. By the time the quartet stage is reached in fertile anthers, there is only disorganized cellular debris in ameiotic anthers.

The final number of sporogenous cells in both fertile and ameiotic anthers is attained in anthers approximately 2.0 mm long. Since the number of archesporial cells in younger anthers is considerably less than the number reached when the ultimate premeiotic mitosis is completed, it follows that the increase in cell number arises from somatic mitosis. At any given time the number of nuclei in mitosis in the young anthers is small, most of the cells being in interphase—*i.e.*, there is no indication of synchronized mitosis in the developing anthers (see Fig. 1a and c). However, in those locules where the ameiotic mitosis is occurring, the majority of the cells are dividing (Fig. 1f). The synchronization observed in this division is comparable to that in true meiosis and is in contrast to the sporadic mitosis observed in the sporogenous cells of premeiotic anthers. From our study of microsporogenesis in ameiotic plants we conclude that in ameiotic anthers meiosis is replaced by a somatic mitosis.

Although the ameiotic mitosis and the first meiotic division differ fundamentally, they share in common a bi-polar acuminate achromatic figure with the spindle fibers converging to a point at each pole (Fig. 2a and b). This is quite unlike the barrel-shaped spindles commonly found in somatic divisions of maize, where the small size of the cells prevents convergence of the spindle fibers. It would appear that the size of the cell dictates the shape of the mitotic apparatus. It is no doubt significant that the spindle figures observed in the plasmodial endosperm divisions where no cell walls are formed are also spindle shaped.

A number of well spread mitotic figures were obtained from squashed normal (*Am Am* and *Am am*) and ameiotic (*am am*) anthers and these

Fig. 3a–e. Premeiotic and meiotic cells from normal plants. a and b Prophase. The chromosomes are more extended than in ordinary somatic prophases. c Metaphase. The chromosomes 6 are designated by arrows. d Anaphase. Three of the four chromosomes 6 are evident. e Meiotic cell in leptonema. Note the larger size of the cell as compared to the mitotic cells. The chromomeres are readily visible. × 720

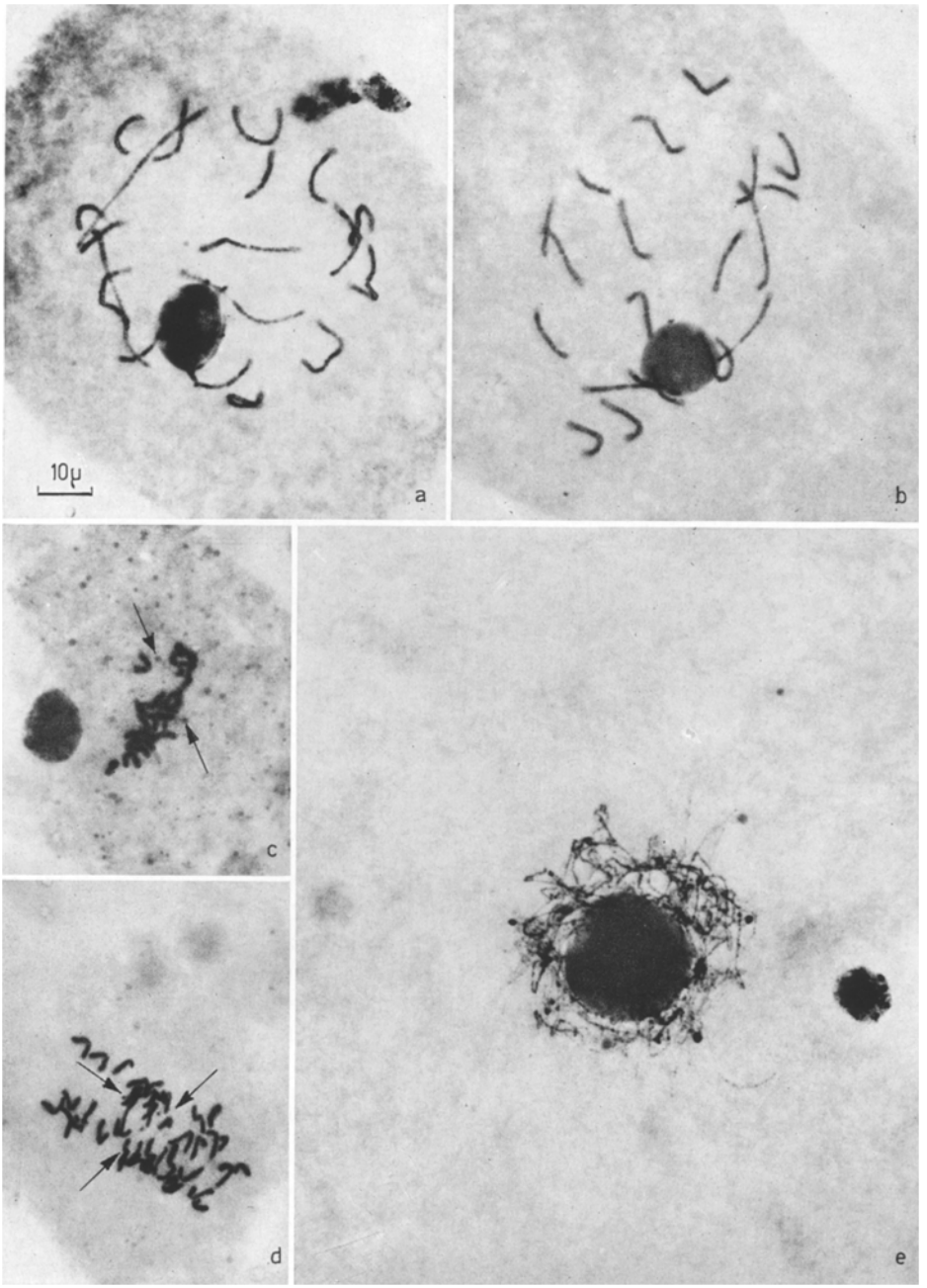

Fig. 3 a–e

were studied in some detail. Since chromosome morphology and behavior were identical in cells undergoing premeiotic and ameiotic mitoses, the following description applies to cells in both divisions. Squashed cells from normal anthers are shown in Fig. 3 while Figs. 4 and 5 illustrate mitoses in ameiotic anthers. The prophase chromosomes are more extended than in ordinary somatic prophases, approaching the length of pachytene chromosomes. However, they do not display the chromomere structure characteristic of meiotic prophase chromosomes. In late prophase the chromosomes were shorter, thicker and more deeply staining (Fig. 4c and d). The metaphase chromosomes were not as contracted as those of meiotic metaphase-I and individual chromatids were not distinguishable. Unusually well spread anaphase figures were obtained, which will be described later. After telophase in normal plants, there is a gradual increase in cell size as the cells enter into meiosis. Leptotene cells (Fig. 3e) are easily distinguishable from those undergoing a premeiotic prophase by both cell size and by the fine beaded structure of the extended chromosomes. No increase in cell size occurs either before or after the ameiotic mitosis and interphase cells following this division often appear somewhat plasmolyzed as degeneration begins.

A number of investigators have observed premeiotic pairing of homologues (Smith, 1942; Battaglia, 1947; Feldman, Mello-Sampayo and Sears, 1966; Brown and Stack, 1968; among others). Inasmuch as Maguire (1967, 1970 personal communication) reported premeiotic association of homologues in maize, it became essential to study chromosome behavior in the mitoses of ameiotic plants and in the premeiotic division of their normal sibs. If homologous chromosomes were paired in the archesporial mitoses of fertile anthers but not in those from ameiotic sibs, this difference might throw some light on the underlying mechanism responsible for the failure of meiosis in ameiotic plants.

The relatively large size and fragile walls of the sporogenous cells made it possible to obtain prophases and anaphases in which recognition of specific chromosomes was possible (Fig. 5b–d). It is in these exceptional figures that a search was made for the pairing of homologous chromosomes. Chromosome 6 by virtue of its satellite is the only chromosome that can be unequivocally distinguished although it is possible on the basis of relative length and arm ratios to tentatively recognize all of the members of the complement in good preparations. Since chromosome 6 is the one chromosome whose recognition did not involve some subjective interpretation, our conclusions regarding somatic pairing are based primarily on the behavior of the two chromosomes 6.

With rare exceptions, only one nucleolus was observed in the archesporial prophases in both normal and ameiotic anthers. At this stage the two chromosomes 6 appeared to be randomly attached to the

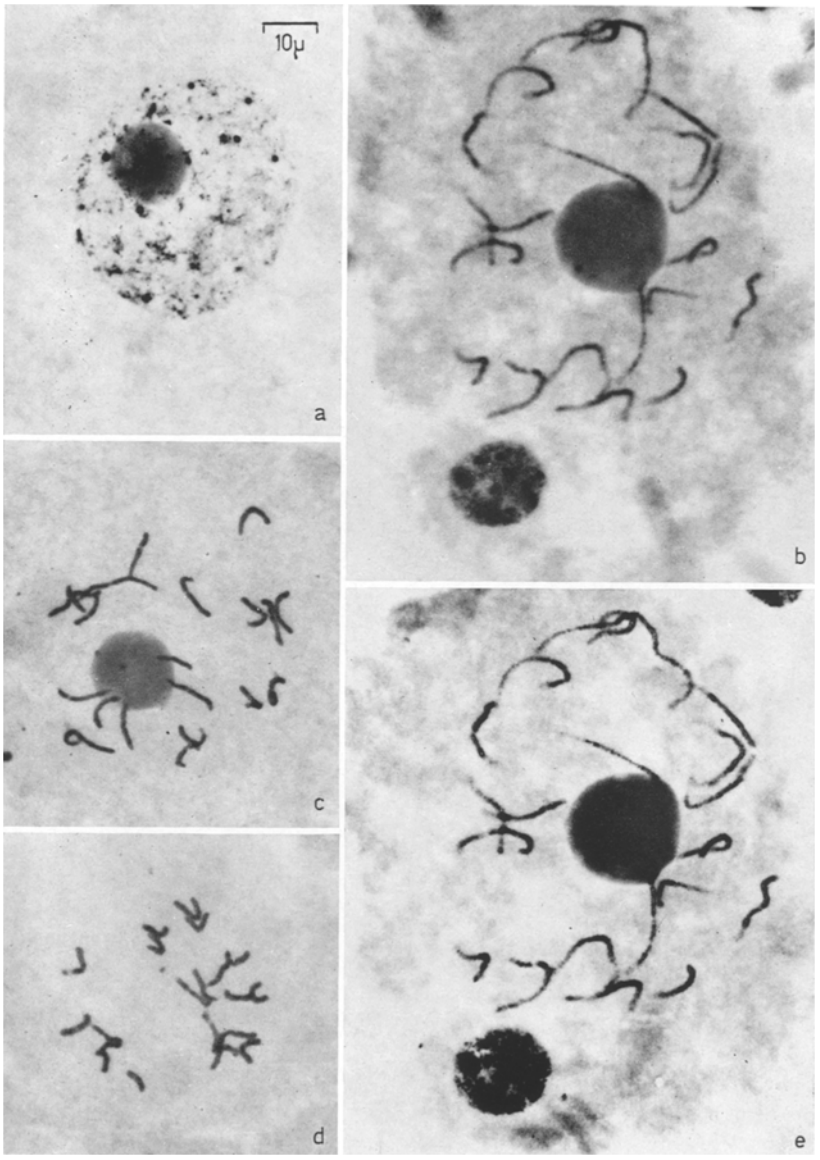

Fig. 4a–e. Cells from ameiotic anthers of different lengths. The majority were in ameiotic mitosis, but those from smaller anthers presumably were from premeiotic cells. a Interphase. b Prophase. The extended condition of the chromosomes is evident. c and d Prophase. In late prophase the chromosomes are shorter, thicker and more deeply staining. e Prophase. Same as b, but illustrates the more deeply staining areas of the chromosomes. × 720

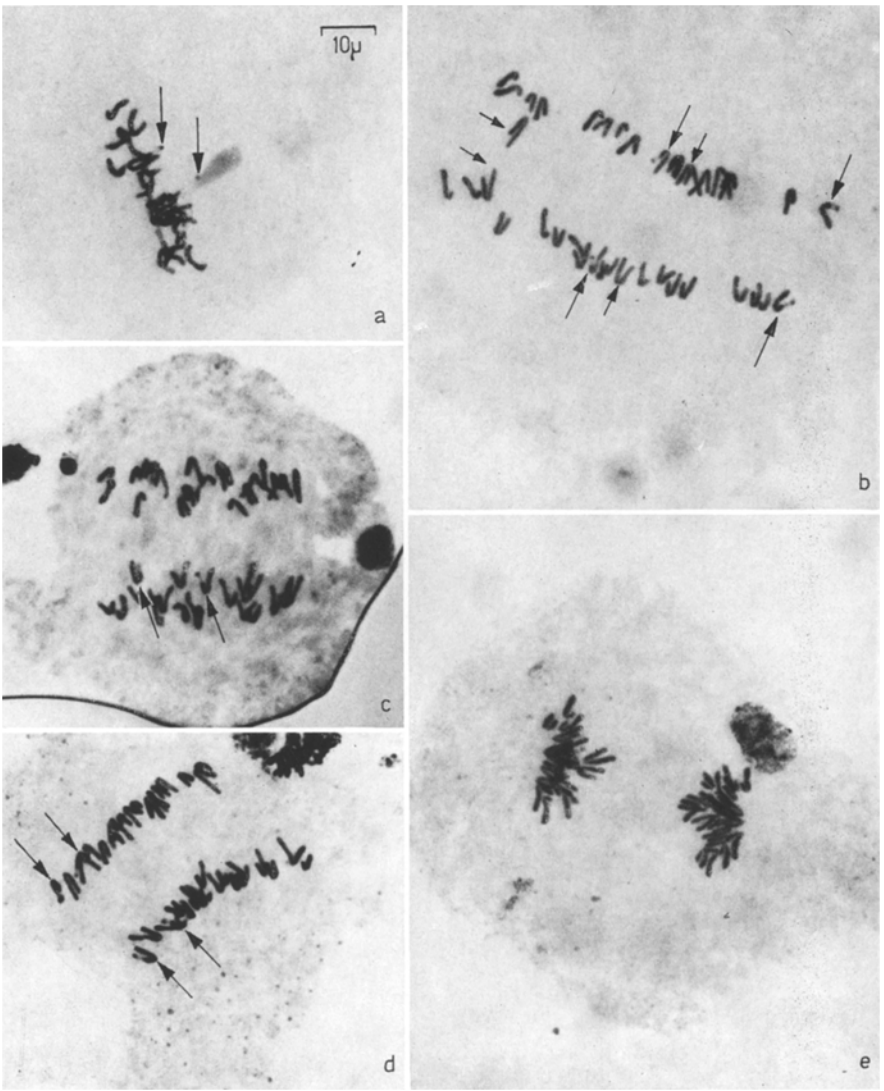

Fig. 5a–e. Cells from ameiotic anthers of different lengths. The majority were in ameiotic mitosis, but those from smaller anthers presumably were from premeiotic cells. a Metaphase. The chromosomes 6 are evident. One of them is attached to the remnant of the nucleolus. b Anaphase. The chromosomes 6 are indicated by long arrows, while the chromosomes 1 are indicated by short arrows. c and d Anaphase. The chromosomes 6 are evident. e Telophase. × 720

body of the nucleolus. Very few polar views of the metaphase spindle were observed and in the side views the only chromosome identifiable was 6, which could be readily distinguished by the satellite (Fig. 5a). The location of the two chromosomes 6 on the spindle suggested a random placement and their position on the metaphase plate is believed to reflect their earlier location in the prophase nucleus.

Anaphases afford the most favorable material for determining if homologous chromosomes tend to be associated in pairs. Not only are the chromosomes well separated, but any marked distortion or displacement of the disjoining chromosome groups caused by the smear or squash technique would be apparent. Since anaphase is of short duration, considerably more prophases and metaphases than anaphase figures were available for analysis, but in 20 clear anaphases from normal anthers and in 18 from ameiotic the two chromosomes 6 passing to each pole appeared to be randomly disposed relative to one another. In the anaphase shown in Fig. 5b, the two chromosomes 1 are indicated by short arrows while the satellited 6's are marked by longer arrows. There is no evidence of somatic association of either homologous pair. The anaphases from normal anthers may not all have been in the ultimate premeiotic division but they unquestionably occurred in cells shortly destined to undergo meiosis. With respect to the prophase, metaphase, and anaphase configurations observed in ameiotic anthers (Figs. 4 and 5), it cannot be said for any specific figure whether it was from the ameiotic mitosis or occurred in a premeiotic mitosis. However, mitotic figures were found in ameiotic anthers of different lengths and, although the majority were in ameiotic mitosis, presumably those from smaller anthers were from premeiotic cells. Significantly, in neither fertile (*Am Am* and *Am am*) nor ameiotic (*am am*) anthers was there any indication of somatic pairing.

Our findings cannot be said to negate the observations of somatic pairing reported in other organisms, but they do suggest that premeiotic pairing is not an indispensable condition. Our observations with maize are not consonant with those of Maguire (1970, personal communication), who claimed that homologous chromosomes were associated in pairs in the last anaphase before the onset of meiosis. We cannot resolve this disagreement. It seems unlikely that premeiotic association is so trivial a matter that races of maize would differ in what must be a meaningful phenomenon.

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