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New Insights Into the Role of the Maize *ameiotic1* Locus

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ABSTRACT

In maize the *am1-1* mutant allele results in both the male and female meiocytes undergoing mitosis in place of the meiotic divisions. A second mutant allele *am1-praI* enables both the male and female meiocytes to proceed to the early zygotene stage of meiotic prophase I before being blocked. Here we report on three new alleles that allow all male meiocytes to undergo mitosis but in female meiocytes approximately one quarter (*am1-2*), one half (*am1-485*), or all (*am1-489*) of them are blocked at an abnormal interphase stage. Previous analysis has shown that *am1-praI* is dominant to *am1-1* in male meiocytes. Cytological analysis of heteroallelic combinations in female meiocytes now indicates a dominance relationship of *am1-praI* > *am1-1* > *am1-2/am1-485* > *am1-489*. The evidence provided by the female phenotypes of the new mutant alleles suggest that, whereas the normal *am1* allele is required for the meiocytes to proceed through meiosis, a partially functional allele may be required for their diversion into a mitotic division. The partial or complete blockage of mitosis in female meiocytes carrying the new *am1* alleles rules out the possibility that the mitotic division of mutant meiocytes reflects a simple default pathway for cells that cannot initiate meiosis. This locus may have a dual function.

SEXUAL reproduction in higher eukaryotes poses a developmental challenge that does not confront yeast and other unicellular eukaryotes. Maize and other multicellular organisms must regulate the shift of a particular group of cells in a tissue from the somatic or vegetative phase of development to the germinal or sporogenous phase of reproduction. This regulated shift from the mitotic sequence to the meiotic sequence occurs in vertebrate testes and ovaries (EDDY 1996) whereas it occurs in the anthers and ovules of maize and other flowering plants (DICKINSON 1994).

The meiocytes of flowering plants are derived from hypodermal cells: In animals there is a germ cell lineage while in plants there is no germ cell line and cell position appears to be determinative of cell fate. In flowering plants the meiotic cells are derived from a special layer of cells, the hypodermal cells, located just below the epidermal layer during early development of the ovule and anther. In most flowering plants only one hypodermal cell in each ovule enlarges to form an archesporial cell, which differentiates directly into the megasporocyte, the megaspore mother cell (MMC) (GIFFORD and FOSTER 1987). The MMC proceeds through meiosis to produce a linear array of four megaspores. Three of these degenerate while the chalazal-most megaspore subsequently undergoes three successive mitotic divisions to yield the seven-celled, eight-nucleate embryo sac (RANDOLPH 1936; HUANG and SHERIDAN 1994). This is the pattern followed by most of the grasses, including maize (COOPER 1937; KIESSELBACH 1949). In some taxa the hypodermal cell divides prior to formation of the MMC (DAVIS 1966). During anther development rows of hypodermal cells undergo a periclinal division to produce an internal sporogenous layer, the archesporial cells, and an external layer, the primary parietal cells. Maize conforms to the monocotyledonous type of anther wall development wherein the sequential divisions of the primary parietal cells give rise to the external wall layers, plus the tapetum internally (KIESSELBACH 1949; DAVIS 1966). The archesporial cells divide one or more times and their cellular progeny differentiate into the microsporocytes (KIESSELBACH 1949). It is evident that, although both the maize female and the male meiocytes are derived from the hypodermal cell layer of ovules and anthers, they differ in their developmental history during the period intervening between hypodermal cell formation and the differentiation of the meiocytes: female meiocytes are derived directly from the hypodermal cell progenitor without any intervening mitosis, but two or more mitotic cell divisions occur between the formation of the hypodermal cells and their male meiocyte derivatives. In both ovules and anthers, the archesporial cells undergo considerable increase in size and change in cell shape as well as an increase in cytoplasmic density. These changes make the archesporial cells easy to identify and undoubtedly reflect profound changes in their programs of gene expression.

The fate of the hypodermal cells is under genetic control: We have identified a new mutation, *multiple archesporial cells1* (*mac1*), which appears to play an im-

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portant role in the switch of the hypodermal cells from the vegetative to the meiotic (sporogenous) pathway in maize ovules (SHERIDAN *et al.* 1996). In the *mac1* mutant ovule several hypodermal cells develop into archesporial cells and the resulting megasporocytes undergo a normal meiosis. Because ears on mutant plants display partial sterility resulting from abnormalities in megaspore differentiation and embryo sac formation, the sporophytic expression of this gene is also important for normal female gametophyte development. Homozygous *mac1* plants are male sterile because of a failure of male meiocytes to undergo meiosis, a failure that appears to result from a defect in the formation of the anther (W. F. SHERIDAN, N. A. AVALKINA and I. N. GOLUBOVSKAYA, unpublished results). We suspect that the different developmental fates of the *mac1* female and male meiocytes are a reflection of their different developmental histories. The *mac1* gene appears to initially act very early in ovules and anthers, in committing the hypodermal cells to the meiotic pathway and to the formation of the archesporial cells that give rise to the meiocytes. At the end of the lengthy developmental sequence, when the meiocytes have completed their enlargement and assumed their characteristic shape and appearance, they are poised for entry into the meiotic divisions. Yet it is apparent that the meiocytes are not irreversibly destined to enter meiotic prophase. Studies of cultured microsporocytes of Lilium and Trillium explanted as late as the late premeiotic G_2 phase have revealed that these meiocytes could still revert to mitosis (STERN and HOTTA 1969; ITO and TAKEGAMI 1982).

The *ameiotic1* locus controls the initiation of meiosis: Genetic and cytological analyses of the *ameiotic1* locus of maize have also indicated that meiocytes that are apparently ready, in all observable aspects, to initiate meiosis and proceed into meiotic prophase I can instead divert into mitosis. Here we present the results of our analysis of three new mutant alleles, which provide new insights into the role of the *ameiotic1* locus. The original mutant allele (*am1-1*) at the *ameiotic1* locus was first reported in 1956 (M. RHOADES, personal communication) and its effects on male meiocytes were characterized by PALMER (1971). The microsporocytes enlarge and appear normal but, at the time during anther development when normal microsporocytes enter into prophase I of meiosis, *am1-1* microsporocytes proceed through a normal-appearing mitotic division and then degenerate (PALMER 1971). When female meiosis was analyzed, it was found that *am1-1* megasporocytes underwent one or more successive mitotic divisions to form a linear array of up to eight cells, which subsequently degenerated. These observations indicated that the *am1-1* locus might control the entry of meiocytes into the sequence of meiotic events, particularly the initiation of prophase I of meiosis (GOLUBOVSKAYA *et al.* 1992). An alternative explanation of the male and female phenotypes of the mutant *am1-1* meiocytes was that the defect in the mutant meiocytes disabled their capacity to depart from the mitotic pattern of cell division and that a normal allele of this locus was required for the exit from the mitotic pattern, rather than for the entry into the meiotic pattern. This question was resolved by the isolation and analysis of a new mutation, the *am1-praI* allele. In both the male and female mutant meiocytes, the meiotic cells initiate meiosis and proceed to the zygotene stage of prophase I, at which point they stop and undergo degeneration. The capacity of the *am1-praI* meiocytes to proceed into prophase I demonstrated that this locus plays an essential role in the initiation of meiosis and entry into the meiotic divisions (GOLUBOVSKAYA *et al.* 1993). The question has remained, however, as to whether the passage of the *am1-1* meiocytes into mitotic divisions reflects a simple default pathway for meiocytes that cannot initiate meiosis.

New alleles provide new insights into the role of the *ameiotic1* locus: We have acquired three new mutations that result in an ameiotic phenotype. A mutation with a male mutant phenotype like that of *am1-1* was reported by CURTIS and DOYLE (1991) and was termed *am2*. Our cytological screening of male sterile mutants identified two additional mutations, *am*-485*, (GOLUBOVSKAYA *et al.* 1993) and *am*-489*, with an ameiotic phenotype. We report here the results of allelism testing wherein these three mutations failed to complement *am1-1* and thus increased to five the number of mutant alleles available for study. We present the results of our comparative analysis of the five mutant *ameiotic1* alleles. We have examined the cytological features of male and female meiocytes homozygous for each of the *am1-2*, *am1-485*, and *am1-489* mutant alleles. The new mutant alleles are more severely defective than the *am1-praI* and *am1-1* alleles and each of the three new alleles exhibits a different phenotype in the female than in the male meiocytes. In addition, we have evaluated the dominance relationships among the five *am1* alleles by examining female meiocytes containing heteroallelic combinations. We discuss the implications of these new findings for understanding the role of this locus in the initiation of meiosis and in controlling the alternative fates of entering either meiosis or mitosis by mutant meiocytes.

MATERIALS AND METHODS

Sources of the new mutants: The two new mutations *am*-485* and *am*-489* were isolated from active Robertson's Mutator stocks during our screening for male sterile mutants. These mutations are therefore likely to be transposon-tagged with a Mutator transposable element. The mutant designated *am2* by CURTIS and DOYLE (1991) is apparently a spontaneous mutation. It was kindly shared with us by GREG G. DOYLE. On the basis of allelism tests with the *am1-1* allele (described in this report) we now designate these three new mutations as *am1-485*, *am1-489*, and *am1-2*, respectively.

TABLE 1

Genetic and cytological data on male meiocytes from allelism tests for five alleles of the *ameiotic1* gene

No.	Cross	Observed segregation of F_1 progeny: No. of analyzed families	Fertile plants	Sterile plants	Total plants	Chi-square value for 3:1	Cytology of microsporocytes: Normal phenotype	Mutant phenotype
1	*am1-1*/+ × *am2*/+	3	45	13	58	0.20[a]	—	—
2	*am1-1*/+ × *am*-485*/+	2	23	7	30	0.43	23	7 aml-1
3	*am1-pral*/+ × *am*-485*/+	2	19	5	24	0.23	19	5 aml-pral
4	*am1-1*/+ × *am*-489*/+	1	6	2	8	0	6	2 aml-1
5	*am*-489*/+ × *am1-1*/+	2	42	14	56	0	42	14 aml-1
6	*am1-pral*/+ × *am*-489*/+	2	16	7	23	0.36	—	—
7	*am2*/+ × *am*-485*/+	1	21	7	28	0	6	2 aml-1
8	*am*-485*/+ × *am2*/+	1	13	5	18	0.07	9	2 aml-1
9	*am2*/+ × *am*-489*/+	1	12	5	17	0.17	—	—
10	*am*-489*/+ × *am2*/+	2	31	12	43	0.18	—	—
11	*am*-485*/+ × *am*-489*/+	3	58	21	79	0.11	27	14 aml-1

[a] The chi-square value was calculated for the goodness of fit of the ratio fertile plants to sterile plants corresponding to the expected 3:1. All of the chi-square values correspond to $P > 0.20$.

Genetic analysis: The allelism tests were performed by crossing heterozygotes (*am1-1*/+ or *am1-pral*/+) as males or females with heterozygotes of each of the new alleles. The progeny of such crosses were grown to flowering and scored for male sterility. The heterozygotes used for the testing were progeny of self-pollinated plants carrying the mutations and known to segregate for mutant plants. Because the genotype (+/+ *vs.* +/mutation) of any particular fertile plant in segregating families was not known at the time of pollination, numerous crosses were performed and their progeny was screened, when conducting each test. All crosses and scoring were performed in Grand Forks, North Dakota, with the mutant alleles propagated in genetic stocks.

Cytological analysis: For the cytological examination of microsporocytes, immature tassels were taken from fertile and male sterile sibling plants and fixed in Farmer's (three parts ethanol: one part glacial acetic acid) fixative. For cytological examination of megasporocytes, the immature ears from normal and mutant sibling plants were fixed for 24 hr at room temperature in FAA fixative (40% formaldehyde: glacial acetic acid: 50% ethanol in a 5:5:90 volume ratio). After 14 hr in 95% ethanol, the fixed samples were stored in 70% ethanol at 4° before analysis (see GOLUBOVSKAYA *et al.* 1992 for details of the dissection and squash techniques of the isolated megasporocytes). Microphotography was carried out with a microcamera MFN 11 using a Biolar microscope and also with a Leica MP548 camera using pseudo-Nomarski optics on a Leica DMRB microscope.

RESULTS

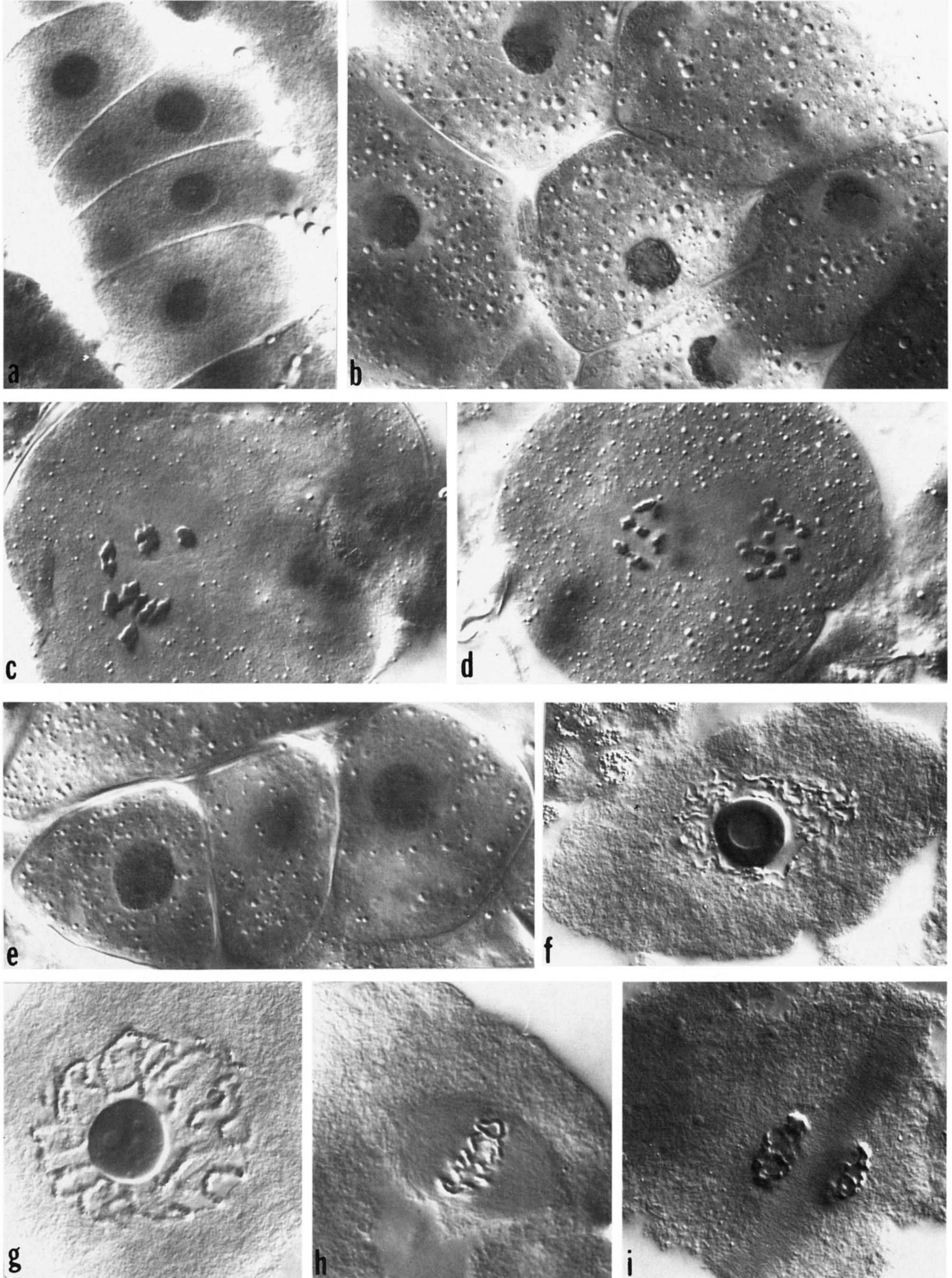
Allelism testing of new mutations with ameiotic phenotypes: Homozygotes of the two previously identified mutations at the *am1* locus, *am1-1* and *am1-pral*, are completely male sterile as evidenced by the failure of anthers to extrude (so no pollen is shed) and are nearly completely female sterile as evidenced by the failure to produce no more than a few kernels when crossed as females by normal pollen. The new mutations, *am2*, *am*-485*, and *am*-489*, exhibited the same sterile phenotypes. All five mutations are maintained and propagated as heterozygotes.

The three new mutations were tested for possibly being new alleles at the *am1* locus by crossing them as heterozygotes with either the *am1-1* allele (crosses with *am2*) or with both the *am1-1* and the *am1-pral* alleles (crosses with *am*-485* and *am*-489*). The F_1 segregation ratios of the progeny of these crosses are shown in Table 1.

The cross of *am1-1*/+ by *am2*/+ yielded three ears (cross no. 1 in Table 1). Kernel samples planted from these ears yielded 58 plants and these segregated as 45 fertile plants and 13 sterile plants, a ratio very close to the 3:1 ratio expected if both parents were heterozygous for a recessive mutation at the same locus. The failure of *am2* to complement *am1-1* demonstrated allelism of *am2* at the *am1* locus and we have therefore designated this mutation *am1-2* and use this gene symbol in subsequent references to it.

Crosses of *am1-1*/+ by *am*-485*/+ and of *am1-pral*/+ by *am*-485*/+ yielded progeny segregating very close to 3:1 ratios of fertile to sterile plants (see crosses nos. 2 and 3 in Table 1). Likewise crosses of *am1-1+*/ by *am*-489*/+ (cross no. 4) and of *am*-489*/+ by *am1-1*/+ (cross no. 5), as well as crosses of *am1-pral*/+ by *am*-489*/+ (cross no. 6) yielded progeny segregating very close to 3:1 ratios of fertile to sterile plants (see Table 1). These genetic data demonstrate that, by their failure to complement the previously identified *am1* mutant alleles, the *am*-485* and *am*-489* mutations are mutations at the *am1* locus; we have designated them *am1-485* and *am1-489*, respectively, and use these gene symbols in subsequent references to them.

In addition to the allelism tests described above, the three new mutations were tested in pair-wise crosses among them. The results of crossing *am2*/+ by or on *am*485*/+ (cross nos. 7 and 8) and crossing *am2*/+ by or on *am*-489*/+ (cross nos. 9 and 10), as well as the


a
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results of crossing *am*-485/+* by *am*-489/+* (cross no. 11), all yielded very similar results as those obtained in the allelism tests described above (Table 1). An examination of the data in Table 1 reveals that all five of the mutations are allelic to each other and therefore alleles of the *am1* locus and that each of the mutant alleles appears to transmit with a frequency equal to that of the normal allele through both the male and female gametes as evidenced by the close fit to the expected 3:1 ratio of fertile to sterile plants for all 11 crosses shown in Table 1.

The results of the segregation analysis in the field (fertile *vs.* sterile plants) indicating allelism of the three new mutations with the *am1* locus were confirmed by cytological examination of male meiocytes obtained from both the fertile and sterile progeny of several of the crosses shown in Table 1. For instance, the 23 fertile plants obtained from cross no. 2 involving *am*-485* were all sampled and in every case their microsporocytes displayed a normal cytological phenotype, with the meiocytes proceeding through meiosis. The seven sterile plants from cross no. 2 were sampled and in every case their microsporocytes all failed to undergo meiosis but were at some stage of mitotic division. Cross no. 4 yielded similar data regarding *am*-489* and cross no. 7 yielded similar data for *am2*. In cross no. 11 *am*-485/+* plants were crossed by *am*-489/+* plants. The progeny segregated 58 fertile plants and 21 sterile plants. All 27 of the fertile plants that were sampled contained microsporocytes that exhibited a normal meiotic phenotype, as can be seen in Figure 1. The Figure 1 chromosomes proceeded through a normal meiotic prophase (Figure 1a and b), 10 bivalents were formed (Figure 1c) and they proceeded with a normal reductional division at anaphase I (Figure 1d). All 14 of the sterile plants that were sampled contained microsporocytes that were normal appearing as predivision interphase meiocytes (Figure 1e) and, as they all proceeded into a normal-appearing mitotic prophase (Figure 1f and g), the chromosomes assembled on a metaphase plate and segregated at mitotic anaphase (Figure 1h) to produce two mitotic telophase nuclei (Figure 1i).

In all cases involving crosses with *am1-praI/+* as one parent the mutant male meiocytes entered meiosis and were blocked in prophase I of meiosis. In all other combinations of heterozygotes for the *am1* mutant alleles the male meiocytes from sterile plants were at some stage of mitotic division. Although it was evident from the cytological examination of the male meiocytes that *am1-praI* is dominant in heteroallelic combination with the other *am1* alleles, because the mutant phenotypes exhibited by heteroallelic combinations of the other four alleles were all the same (all microsporocytes divide mitotically), it was also evident that the dominance relationship among these four alleles could not be discerned by an examination of the male phenotypes.

Female phenotypes of the new mutant alleles in homoallelic combinations: The *am1-2* female meiocytes differed from their male counterparts. Approximately one fourth of the *am1-2* megasporocytes remained in an abnormal interphase but approximately three fourths of the megasporocytes proceeded through one or more mitotic divisions prior to degeneration (Table 2). The behavior and appearance of the chromosomes during the division was that of a typical mitosis (see below). Those megasporocytes that remain in abnormal interphase also degenerate but display no chromosomal condensation or other evidence of initiating either a mitotic or meiotic prophase. It is apparent that in their ovule environment a large proportion of the meiocytes could proceed with a nuclear division but only of a mitotic type.

As was the case for *am1-2*, the *am1-485* female meiocytes displayed a different developmental fate from that of their male counterparts. Whereas approximately one half of the megasporocytes remained in an abnormal interphase (see Figure 2a–c), the remainder of the megasporocytes proceeded through one or more mitotic divisions prior to degeneration. And, as was the case for *am1-2*, their appearance was that of normal mitotically dividing cells (Figure 2d–i). Occasionally three sequential mitoses produced a row of eight cells (Figure 2j). The female meiocytes that remained in an abnormal interphase showed no signs of entering into a mitotic or meiotic prophase prior to their degeneration. See GOLUBOVSKAYA *et al.* (1992) for a description of normal female meiosis in maize.

The *am1-489* female meiocytes displayed a different mutant phenotype from that of the male meiocytes, with most of the megasporocytes remaining in an abnormal interphase as shown in Figure 3. In the case of this mutant allele there was no evidence that an ovule environment could even partially relieve the blockage in proceeding with a nuclear division (Figure 3). The female meiocytes enlarged into elongated pear-shaped cells with dense cytoplasm as is typical of normal megasporocytes (Figure 3a), but as they continued to enlarge they became increasingly vacuolated (Figure 3b–f) and accumulated dark inclusions indicative of degeneration (Figure 3g–j).

FIGURE 1.—Micrographs of microsporocytes from the progeny of the cross *am*-485/+* × *am*-489/+*. (a–d) Normal microsporocytes of fertile segregant plants exhibiting a normal meiotic phenotype. (a) Interphase, a band of microsporocytes squeezed from anther; (b) prophase I of meiosis—zygotene stage; (c) metaphase I with 10 bivalents resulting from the complete synapsis of homologous chromosomes; (d) anaphase I, reductional chromosome segregation with 10 duplicated chromosomes migrating to each spindle pole. (e–i) Mutant microsporocytes of sterile segregant plants exhibiting mitosis in place of meiosis. (e) interphase; (f) early prophase of mitosis; (g) late prophase of mitosis with all 20 chromosomes of maize unsynapsed; (h) anaphase of mitosis; (i) telophase.

TABLE 2

Cytological characteristics of the female meiocytes in homoallelic and heteroallelic combinations of the *ameiotic 1* alleles

Genotype	No. of plants	No. of ovules	Mitotic cell division cycles: No. of normal cells: First division: Int[b]	Pr-Tel	Two cells	Second and third divisions (three to eight cells)	No. of abnormal cells[a] (cells blocked at interphase)	Meiosis	Embryo sac
			Homoallelic combinations						
am1-praI/am1-praI	8	449	0	0	0	0	0 (0)	361	88
am1/am1	5	389	2	173	176	38	0 (0)	0	0
am1-2/am1-2	2	195	42	32	64	8	49 (25)	0	0
am1-485/am1-485	7	333	24	10	95	58	146 (44)	0	0
am1-489/am1-489	4	264	33	0	9	1	216 (82)	0	5
			Heteroallelic combination[c]						
am1-1/am1-2	3	97	9	13	63	2	10 (10)	0	0
am1-1/am1-485	3	207	17	7	115	42	26 (13)	0	0
am1-1/am1-489	5	347	15	2	151	37	142 (41)	0	0
am1-2/am1-485	5	511	146	23	142	21	178 (35)	0	1
am1-485/am1-489	5	297	32	14	81	62	106 (36)	0	2

[a] These cells were readily distinguishable from normal cells in interphase because these cells were highly vacuolated and often displayed evidence of degeneration. Values are number of cells with percentage in parentheses.

[b] Those cells having a normal appearance at interphase of the cell cycle were placed under this heading. However some and possibly all of such cells could be destined to eventually become abnormal and blocked at interphase and would be scored accordingly if examined at a later time. The likelihood that these cells would proceed into mitosis is probably proportional to the frequency of cells scored in a mitotic stage.

[c] The dominance relationship is *am1-praI* > *am1-1* > *am1-2/am1-485* > *am1-489*. The position of *am1-praI* is based on studies of male meiosis while the positions of the remainder of the alleles is based on studies of female meiosis.

Dominance relationship among the *am1* alleles in heteroallelic combinations: The *am1-praI* allele is the least severe of the five *am1* alleles inasmuch as both male and female homozygous *am1-praI* meiocytes are able to proceed into early prophase I of meiosis. Our earlier analysis of male meiocytes with the heteroallelic genotype of *am1-praI/am1-1* revealed that such meiocytes could also advance to early prophase I of meiosis (GOLUBOVSKAYA *et al.* 1993), thereby establishing that *am1-praI* is dominant to the *am1-1* allele.

When *am1-praI/+* plants were crossed with *am1-1/+* plants, analysis of the progeny revealed that the *am1-praI/am1-1* plants were male sterile but their microsporocytes reached early prophase I of meiosis, confirming our earlier observations. The same result was obtained when *am1-praI* was combined with *am1-485*. This result

TABLE 3

Differences in male and female phenotypes of the five alleles of the *ameiotic1* gene

Alleles	Phenotypes of meiocytes: Male	Female
am1-praI	Meiosis stopped at the leptotene-zygotene stage	Meiosis stopped at the leptotene-zygotene stage
am1-1	Mitosis instead of meiosis	Mitosis instead of meiosis
am1-2	All meiocytes have am1-1 phenotype	Three fourths of the meiocytes go through mitosis and one fourth are blocked in interphase
am1-485	All meiocytes have am1-1 phenotype	One half of the meiocytes go through mitosis and one half are blocked in interphase
am1-489	All meiocytes have am1-1 phenotype	All meiocytes are blocked in interphase

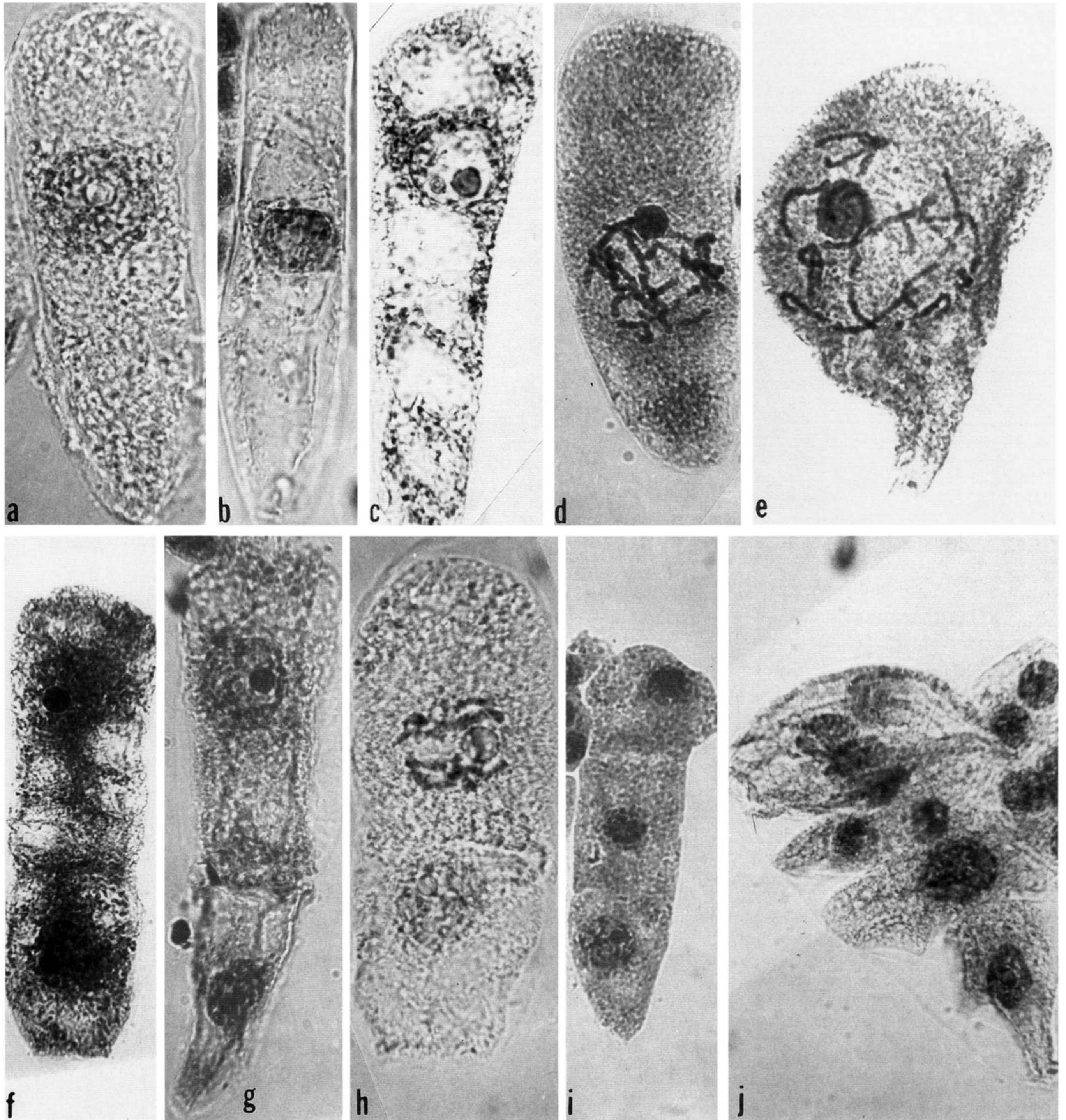

FIGURE 2.—Micrographs of female meiocytes in squash preparations of isolated MMCs from the *am1-485/am1-485* sterile plants. Approximately one half of the female meiocytes remain blocked at an abnormal interphase and become vacuolated (a–c), while the remainder enter prophase with typical appearing mitotic chromosomes visible (d and e), complete their division (f), and proceed with a second and third round of mitotic cell divisions (g–i), occasionally resulting in a linear array of eight cells (j).

demonstrated that *am1-praI* was dominant to *am1-1* and *am1-485*. Because *am1-1* is dominant to the other three alleles (see below) it is evident that *am1-praI* is dominant to all the other four alleles in male meiocytes. It was apparent that cytological analysis of heteroallelic combinations among the other four mutants (other than *am1-praI*) would not be informative when performed with male meiocytes because they all exhibit an identical phenotype as homozygotes (Table 3). However, the observation that all of the mutant alleles display different female phenotypes from *am1-1* and *am1-praI* and from each other (except for *am1-2* and *am1-485*, which

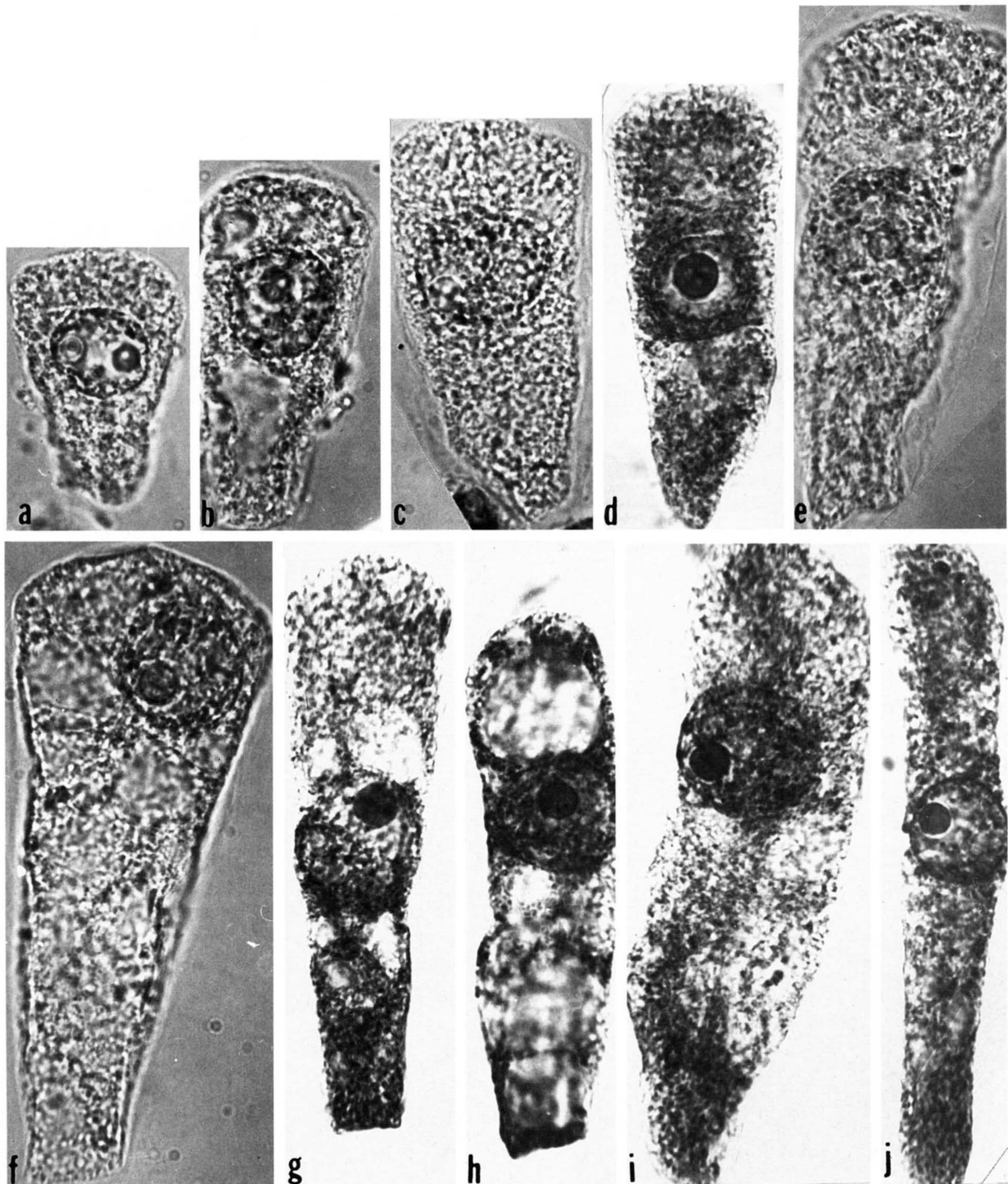

FIGURE 3.—Micrographs of female meiocytes in squash preparations of isolated MMC from the *am1-489/am1-489* sterile plants. (a–j) MMCs exhibit a block of mitotic and meiotic cell divisions. (a) The normal appearing archesporial cell has enlarged and widened at its apical end and appears ready to enter into meiosis; (b–f) growth and continued enlargement of the MMCs is evident, but they become increasingly vacuolated; (g–j) the cytoplasm develops darker staining regions and the MMCs become abnormal in shape, indicating the onset of degeneration of the MMCs.

are similar) indicated that dominance relationships could be evaluated by examining the female phenotypes of heteroallelic combinations among the four alleles (other than *am1-praI*).

Although *am1-1* is recessive to *am1-praI* it was found to be dominant to the three new alleles. This was evidenced by the observations that, when *am1-1* was combined with *am1-2*, *am1-485*, or *am1-489*, a large proportion of the megasporocytes underwent mitosis, as is typical of *am1-1* homozygotes (Table 2). For example, when

am1-1 was combined with *am1-489*, the *am1-1/am1-489* megasporocytes displayed a phenotype similar to either that observed in *am1-489/am1-489* or observed in *am1-1/am1-1* megasporocytes, with ~40% of the meiocytes remaining blocked in interphase and ~60% of them proceeding through mitosis (Figure 4). Those female meiocytes blocked at interphase had the same vacuolated, abnormal appearance (Figure 4a and b) as is typically displayed by *am1-489* homozygotes (compare with Figure 3); while those that divided proceeded with a normal mitotic prophase (Figure 4c) and metaphase (Figure 4d) to produce two normal-appearing daughter cells (Figure 4e), as is typically displayed by *am1-1* homozygotes. When the *am1-485* allele was combined with the *am1-489* allele essentially the same result was obtained as seen when *am1-485* was combined with the *am1-2* allele; approximately one third of the megasporocytes remained blocked in interphase but the other two thirds proceeded through a mitotic division (see Figure 4). The female meiocytes that remained in an abnormal interphase were highly vacuolated and displayed no signs of entering either a mitotic or a meiotic prophase (Figure 4f and g). Those meiocytes that underwent mitosis proceeded with a normal appearing mitotic early prophase (Figure 4h) and later prophase (Figure 4i) and underwent division to produce normal-appearing daughter cells (Figure 4j). These results established that *am1-485* is dominant to the *am1-489* allele and also demonstrate that the *am1-1* allele is dominant to the *am1-489* allele.

When the results of the cytological analysis of the heteroallelic combinations are taken as a whole (Table 2), they reveal a dominance relationship where *am1-praI* is dominant to the other four alleles, *am1-1* is recessive to *am1-praI* but dominant to the other three alleles, *am1-2* and *am1-485* are recessive to *am1-praI* and *am1-1* but are about equal to each other and are both dominant to *am1-489*, and the *am1-489* allele is recessive to the other four alleles. The dominance relationship is *am1-praI* > *am1-1* > *am1-2/am1-485* > *am1-489*. It should be noted that the dominance position of the *am1-pra1* allele is limited to male meiosis (Table 1 and Golubovskaya *et al.* 1993) while the remaining positions in the dominance relationship are based on studies with heteroallelic combinations in female meiocytes (Table 2).

An examination of the data in Table 2 reveals that not in every case where dominance was exhibited in the heteroallelic combination was the dominance complete. In some cases the phenotype observed was intermediate between that typical of the two alleles being tested. This was the case for the combination of *am1-1/am1-489*. Whereas *am1-1/am1-1* homozygotes had 0% female meiocytes blocked at an abnormal interphase and *am1-489/am1-489* homozygotes had 82% of them blocked, the *am1-1/am1-489* heteroallelic combination had 41% of the meiocytes blocked (Table 2). In other cases the megasporocytes developed to nearly the same extent and with the same appearance as that exhibited by megasporocytes homozygous for the dominant allele.

DISCUSSION

Female meiocytes are more severely affected than the male by the new *am1* alleles: The study of maize meiotic mutants has revealed that, generally, male meiocytes are more severely affected than are female meiocytes. This is evidenced by the observation that, whereas male sterility is usually complete, there may be some female fertility (Golubovskaya 1989; Golubovskaya *et al.* 1992). The results reported herein are, therefore, somewhat unexpected in that the three new alleles (*am1-2, am1-485,* and *am1-489*) condition a more severe phenotype in ovules than in anthers: although all of the *am1-2* or *am1-485* microsporocytes undergo a mitotic division only a portion of the *am1-2* or *am1-485* megasporocytes undergo mitosis; and, whereas all of the *am1-489* microsporocytes undergo a mitotic division, none of the *am1-489* megasporocytes divided (Table 3).

An explanation for the greater severity of expression of these mutant alleles in ovules than in anthers possibly may be found in considering the relative abundance of meiocytes in these two reproductive organs. An anther is comprised of four locules and each locule normally contains >100 microsporocytes while an ovule normally contains only a single megasporocyte (Kiesselbach 1949). Furthermore, the microsporocytes form a syncytium within each locule of the anther from the beginning of meiosis throughout prophase I of meiosis because the meiocytes are all interconnected by cytoplasmic bridges (Heslop-Harrison 1966).

The *am1-2, am1-485,* and *am1-489* alleles are recessive mutations that do not allow microsporocytes to enter meiotic prophase but do allow them to proceed through a mitotic division. This capacity to allow these meiocytes to proceed through a nuclear division, even though it is a mitotic rather than a meiotic division, suggests that these alleles do produce a gene product, although an abnormal one. The level of mutant gene product is likely to vary somewhat among the microsporocytes but, among the many meiocytes in a locule, some of them are likely to produce an amount that exceeds the threshold required to activate the division process. According to this hypothesis the diffusion of the gene product throughout the cytoplasmic continuum could result in the activation of all the meiocytes within a locule so that they would undergo a synchronous mitotic division. It is also possible that the differences in the behavior of the male and female meiocytes containing the new alleles is a reflection of the differences of the developmental history of meiocytes in anthers and ovules. These differences might be significant in explaining why all of the meiocytes in anthers of

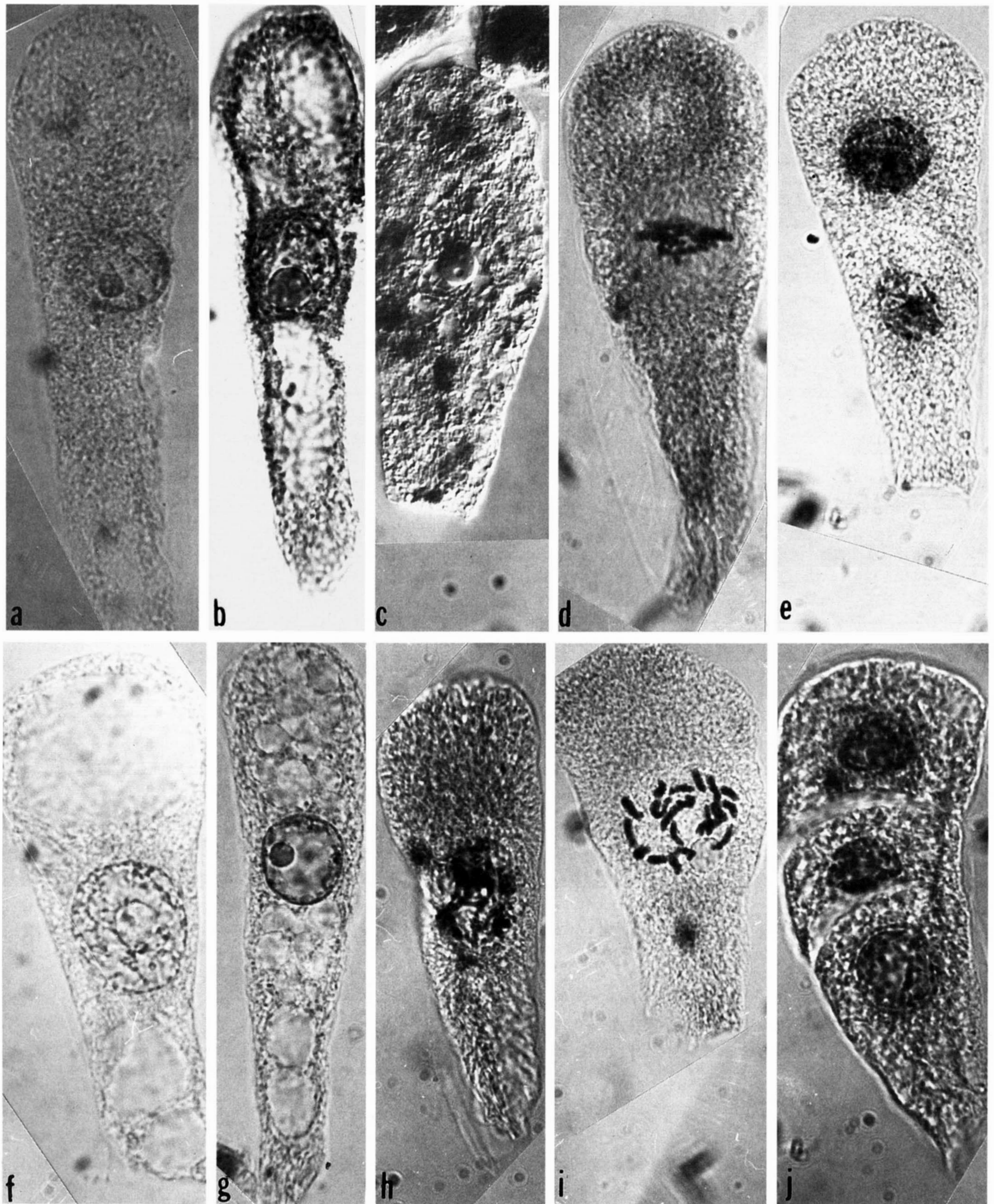

FIGURE 4.—Micrographs of female meiocytes in squash preparations of isolated MMCs. From the heteroallelic *am1-1/am1-489* sterile plants (a–e). Some MMCs (~40%) exhibit the block at an abnormal interphase with vacuolization and densification of the cytoplasm (a and b), while the remainder (~60%) of the MMCs undergo a normal-appearing mitotic prophase (c) and metaphase (d), to produce two normal-appearing daughter cells (e). From the heteroallelic *am1-485/am1-489* sterile plants (f–j). Some MMCs (~36%) are blocked at an abnormal interphase and become highly vacuolated (f and g) while others (~64%) proceed through a mitotic early prophase (h), and late prophase (i) and go on to divide to produce two or more normal-appearing daughter cells (j).

am1-489/am1-489 plants underwent mitosis but none of those in their ovules underwent mitosis.

Significance of the dominance relationship: All five mutant *am1* alleles in the homozygous state result in abnormal behavior of male meiocytes but in all five cases the microsporocytes can either enter meiotic prophase I (*am1-praI*) or proceed with a mitotic division (*am1-1, am1-2, am1-485, am1-489*). These behaviors indicate that all of the alleles enable the meiocytes to progress out of interphase and enter the M phase of the cell cycle. If the passage through the G_2/M checkpoint requires the activity of the *am1* locus, then the male meiocyte phenotypes suggest that all five alleles must be active at some minimal level and are, therefore, not null alleles. The tests of heteroallelic combinations of the different *am1* alleles reveal that a single dose of the dominant mutant allele is sufficient to enable the female meiocytes to express a phenotype that is at or approaching that typical of that allele. But a dosage effect might be exhibited by some of the alleles. The observation that the *am1-1/am1-1* female meiocytes all proceed through a normal mitotic cell division cycle while only ~60% of the *am1-1/am1-489* female meiocytes do so suggests that *am1-1* might exhibit a positive dosage effect. This could be tested by examining mutant female meiocytes from plants hypoploid for the *am1-1* and the other *am1* alleles.

Where in the cell cycle do the new *am1* alleles block the meiocytes? There is no uncertainty regarding the phase of the cell cycle where the meiocytes are blocked by the *am1-praI* allele: both male and female meiocytes are blocked during prophase I of meiosis. Likewise, their is no uncertainty regarding the *am1-1* allele: both male and female meiocytes fail to enter into prophase I of meiosis but they instead proceed with one to three mitotic divisions and then degenerate. It is noteworthy that the number of cellular divisions in the *am1-1* female meiocytes can be as many (three) as occurs during embryo sac formation. But in the latter situation cellularization is delayed until after the third mitosis (HUANG and SHERIDAN 1994).

For the three newly identified *am1* alleles the situation is not so clear. While all three alleles enable the male meiocytes to divide mitotically, either approximately one half (*am1-2, am1-485*) or all (*am1-489*) of the female meiocytes are blocked at interphase. Whether these blocked megasporocytes are stopped at the G_1, at the S, or at the G_2 phase of the cell cycle is not evident. The meiocytes that are blocked become increasingly vacuolated and eventually degenerate. Because the cellular progeny of the meiocytes that are not blocked but that proceed with mitosis also degenerate, as do the *am1-praI* meiocytes blocked at meiotic prophase I, it is likely that a program of cell death is activated in all of the mutant meiocytes by their failure to pass a meiotic checkpoint.

It appears likely that the blocked meiocytes are not stopped during either a *premeiotic* S or G_2 phase. This conclusion is based on the observation that the *am1-2, am1-485,* and *am1-489* alleles enable the male meiocytes to undergo a mitotic division and, in the case of the *am1-2* and *am1-485* female meiocytes, those female meiocytes that are able to divide do so mitotically. In both sexes, those meiocytes that divide mitotically are likely to have passed through the *premitotic* S and G_2 phases in preparation for mitosis and it is likely, therefore, that the female meiocytes that fail to divide are blocked in a premitotic interphase (either in S phase or G_2 phase). It is conceivable, of course, that the meiocytes of all of the *am1* alleles pass through a *premeiotic* S phase and those cells that are not blocked in either the S phase or G_2 phase undergo a switch to a mitotic developmental pathway and then proceed into a mitotic division. This would undoubtedly require numerous biochemical alterations in these cells, including changes in chromosome composition and behavior (JOHN 1990; STERN 1990).

It is unlikely that the blocked meiocytes are stopped in the G_1 phase. In the cereals all of the female meiocytes experience a lengthy G_1 period of several days, which extends from the time that the hypodermal cell is formed through the period when it enlarges to form an archesporial cell and continues through the lengthy period of maturation of this cell into the megasporocyte (BENNETT *et al.* 1973; BENNETT 1977). There is no intervening mitosis between the formation of the hypodermal cell and the onset of meiosis in the mature maize megasporocyte (COOPER 1937; KIESSELBACH 1949; SHERIDAN *et al.* 1996). Our observations indicate that the mutant female meiocytes are normal in appearance up to the time that their normal counterparts would enter meiotic prophase I. It seems likely that for all of the *am1* mutant alleles (except *am1-praI*), when the meiocytes exit G_1 they proceed with a mitotic-type S phase. The appearance of the chromosomes in the mitotically dividing meiocytes is that of completely normal mitotic chromosomes and this normalcy suggests that chromosome replication in a mutant meiocyte is like that of a normal cell destined for a mitotic division. Whether the blocked meiocytes proceed entirely through an S phase can be assayed by cytophotometric analysis of the DNA levels in these cells. If the blocked meiocytes have reached a full 4N level of DNA, then they will have completed the S phase and are therefore blocked in the G_2 phase.

The role of the *am1* gene in controlling the developmental fate of meiocytes: The role of the *ameiotic1* gene appears to be to control the switch from the mitotic cell cycle to the meiotic cell cycle in meiocytes and their initiation of meiotic prophase I. This view is based upon the observations that meiocytes containing a normal *am1* allele proceed through a normal set of meiotic divisions; those meiocytes lacking a normal allele but possessing an *am1-praI* allele proceed to enter meiotic

prophase I and progress as far as the early zygotene stage of meiosis, while those meiocytes carrying only the *am1-1* allele or the new alleles proceed with one or more mitotic divisions (*am1-2* or *am1-485*) or divide mitotically only in the male but are blocked from dividing in the female meiocytes (*am1-489*).

How might this gene control the switch from the mitotic pathway to the meiotic pathway? It may be that the *ameiotic1* gene codes for a protein that is unique to meiocytes and that controls a group of genes that are activated in meiocytes but remain silent in cells that will undergo mitosis.

We have no information as to the nature of the product of the *ameiotic1* gene. Although cell cycle phase-specific cyclin-dependent kinase variants have been reported that are coded for among six different *cdc2* homologous genes in alfalfa (Magyar *et al.* 1997), and four cyclins that form three distinct groups of mitotic cyclins have been cloned in maize (Renaudin *et al.* 1994), we are not aware of any evidence of a meiosis-specific cyclin or meiosis-specific cyclin-dependent kinase in any higher plant (Jacobs 1995; Pines 1995, 1996). But if the *am1* locus does code for a protein product, it is possible to consider how such a single gene product might function to control both the meiotic and the mitotic sequences. The two alternative fates of the meiocytes, entering meiosis or proceeding with mitosis, depending on their allelic constitution, is at least consistent with such a dual-control function for the product of this locus.

Possibly the fully translated sequence of the normal allele results in a protein that functions to direct the meiocytes into a premeiotic S phase and onward into meiosis. A somewhat truncated form resulting from a mutation may be sufficient to direct the cells into a meiotic S phase but one that is defective, so that the cells cannot pass the zygotene stage. Even more severely truncated forms of the protein resulting from more upstream mutations could act to direct the cells into mitosis instead of meiosis. The blockage of mitosis in female meiocytes carrying the new *am1* alleles is particularly significant inasmuch as its occurrence rules out the possibility that the mitotic divisions of mutant meiocytes reflect a simple default pathway for cells that cannot initiate meiosis.

An understanding of the role of the *ameiotic1* gene in controlling the initiation of meiosis is likely to come only from its molecular analysis. It is an especially interesting gene both because of its importance for the control of the meiotic pathway and because of the variety of phenotypes expressed by its different alleles.

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