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The zygote and proembryo of alfalfa: quantitative, three-dimensional analysis and implications for biparental plastid inheritance

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Summary. Genetic studies have demonstrated biparental inheritance of plastids in alfalfa. The ratio of paternal to maternal plastids in the progeny varies according to the genotypes of the parents, which can be classified as strong or weak transmitters of plastids. Previous cytological investigations of generative cells and male gametes have provided no consistent explanation for plastid inheritance patterns among genotypes. However, plastids in the mature egg cells of a strong female genotype (6-4) were found to be more numerous and larger than in mature eggs of a weak female genotype (CUF-B), and the plastids in 6-4 eggs are positioned equally around the nucleus. In CUF-B, the majority of plastids are positioned below (toward the micropyle) the mid level of the nucleus, which is the future division plane of the zygote. Since only the apical portion of the zygote produces the embryo proper, plastids in the basal portion were predicted to become included in the suspensor cells and not be inherited. In the present study, we examined zygotes and a two-celled proembryo from a cross between CUF-B and a strong male genotype (301), a cross that results in over 90% of the progeny possessing paternal plastids only. Our results indicate that the distribution of plastids observed in the CUF-B egg cell is maintained through the first division of the zygote. Further, paternal plastids are similarly distributed; however, within the apical portion of the zygote and in the apical cell of the two-celled proembryo, the number of paternal plastids is typically much greater than the number of maternal plastids. These findings suggest that maternal and paternal plastid distribution within the zygote is a significant factor determining the inheritance of maternal and paternal plastids in alfalfa.

Keywords: *Medicago sativa*; Plastid distribution; Plastid inheritance; 3-D reconstruction; Zygote; Embryo.

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Introduction

Alfalfa has been shown to exhibit biparental inheritance of plastids (Smith et al. 1986) and chloroplast DNA (Lee et al. 1988, Schumann and Hancock 1989, Masoud et al. 1990). The degree of transmission of paternal and maternal plastidy is genotype specific but, unlike other species exhibiting biparental plastid inheritance, there is consistently a strong paternal bias in alfalfa (Smith 1989, Schumann and Hancock 1989, Masoud et al. 1990). Cytological studies have shown that among genotypes characterized as strong, inter-

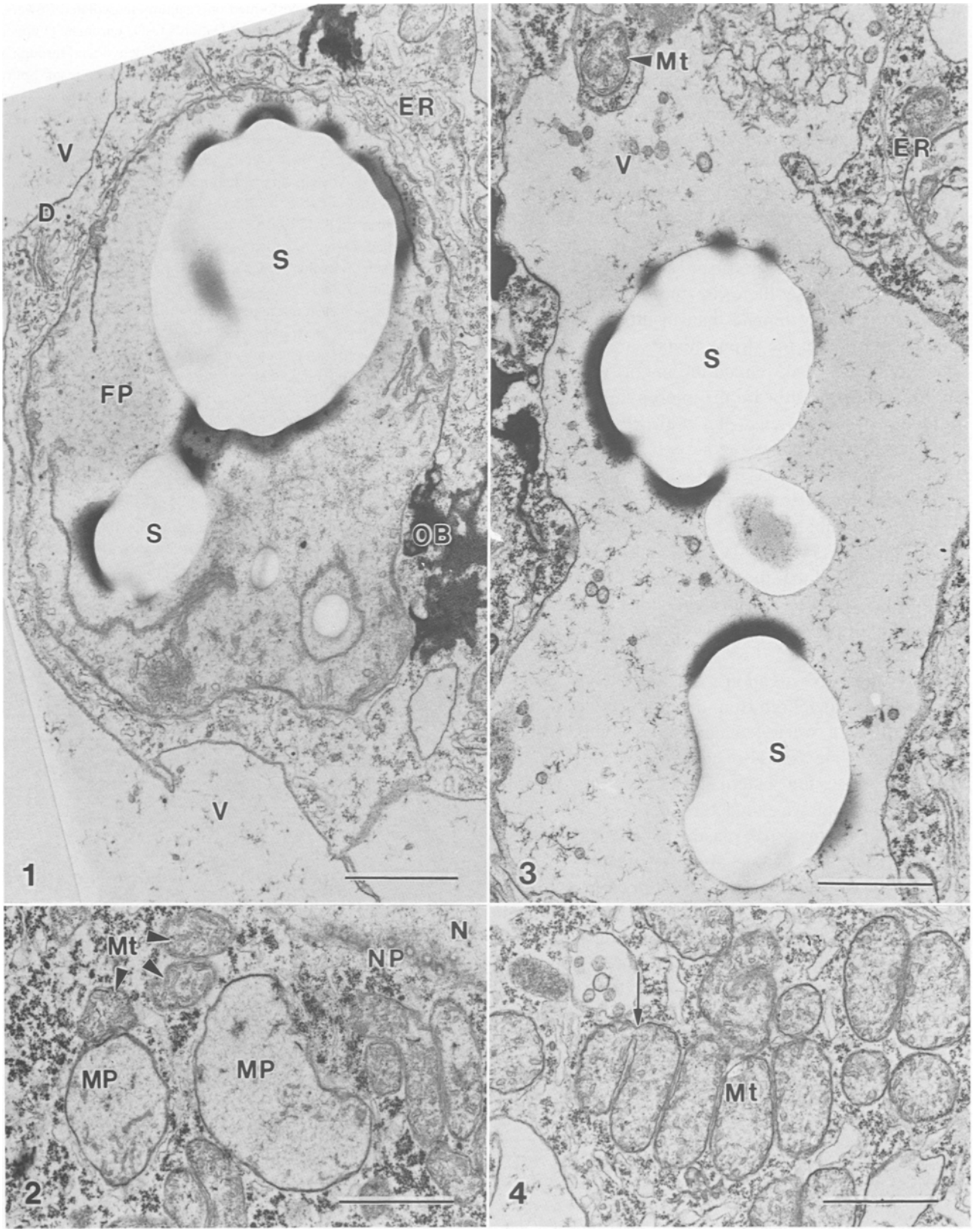
Figs. 1–4. Ultrastructural features of cytoplasmic components within the zygote and two-celled proembryo of alfalfa. Bars: 1 μm

Fig. 1. A large female plastid (*FP*) is shown within the fertilized egg. It contains starch grains (*S*) and a few internal membranes, and is in close proximity to endoplasmic reticulum (*ER*), a dictyosome (*D*), a vacuole (*V*), and osmiophilic bodies (*OB*)

Fig. 2. Male plastids (*MP*) within the zygote. These organelles are characterized by their smaller size compared to the maternal plastids, and the lack of starch grains. *Mt* Mitochondria, *N* nucleus, *NP* nuclear pores

Fig. 3. Starch grains (*S*) within a vacuole (*V*) of the two-celled proembryo. Presumably, this condition has resulted from the breakdown of maternal plastids by an autophagic vacuole. *ER* Endoplasmic reticulum, *Mt* mitochondrion

Fig. 4. Stacks of mitochondria as seen within the prophase zygote and both cells of the proembryo. Note that the mitochondrial matrix is continuous between some mitochondria of the stack (unlabelled arrows). *Mt* Mitochondria


ER
V
D
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OB
V
1
Mt
V
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NP
MP
MP
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Mt
4

mediate, or weak transmitters of paternal plastids there is no consistent relationship between the mean number of plastids or plastid nucleoids per generative cell and plastid inheritance patterns (Zhu et al. 1990, 1991; Shi et al. 1991). Moreover, brother sperm cells are essentially alike with respect to plastid numbers (Zhu et al. 1992).

Cytological studies of mature, unfertilized egg cells of genotypes characterized as weak or strong females, in terms of plastid transmission, reveal that plastids in the strong female are significantly larger than plastids in the weak female; there are more plastids in egg cells of the strong female than in the weak female; and the plastids in the strong female are perinuclear, whereas in the weak female the majority of plastids are located below (toward the micropyle) the equatorial plane of the nucleus (Zhu et al. 1993). In alfalfa, the first division of the zygote is transverse and unequal. Only the smaller apical cell of the two-celled proembryo gives rise to the embryo proper; the larger basal cell contributes to the suspensor, which eventually degenerates (Cooper 1935). Therefore, Zhu et al. (1993) suggested that the distribution, size, and number of plastids in the mature egg cell may influence the pattern of plastid inheritance among genotypes of alfalfa.

In the present investigation we analyzed zygotes and a two-celled proembryo from the cross between genotype CUF-B, a weak female, and genotype 301, a strong male. This cross results in over 90% of the progeny possessing paternal plastids only (Smith 1989). The goal was to determine: (1) whether the distribution of maternal plastids in the egg cell is maintained in the zygote after fertilization, (2) the distribution of paternal plastids within the zygote and two-celled proembryo, (3) whether paternal and maternal plastids maintain their own grouping or become mixed, and (4) the relative numbers and sizes of plastids of male and female origin. These features are discussed within the context of how they may influence plastid inheritance patterns in alfalfa.

Materials and methods

Alfalfa (*Medicago sativa* L.) plants used in this study were grown and maintained under the same conditions as those used in earlier investigations (Smith et al. 1986; Smith 1989; Zhu et al. 1990, 1993). Genotypes CUF-B and 301 were selected as the maternal and the paternal parents, respectively. Genotype CUF-B is considered a weak female and 301 a strong male because when crossed in this direction over 90% of the progeny contain paternal plastids only (Smith 1989).

Ovules were dissected under moist conditions 32, 48, and 52 h after hand pollinations were performed on vacuum-emasculated flowers. Ovules were fixed in cold (on ice) Dalton's OsO_4-chromate (Juniper et al. 1970) for 3.5 h, rinsed in distilled water, dehydrated through a graded ethanol-acetone series, and embedded in Spurr's resin (Spurr 1969). Embedded ovules were serially semithin sectioned at 3 µm for observation with differential interference contrast microscopy. Pertinent semithin sections were re-embedded (Mogensen 1971) and serially ultrathin sectioned. Ultrathin sections were collected on single slot, formvar coated, carbon-stabilized grids, stained with uranyl acetate and lead citrate (1 h in each) in an LKB Ultro-stainer, and viewed with a JEOL 1200 EX II transmission electron microscope. Primary images were recorded on Kodak electron microscope film no. 4489, then printed on Kodak Polycontrast III, RC printing paper. For three-dimensional reconstruction and quantitative analyses, relevant structures on each micrograph of a series were traced onto acetate transparencies, one transparency for each micrograph. Data were entered into an IBM-AT computer system for quantification as previously described (Young et al. 1987; Rusche 1990; Zhu et al. 1990, 1991). Final reconstructions, based on every third section, were created on a Silicon Graphics Personal Iris 4D-25 workstation using the Wavefront software program (Wavefront Technologies, Santa Barbara, CA).

Although ovules from all three collection times were semithin sectioned and examined at the light microscope level, the ovules used in this study were selected from the collection made 48 h after pollination, since several strategic stages of maturity were found. We report here on four stages: zygotes in interphase, prophase, and telophase; and the two-celled proembryo.

Results

Ultrastructure

In all stages studied, the cytoplasm consists of a complement of plastids, mitochondria, endoplasmic reticulum, dictyosomes, ribosomes and osmiophilic bodies as illustrated in Figs. 1–4.

Zygotes

The most prominent features of the zygote are the chalazally positioned nucleus (Figs. 5, 6, 8, and 10–12), and the large micropylar vacuole. The latter contains cytoplasmic inclusions as well as invaginations of cytoplasm (Figs. 5, 6, and 8). Plastids are irregularly shaped, have a double-membraned envelope, contain few internal membranes and, occasionally, cytoplasmic invaginations are present. Endoplasmic reticulum is commonly closely associated with the plastids (Fig. 1). Two types of plastids occur: large plastids containing starch grains (amyloplasts; Fig. 1) and smaller, starch-free plastids (proplastids; Fig. 2). Based upon results from previous studies (Zhu et al. 1992, 1993) of sperm and egg cell plastids of the genotypes used in this study, we interpreted the large amyloplasts as maternal plastids and the smaller proplastids as paternal plastids.

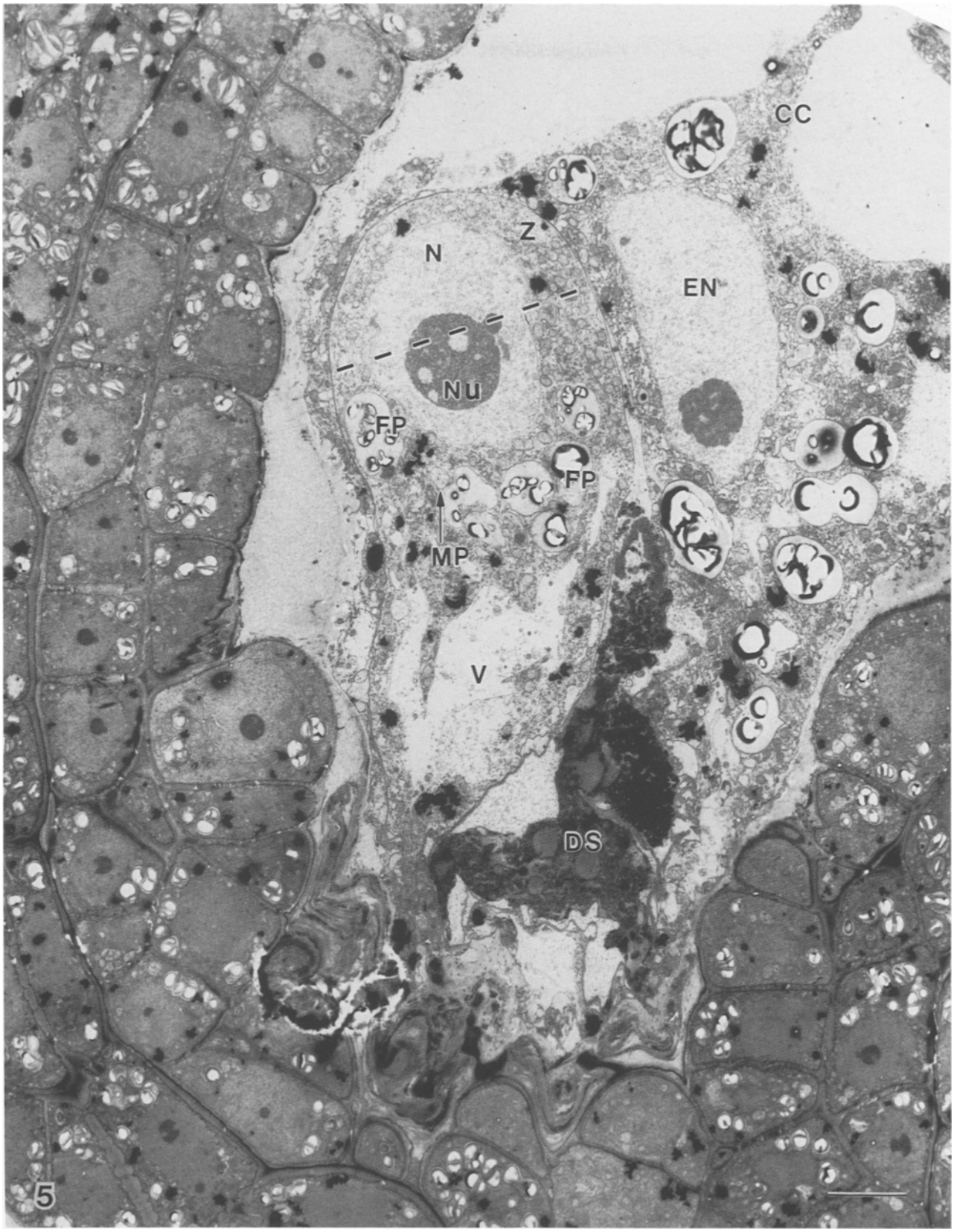


Fig. 5. Electron micrograph of a near median longisection through an alfalfa zygote (*Z*) at interphase. Note the numerous female plastids (*FP*) located below (toward the micropyle) the projected future division plane of the zygote (dashed line). A reconstruction of this zygote is shown in Fig. 10. *CC* Central cells, *DS* degenerated synergid, *EN* free nuclear endosperm, *MP* male plastid, *N* nucleus, *Nu* nucleolus. Bar: 5 μm

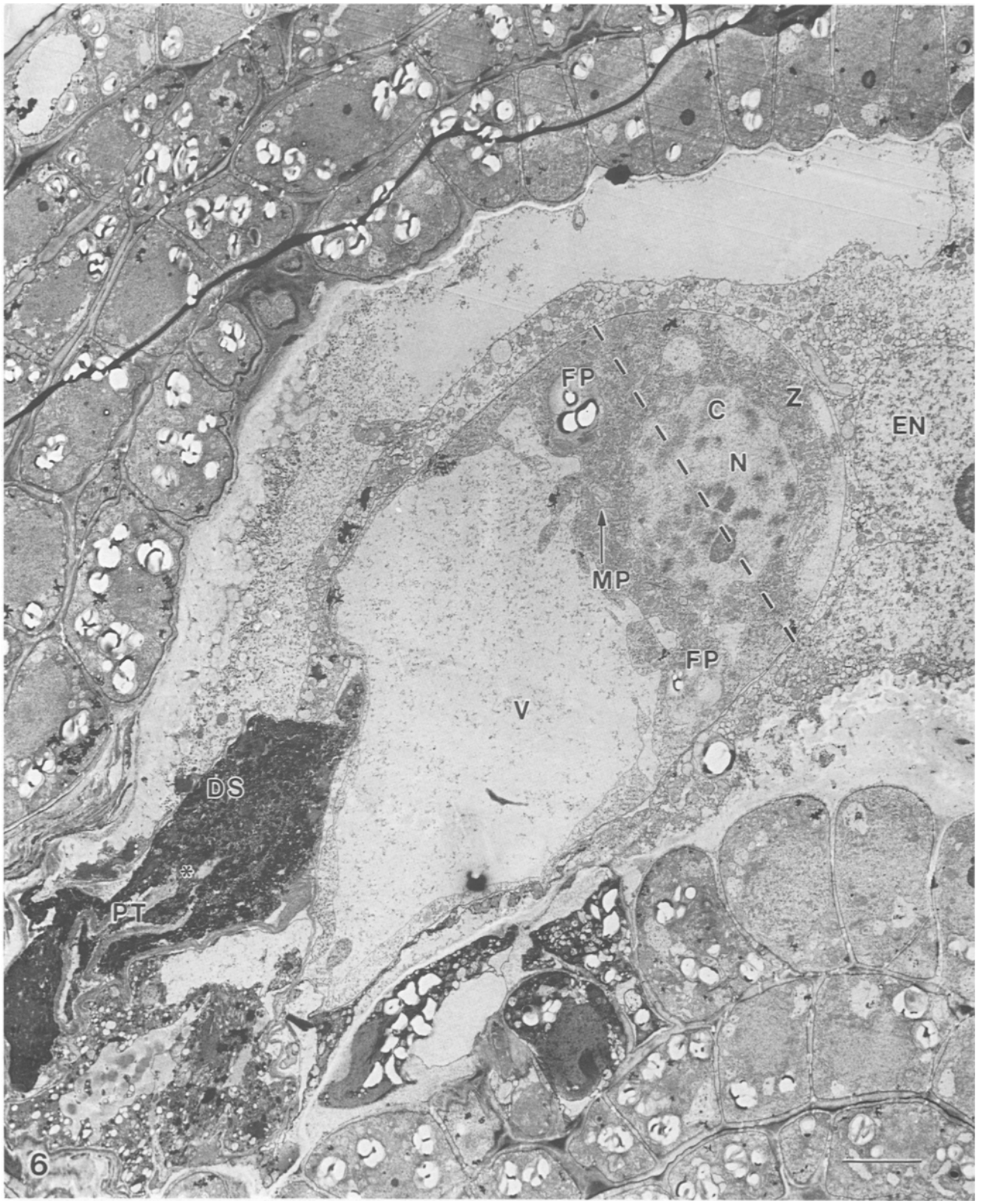
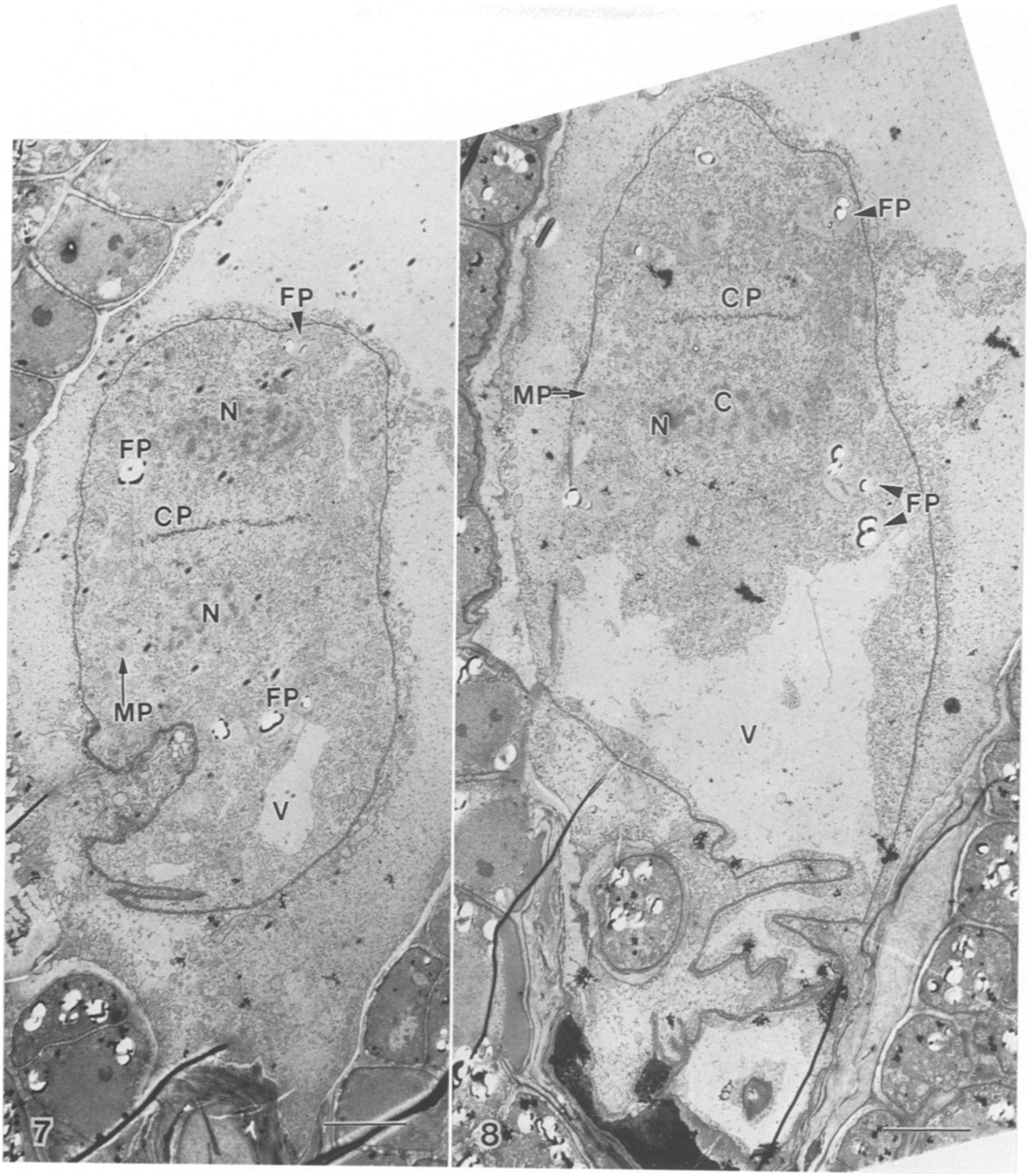


Fig. 6. Electron micrograph of a near median longisection of an alfalfa zygote (*Z*) at prophase. Two female plastids (*FP*) and a male plastid (*MP*) are seen in this section. The dashed line represents the projected future division plane of the zygote. A discharged pollen tube (*PT*) with a terminal pore (asterisk) is seen within the degenerated synergid (*DS*). A reconstruction of this zygote is shown in Fig. 11. *C* Condensed chromosomes, *EN* free nuclear endosperm, *N* nucleus, *V* vacuole. Bar: 5 µm



Figs. 7 and **8.** Electron micrographs of two longisections through the alfalfa zygote at telophase. A reconstruction of this cell is shown in Fig. 12. Bars: 5 μm

Fig. 7. The developing cell plate (*CP*) is seen between portions of the two newly formed nuclei (*N*). Female (*FP*) and male plastids (*MP*) are present in this section. Only a portion of the large micropylar vacuole (*V*) is visible

Fig. 8. Near median longisection showing a larger portion of the basal nucleus (*N*) than in Fig. 7, the forming cell plate (*CP*), and the micropylar vacuole (*V*). Female (*FP*) and male plastids (*MP*) are present. *C* Chromosomes

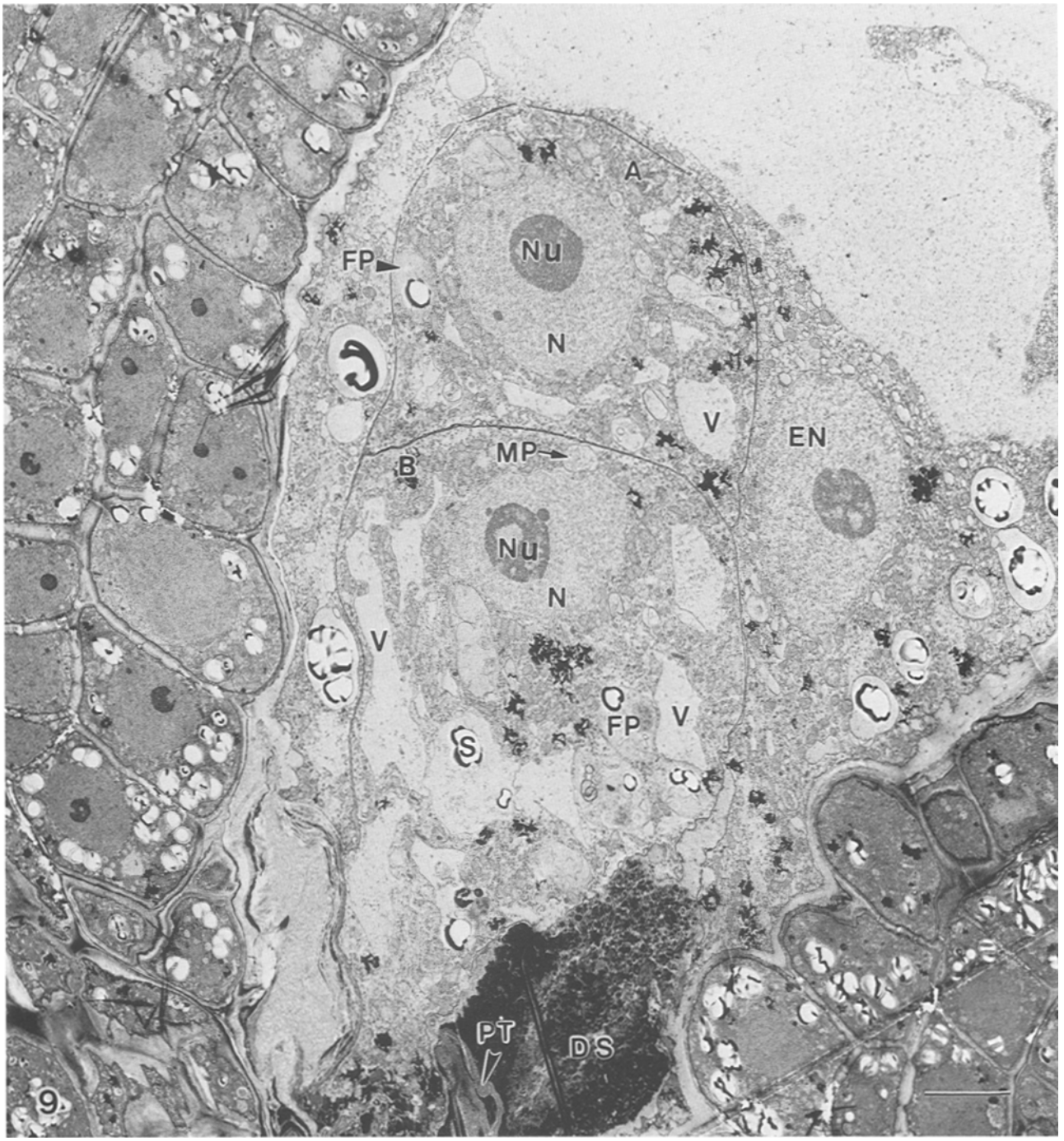


Fig. 9. Electron micrograph of a near median longisection of a two-celled proembryo of alfalfa. Note the smaller apical cell (*A*) and the larger basal cell (*B*). Each cell contains several small vacuoles (*V*) and a nucleus (*N*) with a conspicuous nucleolus (*Nu*). Female (*FP*) and male plastids (*MP*) are also seen. *EN* Free nuclear endosperm, *DS* degenerated synergid, *PT* pollen tube, *S* starch grain. A reconstruction of this proembryo is shown in Fig. 13. Bar: 5 μm

In contrast to the plastids, mitochondria are smaller, have numerous cristae, and have no cytoplasmic intrusions (Figs. 2–4). Their shape ranges from discoid to tubular or reticulate. In the prophase stage zygote we observed "stacks" of up to 8 mitochondria in the apical and basal regions of the cell. Continuity between elements of the stacks was sometimes observed (Fig. 4). In each zygote reconstructed, a pol-

Table 1. Comparison of quantitative data from three alfalfa zygotes and a two-celled proembryo

	Zygotes			Two-celled proembryo		
	interphase	prophase	telophase	apical	basal	total
Protoplast						
Volume[a]	17155.9	28390.9	20968.9	8768.5	18832.9	27601.4
Surface area[b]	3664.7	4412.5	3989.9	2212.6	3787.7	6000.3
Nucleus						
Volume	2151.7	2196.5	622.5[c]	1139.5	1216.4	2355.9
Surface area	767.1	783.9	474.9[c]	486.1	551.2	1037.3
Nucleolus						
Volume	255.3	281.4	–	118.7	119.0	237.7
Surface area	242.8	257.7	–	114.6	157.1	261.7
Maternal plastids						
Total volume	1295.0	1231.5	1054.8	424.8	1465.1	1889.9
Volume/plastid	48.0	68.4	20.7	53.1	52.3	52.5
Surface area	1762.2	1351.6	1926.9	493.1	1702.0	2195.1
Number	27	18	51	8	28	36
apical	3	1	13	–	–	–
basal	24	17	38	–	–	–
Paternal plastids						
Total volume	100.1	50.0	207.0	113.7	194.1	307.8
Volume/plastid	1.8	1.4	4.7	3.6	3.8	3.7
Surface area	349.6	222.3	589.6	328.0	574.5	902.5
Number	57	37	44	32	51	83
apical	22	14	14	–	–	–
basal	35	23	30	–	–	–

[a] Volume in μm^3
[b] Surface area in μm^2
[c] Sum of two newly formed nuclei

len tube had discharged within the degenerated synergid, as illustrated in Fig. 6, and free-nuclear endosperm was present (Figs. 5 and 6).

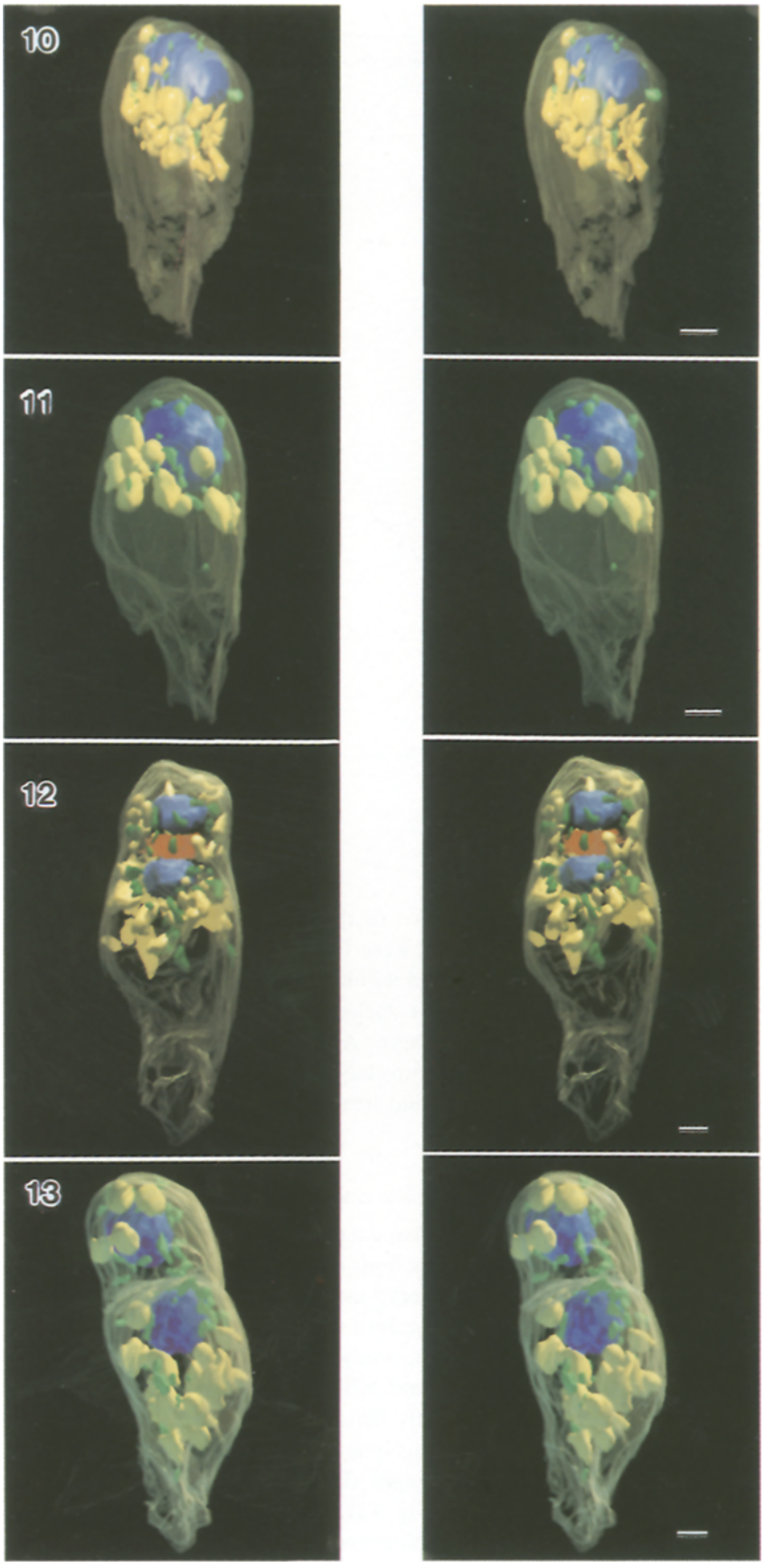
Proembryo

The basal cell of the two-celled proembryo reconstructed had a volume 2.15 times larger than that of the apical cell (Table 1). The nucleus of the apical cell was centrally located, whereas that of the basal cell was more chalazal (Figs. 9 and 13). Each cell had an extensive vacuole system consisting of several smaller vacuoles lateral to the nucleus or in the micropylar part of the cell (Fig. 9). Plasmodesmata were present between the cells. Both maternal and paternal plastids were recognizable in the proembryo cells, as described for the zygotes. All but two of the maternal plastids in the apical cell and all but one in the basal cell were found immediately adjacent to, or within 0.2 μm of a vacuole. Starch grains, unassociated with a plastid membrane system, were seen within vacuoles (Fig. 3). Such starch grains occur singly, or in clusters of two or three, as is the case in plastids. Starch grains were found within one vacuole of the apical cell and within five vacuoles of the basal cell. Mitochondrial stacks (Fig. 4) were seen in both cells of the proembryo. A pollen tube had penetrated and discharged into the degenerated synergid of this embryo sac, and free-nuclear endosperm was present (Fig. 9).

Quantitative data and distribution of plastids

The quantitative data obtained from computer-generated reconstructions of three zygote stages and a two-celled proembryo are presented in Table 1. Because only one example of each stage was reconstructed, the data cannot be analyzed statistically; however, there is a clear trend with regard to plastid distribution within the cells. This distributional pattern is evident in three-dimensional reconstructions showing each of the zygote stages (Figs. 10–12) and the two-celled proembryo (Fig. 13).

In all stages observed, including numerous examples



Figs. 10–13. Stereo pairs of computer-generated reconstructions of zygotes (Figs. 10–12) and a proembryo (Fig. 13) of alfalfa. Within the transparent outer limits of the cells, the distribution of the yellow female plastids can be seen to be primarily below (toward the micropyle) the mid transverse division plane of the nucleus (blue) in the zygotes, and mostly within the basal cell of the proembryo. The green male plastids are also more numerous in the basal than in the apical regions, but more male than female plastids are present in the apical areas. Bars: 5 μm

Fig. 10. Zygote at interphase. An electron micrograph of a near median longisection of this cell is shown in Fig. 5

Fig. 11. Zygote at prophase. An electron micrograph of a near median longisection of this cell is shown in Fig. 6

Fig. 12. Zygote at telophase. Electron micrographs of longisections of this cell are shown in Figs. 7 and 8. Orange area represents the developing cell plate

Fig. 13. Two-celled proembryo. The nuclei are shown in blue. A near median longisection of this proembryo is shown in Fig. 9

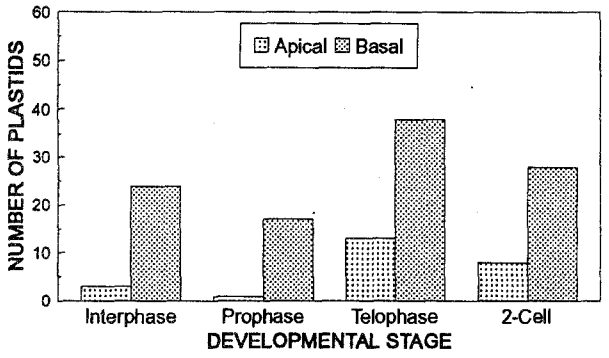


Fig. 14. Comparison of maternal plastid numbers and distribution within the apical and basal portions of the zygotes and apical and basal cells of the proembryo. At each stage, the number of maternal plastids in the apical regions is considerably less than that in the basal areas. Data derived from Table 1. See text for an explanation of apical and basal portions

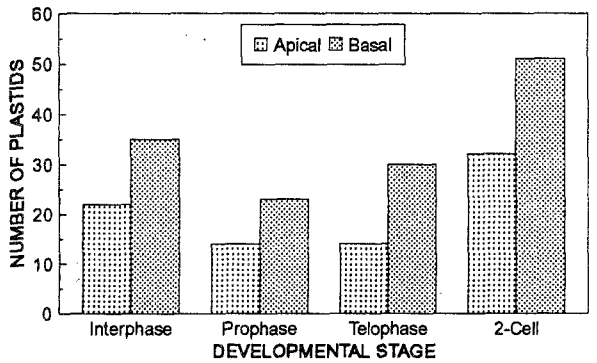


Fig. 15. Comparison of paternal plastid numbers and distribution within the apical and basal portions of the zygotes and apical and basal cells of the proembryo. At each stage, the number of paternal plastids in the apical regions is less than that in the basal areas, but not to as great a degree as in the case of the maternal plastids (Fig. 14). Data derived from Table 1. See text for an explanation of apical and basal portions

at the light microscopic level that were not quantified, the distribution of maternal plastids is essentially the same as that observed in the unfertilized egg cells of genotype CUF-B (Zhu et al. 1993). If a projected division plane is drawn through the zygote nucleus (based on early embryogenic studies by Cooper 1935), the pre-metaphase cell can be divided into a chalazal or apical portion and a micropylar or basal portion (Figs. 5 and 6) (Zhu et al. 1993). The cell plate clearly divides the telophase stage cell into an apical and basal portion (Figs. 7 and 8). In each stage, the number of maternal plastids is greater in the basal part of the zygote and proembryo than in the apical

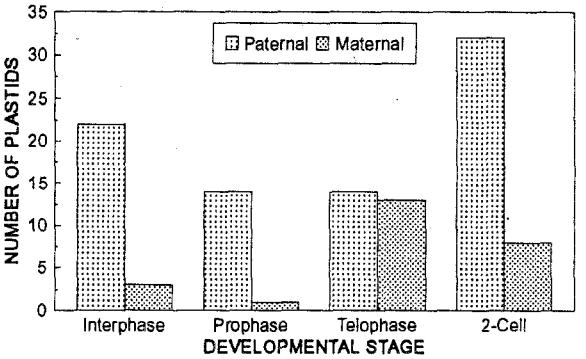


Fig. 16. Comparison of maternal and paternal plastid numbers within the apical portion of the zygote and apical cell of the proembryo. At each stage, except the telophase zygote, there are many more paternal than maternal plastids. Data derived from Table 1. See text for an explanation of apical and basal portions

region as illustrated graphically in Fig. 14. Figure 15 shows that this same distributional pattern holds for the paternal plastids; however, within the apical region of each example, except the telophase cell, the number of paternal plastids is considerably greater than the number of maternal plastids as shown in Fig. 16.

Discussion

That double fertilization had occurred in all of the ovules utilized in this study is evidenced by the presence of a discharged pollen tube within the degenerated synergid of each embryo sac, and by the presence of free-nuclear endosperm in each example. The general organization and ultrastructure of the zygotes of this study are typical of that of most angiosperms investigated (Natesh and Rau 1984). The pyriform cell has a distinct polarity with the nucleus and most of the cytoplasm located in the chalazal portion, and a large vacuole occupying most of the rest of the cell. The groups of stacked mitochondria observed in the prophase zygote and the two-celled proembryo of this study have not been previously reported to our knowledge. No such mitochondrial configurations were observed in two ultrastructural studies of *Medicago* embryogenesis (Sangduen et al. 1983a, b); but these studies concentrated primarily on later stages of embryo development than did our study. The significance of such mitochondrial organization is not apparent at this time.

Another apparently unique feature of the alfalfa zygote, at least in certain genotypes, is the high concentration of plastids below (toward the micropyle)

the mid transverse plane of the nucleus. The usual distribution of plastids, as well as the other components of the cytoplasm, in the angiosperm zygote is perinuclear (Natesh and Rau 1984). In a report based on a light microscopic study of embryogenesis in *Medicago sativa*, Cooper (1935) illustrated a similar distributional pattern of plastids as we observed; however, no mention of this plastid distribution was made, and its potential significance regarding plastid inheritance was not recognized. The genotype used in Cooper's study was not identified.

Previous studies have shown that the egg cell plastids of alfalfa genotype CUF-B are many times larger than the plastids in the sperm cells of genotype 301. In addition, nearly all of the plastids in the CUF-B egg cell contain starch grains, whereas the sperm cell plastids contain no starch grains (Zhu et al. 1992, 1993). Thus, in this study we interpreted the larger plastids containing starch grains, the amyloplasts, to be of maternal origin and the smaller plastids without starch grains, the proplastids, to be of paternal origin. The plastids identified as maternal in the present study have, on average, volumes that are 14 times greater than those of the plastids identified as paternal (Table 1). The same criteria, i.e., volume differences and the presence or absence of starch grains, were also used to distinguish between maternal and paternal plastids within the zygotes of *Oenothera erythrosepala* (Meyer and Stubbe 1974). However, the maternal plastids in this species had a mean volume only 8 times larger than that of the paternal plastids.

Zhu et al. (1993) have suggested that a contributing factor to alfalfa genotype CUF-B being a poor maternal plastid transmitter is the fact that the plastids within unfertilized egg cells of this genotype are positioned primarily below the future division plane of the zygote. Three-dimensional reconstructions derived from serial ultrathin sections of five immature and five mature egg cells show that this pattern of plastid distribution is established in the young egg cell (Zhu et al. 1993). Zhu et al. (1993) predicted that those plastids located below the projected division plane of the zygote would ultimately become included in the basal (micropylar) cell of the two-celled proembryo and, consequently, not be inherited because the descendants of this cell become part of the suspensor, which eventually degenerates. In contrast, plastid distribution within egg cells of a strong female plastid transmitter (genotype 6-4) were found to be positioned equally around the nucleus and, thus, many more maternal plastids would likely be included in the apical cell of the two-celled proembryo and be inherited by the next generation (Zhu et al. 1993).

The results of the present study lend strong support to the hypothesis that plastid inheritance patterns in alfalfa are influenced by plastid distribution within the unfertilized egg cell. We have shown that the plastid distribution established in the egg cell of CUF-B is maintained throughout zygote maturation, and is reflected in the number of maternal plastids contained in the apical and basal cells of the two-celled proembryo.

Our results further demonstrate that paternal plastids become distributed in a pattern similar to that of the maternal plastids after fertilization. However, the differential between the numbers of paternal plastids in the apical and basal portions is not as extreme as in the case of maternal plastids (Table 1; Figs. 14 and 15).

Even though both maternal and paternal plastids are distributed similarly within the zygote and two-celled proembryo and become largely mixed rather than remaining in separate groups, there still remains, in all but the telophase stage zygote of the present study, considerably more paternal than maternal plastids in the apical position (Table 1; Fig. 16). The greater proportion of paternal to maternal plastids in the apical cell of the two-celled proembryo is consistent with a genetic study that demonstrates a strong paternal bias with regard to plastid inheritance resulting from the cross of the present study (CUF-B $\times$ 301; Smith 1989). The nearly equal numbers of maternal (Fig. 14; Table 1) and paternal (Fig. 15; Table 1) plastids in the apical portion of the telophase stage zygote may represent an example of a progeny that would possess both maternal and paternal plastids. Alternatively, since some of the derivatives of the apical cell also contribute to the suspensor (Cooper 1935), some or all of the maternal plastids could still be sorted into a determinate cell lineage. Other workers have speculated that plastid distribution within the zygote of certain species may play a deciding role in biparental plastid inheritance (Meyer and Stubbe 1974, Smith 1989, Tilney-Bassett and Almouslem 1989), but this is the first documentation of such a phenomenon.

Mechanisms controlling the pattern of plastid distribution within the egg, zygote and proembryo of alfalfa are not known, but a cytoskeletal involvement is likely. A microtubular network has been observed in

the various cells of the embryo sac in *Arabidopsis* (Webb and Gunning 1991), *Nicotiana* (Huang and Russell 1992), *Plumbago* (Huang et al. 1993), and *Zea mays* (Huang and Sheridan 1994). Maternal plastids may travel and distribute themselves along a microtubular array. Following gametic union, the paternal plastids may use the same microtubular network. The cytoskeletal network of the egg cell and zygote of alfalfa is currently being investigated.

Based upon counts from three pairs of reconstructed sperm cells from alfalfa genotype 301, each sperm cell contains approximately 70 plastids (Zhu et al. 1992). The zygotes of the present study were found to contain 57, 37, and 44 (Table 1) paternal plastids, indicating perhaps that some paternal plastids were not identifiable within the zygotes, that they were excluded from the egg cell at the time of syngamy, or that they had degenerated or been discarded from the sperm cells before fertilization. The higher number of male plastids (83; Table 1) within the two-celled proembryo could mean that the male plastids have multiplied by this stage but, of course, sampling error alone may account for the differences in plastid numbers we observed among stages and additional studies are needed to clarify this.

In the interphase and prophase stage zygotes, we found 27 and 18 maternal plastids, respectively (Table 1). In the telophase zygote we identified 51 maternal plastids, and in the two-celled proembryo a total of 36 maternal plastids were found (Table 1). This could mean that the maternal plastids multiply during zygote maturation, then decrease in number after the first zygotic division. However, counts from many more examples are needed before any conclusions can be drawn with regard to the dynamics of maternal plastid numbers during zygote maturation and early embryogeny in alfalfa.

Starch grains within vacuoles were found in the two-celled proembryo (Fig. 3) but not in the zygotes or unfertilized egg cells of genotype CUF-B, indicating that degradation of maternal plastids may begin during this stage. The close association of maternal plastids with the endoplasmic reticulum and vacuoles observed in this study is consistent with a process involving the formation of autophagic vacuoles, derived from endoplasmic reticulum membranes, in which the plastids are degraded by hydrolytic enzymes (Wink 1993). Such plastid degeneration could further affect plastid inheritance patterns. A low number of plastids and apparent plastid degeneration within the eggs of *Daucus muricatus* have been implicated in paternal plastid inheritance in the cross *D. muricatus* × *D. carota* (Hause 1991, Boblenz et al. 1990). Differential multiplication rates between maternal and paternal plastids during embryogenesis is considered to be an important determinant in biparental plastid inheritance frequencies in *Oenothera* (Chiu et al. 1988, Chiu and Sears 1993). Preferential degeneration of maternal or paternal plastids, or their DNA, is thought to be unlikely for this plant (Chiu et al. 1988), since a quantitative study of *Oenothera* zygotes showed no sign of plastid degeneration, and plastids from both parental types increased during zygote maturation (Meyer and Stubbe 1974). However, as seen in the present study, preferential plastid degeneration could occur during later stages of embryogenesis.

Shi et al. (1991) found that a large proportion of the generative cell plastids in alfalfa genotype 301 do not contain DNA detectable by the DNA-specific fluorochrome, DAPI. This finding suggests that DNA degradation or, at least, modification occurs within many paternal plastids prior to fertilization. It also raises the question of whether a mechanism resulting in maternal plastid DNA degradation/modification occurs within the egg, zygote and/or embryo of alfalfa. Any such process, whether acting on paternal or maternal plastids, could have compounding effects on plastid inheritance frequencies. Studies to determine the DNA content of plastids during alfalfa embryogenesis are in progress.

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