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Quantitative cytology of the alfalfa generative cell and its relation to male plastid inheritance patterns in three genotypes

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Summary. Studies utilizing restriction analysis of plastid DNA, as well as those employing chlorophyll-deficient mutants, have shown a high frequency of paternal plastid transmission in alfalfa. Recent research has also shown that plastid inheritance patterns among alfalfa genotypes and are under genetic control. In a previous study we were unable to detect any correlations between qualitative, three-dimensional ultrastructure of generative cells and male plastid transmission strength in certain genotypes. In the present study we used serial ultrathin sectioning, computerized reconstruction and quantitation, and stereology to further analyze generative cells within mature pollen. Measurements included volumes and surface areas of cells, nuclei, and organelles, as well as organelle number and distribution. Three genotypes were investigated, one that is a strong transmitter of male plastids (genotype 301), one that is a weaker transmitter of male plastids (genotype 7W), and a third that is an even weaker male plastid transmitter (genotype MS-5). Our results show that genotype MS-5 has significantly fewer plastids/generative cell than either of the other genotypes, which may account for it being a relatively poor transmitter of male plastids. However, plastid number does not explain known differences in male plastid inheritance between genotypes 301 and 7W, since plastid number does not differ significantly between these two genotypes. Regarding the other features of generative cells measured in this study, no consistent correlations were found that might account for differences in male plastid inheritance patterns between genotypes. Plastid distribution is equal in each end of the spindle-shaped generative cell in all genotypes studied. Similar relative results were found with regard to mitochondria within generative cells; however, comparative genetic data are not available on mitochondrial transmission patterns in alfalfa genotypes.

Key words: *Medicago sativa* – Biparental cytoplasmic inheritance – Plastid transmission – Three-dimensional reconstruction

Introduction

Several recent investigations have demonstrated a predominance of paternal plastid inheritance in alfalfa. Similar results were obtained from studies utilizing analysis of plastid DNA restriction fragment length polymorphisms (Lee et al. 1988; Schumann and Hancock 1989; Masoud et al. 1990) and those using chlorophyll-deficient mutants (Smith et al. 1986; Smith 1989 b).

In a previous study we examined qualitative aspects of alfalfa generative cell structure and three-dimensional organization. We found that generative cells have basically similar morphology in the genotypes investigated. Althoug certain genotypes were known from genetic studies to be "strong" or "weak" transmitters of male plastids (Smith 1989 b), no correlations between generative cell structure and plastid transmission behavior were observed (Zhu et al. 1990).

Here we report quantitative data derived from computer-generated reconstructions of nine mature generative cells (three from each of three genotypes), as well as morphometric data based upon 75 generative cells (25 from each of the three genotypes). The objective of this study was to further characterize the generative cells of alfalfa to determine whether generative cell structure is related to relative male plastid transmission strength in a given genotype.

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Table 1. Quantitative data from three mature generative cells from each of three alfalfa genotypes[1]

	MS-5 (n=3)		301 (n=3)		7W (n=3)	
	Mean	SD	Mean	SD	Mean	SD
Protoplast						
Volume[2]	133.87[a4]	8.34	160.32[a]	46.17	206.43[a]	41.04
Surface area[3]	213.70[a]	26.92	215.00[a]	51.94	235.77[a]	39.86
S/V	1.60[a]	0.25	1.35[ab]	0.08	1.15[b]	0.11
Nucleus						
Volume	55.47[b]	2.25	72.26[ab]	21.65	90.71[a]	12.69
Surface area	76.83[a]	6.26	99.23[a]	25.10	103.12[a]	12.14
S/V	1.39[a]	0.16	1.38[a]	0.09	1.14[a]	0.14
% VOP	41.40[a]	1.06	45.03[a]	1.06	44.26[a]	2.58
Plastid						
Volume	12.45[a]	1.80	12.69[a]	4.72	16.67[a]	1.22
V/Plastid	0.21[a]	0.07	0.07[b]	0.03	0.07[b]	0.03
Surface area	155.70[b]	22.13	189.81[b]	60.41	276.75[a]	21.96
S/Plastid	2.52[a]	0.10	1.08[b]	0.28	1.13[b]	0.31
S/V	12.84[a]	3.46	15.26[a]	1.20	16.66[a]	1.89
Number	62.00[b]	10.44	174.67[ab]	26.31	264.67[a]	107.60
% VOP	9.27[a]	0.74	7.76[a]	0.79	8.22[a]	1.17
% VOC	15.82[a]	1.03	14.14[a]	1.62	14.82[a]	2.61
Mitochondrion						
Volume	0.17[c]	0.07	0.80[b]	0.24	1.56[a]	0.44
V/Mitochondrion	0.03[a]	0.03	0.02[a]	0.001	0.001[a]	0.001
Surface area	3.39[b]	1.31	16.76[a]	4.97	27.89[a]	8.57
S/Mitochondrion	0.68[a]	0.49	0.37[a]	0.21	0.26[a]	0.08
S/V	20.37[a]	0.34	18.98[a]	2.61	18.03[a]	2.98
Number	6.67[c]	4.62	54.00[b]	26.21	110.33[a]	21.13
% VOP	0.13[c]	0.05	0.51[b]	0.12	0.75[a]	0.15
% VOC	0.21[c]	0.09	0.92[b]	0.18	1.35[a]	0.29

[1] Data are computer-generated from reconstructed generative cells
[2] Volume is expressed as μm^3
[3] Surface area is expressed as μm^2
[4] Means within a row followed by a different letter are significantly different ($P<0.05$) according to Waller-Duncan K-ratio test
V=volume; S=surface area;
% VOP=percent volume of protoplast
% VOC=percent volume of cytoplasm, where cytoplasmic volume=V of protoplast −V of nucleus

Materials and methods

Materials for this study were taken from the same fixations used in a previous investigation on the qualitative ultrastructure of alfalfa generative cells. Details of ancestry of the genotypes studied, plant growing conditions, and tissue processing schedules have been described previously (Smith et al. 1986; Smith 1989b; Zhu et al. 1990). All data reported here were taken from generative cells within mature, dehisced pollen, and only flowers from green sectors (nonmutant) were used.

Using a chlorophyll-deficient mutant as a marker, Smith (1989b; unpublished results) has characterized certain genotypes according to their relative plastid transmission strength when used either as male or female parents. Genotypes 301 and 7W were selected for this study because of their high fertility as paternal parents and because they exhibit significantly different plastid transmission behavior. When genotype 301 is crossed as the male parent with a weak female (CUFB), 96% of the progeny contain paternal plastids, with over 90% of the progeny having only male plastids. Crosses with genotype 7W as the male parent and CUFB as the female parent produce 97% progeny containing some male plastids, but only an average of 62% of the progeny have purely male plastids (Smith 1989b). The third genotype used in this study, MS-5, has been studied less extensively as a male parent, but preliminary results indicate that MS-5 is a much poorer transmitter of male plastids than is 301. In crosses with a moderately weak female, 53.8% of progeny had purely paternal 301 plastids, whereas only 9.5% of progeny had purely paternal MS-5 plastids (S. E. Smith, unpublished results).

All measurements taken from the nine generative cells reconstructed from serial ultrathin sections were computer-generated using the program of Young et al. (1987). Data, other than number of organelles, for comparing the two ends of the generative cells were obtained by using the computer to divide the generative cells into their two longest halves; that is, beginning at the center of the nucleus and continuing to one end of the cell, the data were totaled for that half of the cell. The same procedure was used for the other half of the cell. The half with the more tapered end (Zhu et al. 1990) was designated half B, the other as half A. In order to be able to use the above procedure, it is necessary for the cells to have been originally sectioned in a near transverse plane; therefore, it was only possible to obtain values of percent volume of protoplast and percent volume of cytoplasm for the organelles (plastids and mitochondria) of two generative cells in MS-5 and one generative cell each in 301 and 7W.

Table 2. Results of stereological analysis of plastids and mitochondria in generative cells from each of three alfalfa genotypes[1]

Genotype	Plastid		Mitochondria	
	Volume density (%)[2]	Volume[3]	Volume density (%)[2]	Volume[3]
MS-5	29.42±18.40	23.06±14.34	1.29±3.23[a]	1.00±2.50[a]
301	26.26±14.48	23.45±14.02	3.83±5.25	3.37±4.75[b]
7W	28.87±16.27	33.41±20.12[c]	4.12±6.17	4.77±7.25

[1] Data were derived from 25 generative cells from each genotype (total of 75 cells)
[2] Volume density was based upon relative percent volume of cytoplasm
[3] Values were derived by converting volume density to absolute cytoplasmic volume calculated from Table 1, i.e. (volume of protoplast − volume of nucleus) × volume density % = absolute organellar volume
[a] Significantly less than either 301 or 7W ($P<0.05$)
[b] Significantly larger than MS-5 and significantly less than 7W ($P<0.05$)
[c] Significantly larger than either MS-5 or 301 ($P<0.05$)

Morphometric data were obtained from random ultrathin sections of 75 generative cells (25 from each of the three genotypes). Grids on transparent plastic overlays were randomly placed over photographic prints of each section, and point counts were made according to the procedures described by Weibel (1979) and Steer (1981). Data presented are expressed as relative percent volume of cytoplasm. Analysis of variance and a Waller-Duncan *K*-ratio test were conducted for comparisons of differences between mean values among genotypes. Proportional data were transformed into square roots before analysis.

Results

Generative cell and nucleus

No significant differences were detected between the three genotypes in mean generative cell volume or surface area (Table 1). Nuclear volume (Table 1) is significantly greater in 7W than in MS-5; however, the differences between mean nuclear volumes of 7W and 301 and between MS-5 and 301 are not significant. Nuclear surface areas do not differ significantly between the three genotypes (Table 1).

Plastids

The mean plastid number/cell is significantly lower in MS-5 (62.0) than in either 7W (264.7) or 301 (174.7), but the differences between 7W and 301 are not significant (Table 1).

No significant differences were found between the genotypes in mean total plastid volume/generative cell using data from reconstructed cells (Table 1). However, when the volume density of plastids (relative percent volume of cytoplasm) is converted to absolute plastid volume/generative cell, it is found to be significantly larger in 7W than in either 301 or MS-5, but no significant differences in mean absolute plastid volume/generative cell occur between 301 and MS-5 (Table 2). No significant differences (Table 1) were found among the three genotypes in the percent of the cytoplasmic volume occupied by plastids: 301 = 14.1%, 7W = 14.8%, MS-5 = 15.8%. A similar result was obtained from the morphometric analysis (Table 2); that is, no significant differences were found in the relative percent volume of cytoplasm occupied by plastids among the three genotypes: 301 = 26.2%, 7W = 28.8%, MS-5 = 29.4%.

Total plastid surface area/generative cell is significantly greater in 7W (276.7 μm^2) than MS-5 (155.7 μm^2; Table 1). The mean volume/plastid is significantly larger in MS-5 (0.2 μm^3), than in 301 (0.07 μm^3) or 7W (0.07 μm^3).

Mean surface area/pastid (Table 1) is also significantly greater in MS-5 (2.52 μm^2) than in 301 (1.08 μm^2) or 7W (1.13 μm^2). However, genotypes 301 and 7W do not differ significantly in this trait (Table 1).

The number, volume, surface area, and other aspects of plastids compared within each end of the generative cell do not differ significantly in any genotype (Table 3).

Mitochondria

Mean mitochondrial number/generative cell (Table 1) differs significantly among all three genotypes, with 7W having the most (110.3), followed by 301 (54.0) and MS-5 (6.7). Nevertheless, the mean total volume of mitochondria/generative cell (Table 1) is not significantly different in 301 (0.80 μm^3) and 7W (1.56 μm^3), but is significantly less in MS-5 (0.17 μm^3; Table 1) than in either of the other two genotypes. A similar result was obtained from the stereological analyses when volume density of mitochondria/generative cell was converted to absolute volume of mitochondria/generative cell; that is, the mean for MS-5 is significantly less than that of either 7W or 301, but no significant differences were found between 7W and 301 (Table 2).

The mean volume or surface area/mitochondrion does not differ significantly among genotypes (Table 1). However, mean total surface area of mitochondria/generative cell (Table 1) is significantly larger in 7W (27.9 μm^2) and 301 (16.8 μm^2) than in MS-5 (3.4 μm^2).

The percent of cytoplasmic volume occupied by mitochondria is significantly less in MS-5 than in either 301 or 7W, but does not differ significantly between 301 and 7W (Table 1). Results were the same in relative terms using data from reconstructions or from stereology (Tables 1 and 2).

Comparisons of generative cell halves show no significant differences in any of the features measured except for mitochondrial number, which differs significantly in MS-5 (Table 3).

Table 3. Comparison of plastid and mitochondria in each half of the generative cell in three genotypes [1]

	MS-5 (n=3)		301 (n=3)		7W (n=3)	
	Half A	Half B	Half A	Half B	Half A	Half B
Plastids						
Volume [2]	6.17± 0.50	6.28±1.64	5.70± 2.00	6.99± 2.73	8.41± 0.44	8.26± 0.79
Surface area [3]	76.55±20.37	79.16±7.93	85.47±17.97	104.35±43.08	141.64±15.90	135.11±13.67
S/V	12.50± 3.60	13.13±3.43	13.95± 1.16	14.78± 1.25	16.91± 2.50	16.39± 1.56
Number	29.67± 8.54	32.00±3.61	76.67±11.50	88.67± 3.79	144.00±27.62	136.33±21.22
% VOP	9.31± 0.40	8.37±0.08	7.68	9.16	7.26	9.26
% VOC	15.29± 0.80	14.64±0.22	15.07	16.45	12.00	20.94
Mitochondria						
Volume	0.07± 0.09	0.20±0.14	0.35± 0.09	0.45± 0.20	0.75± 0.08	0.80± 0.36
Surface area	1.38± 1.38	3.75±2.48	7.66± 1.88	9.11± 4.04	14.17± 3.87	13.73± 5.36
S/V	13.69±11.87	21.13±3.72	21.93± 0.63	20.23± 1.81	18.66± 3.55	17.65± 2.82
Number	2.00± 2.64*	4.67±2.08*	26.33±13.87	30.67±19.66	56.33±15.14	59.33± 6.81
% VOP	0.06± 0.08	0.12±0.08	0.44	0.33	0.75	1.10
% VOC	0.10± 0.14	0.39±0.40	0.87	0.59	1.23	2.44

[1] Data are computer-generated from reconstructed generative cells. Values represent mean ± standard deviation
[2] Volume expressed in μm^3
[3] Surface area expressed in μm^2
S = surface area; V = volume
% VOP = percent volume of protoplast; % VOC = percent volume of cytoplasm where cytoplasm volume = V of protoplast − V of nucleus. Due to the plane of sectioning, it was only possible to derive these values from two cells of MS-5 and one cell each of 301 and 7W
* Mean mitochondrial number differs significantly between the two cell halves in genotype MS-5 ($P < 0.05$)

Discussion

In approximately two-thirds of the flowering plants investigated, plastid inheritance is strictly maternal. In those species exhibiting biparental plastid inheritance, there is typically a predominance of maternal plastid transmission. In alfalfa, however, there is high transmission of paternal plastids (Kirk and Tilney-Bassett 1978; Sears 1980; Corriveau and Coleman 1988; Schumann and Hancock 1989; Smith 1989a, b; Masoud et al. 1990). Several hypotheses have been presented to explain the observed differences in plastid transmission behavior including the following:

(1) Differential input of plastids. Typically, the plastids are excluded from the generative cell during the first microspore division, or they may degenerate or be sloughed off during generative cell or sperm cell maturation (Clauhs and Grun 1977; Hagemann and Schröder 1989; Mogensen and Rusche 1985). In one species the two sperms of a pair differ greatly in their plastid number (Russell 1984). Exclusion of male plastids during gametic fusion is another possibility (Mogensen 1988).

(2) Degradation of plastid DNA. This may occur during pollen development (Miyamura et al. 1987; Corriveau and Coleman 1988) or, perhaps, differentially within the zygote (Sager and Kitchin 1975).

(3) Gene conversion. In this mechanism it is postulated that recombination occurs between different plastomes in the zygote, with subsequent DNA repair through the use of the strong plastome as the template (Sager 1972; Tilney-Basset and Birky 1981; Chiu et al. 1988).

(4) Differential multiplication rates between plastids from each parent within the zygote and embryo (Chiu et al. 1988).

(5) Differential sequestering of plastids into the apical and basal cells of the two-celled embryo, with the plastids of the basal cell ending up in the suspensor (Chiu et al. 1988).

As expected from the results of genetic studies on alfalfa, all generative cells of the three genotypes of the present study contain large numbers of plastids, and considerable variation in the number of plastids per generative cell occurs between genotypes. However, contrary to what might be expected, only one of the weak males (MS-5) contains significantly fewer plastids per generative cell (mean of 62 plastids) than either of the other males. The other weak male (7W) has the largest mean number of plastids per generative cell (over 250 plastids) among all three genotypes, although the number is not significantly greater in 7W than in 301, which has a mean of 175 plastids/generative cell. These results may indicate that, even though 7W is a weaker male than 301, it is still much stronger than MS-5. The difference in male plastid input potential between 301 and 7W is not sufficient to override other factors affecting plastid inheritance such as those listed above. The significantly lower plastid input potential of MS-5 may be below a critical threshold

level that results in a lower frequency of male plastid transmission regardless of other factors. Alternatively, it is possible that the actual number of plastids, as determined by electron microscopy, does not reflect the number of plastid genomes present, i. e., perhaps not all of the plastids of 7W generative cells contain DNA. There is evidence from several species of the Cucurbitaceae that the number of mitochondria may be greater than the number of mitochondrial genomes in certain cells (Bendich and Gauriloff 1984). It is also possible that male plastid number changes sometime after the stage studied here, or that male plastids are excluded differentially (according to genotype) at the time of gametic fusion.

A pattern similar to that seen in the number of plastids per generative cell is also seen with regard to generative cell size (volume and surface area), generative nuclear size (volume and surface area), and total plastid amount (total plastid volume and total plastid surface area/generative cell). That is, all of these parameters are greatest in genotype 7W and smallest in MS-5. However, none of the differences appears to be significant between 7W and 301, except for the absolute plastid volume calculated from a combination of reconstruction and stereological data (Table 2), and for this parameter, the values for 301 and MS-5 are nearly identical (Table 2). Thus, it does not appear that these physical features of generative cells are associated with plastid transmission strength.

The mean size (volume and surface area) of the individual generative cell plastids is very similar in 7W and 301 (Table 1), yet male plastid transmission behavior is quite different in these two genotypes. Therefore, plastid size is not a determining factor in this case. The presence of significantly larger plastids in genotype MS-5 generative cells is curious, since MS-5 is apparently the least successful transmitter of male plastids among the three genotypes. Whether the larger plastids of MS-5 are causally related to its male plastid transmission behavior cannot be determined from the results of this study.

We measured plastid distribution within generative cells because, at least in one species, *Plumbago zeylanica* (Russell et al. 1988), polarization of the plastids at one end of the cell leads to one of the sperms of a pair receiving few, if any, plastids. Dimorphic sperm cells with regard to plastids could be related to male plastid transmission behavior if double fertilization were regulated such that preferential fusion occurred between a given sperm and the egg cell. In *Plumbago zeylanica*, the plastid-rich sperm nearly always fertilizes the egg (Russell 1985). In the present study we found no significant differences in plastid distribution within the generative cells of any of the genotypes. Consequently, we do not expect that sperm dimorphism with regard to plastids will be found in alfalfa. Likewise, the other quantitative measurements comparing the two ends of the generative cells (Table 3) would not appear to be sufficiently different to lead to sperm dimorphism in alfalfa.

The same general trends found for plastids are true for mitochondria of alfalfa generative cells (Tables 1–3). However, it is difficult to assess the mitochondrial data at this point, since no genetic comparisons have been made among genotypes with regard to mitochondrial transmission behavior. Schumann and Hancock (1989) did not detect male transmission of DNA restriction fragments between plants from two subspecies of alfalfa. However, studies of the transmission of large RNA molecules, apparently located within mitochondria, suggest that biparental inheritance of mitochondria may occur in alfalfa (Fairbanks et al. 1988). Based upon potential input alone, data from the present study suggest that genotype MS-5 may be deficient in mitochondrial number (mean of 6.6 mitochondria/generative cell), whereas 301 and 7W potentially have greater capacity for paternal mitochondrial inheritance (mean of 54.0 and 110.3 mitochondria/generative cell, respectively).

Obtaining very similar relative results from the two methods used in this study, i.e., reconstructions from serial ultrathin sections and stereological analysis, is gratifying. However, the differences in actual values are puzzling at this point, especially since we have checked the computer reconstruction program against objects of known volumes. In other studies that have applied these same two approaches, similar results were obtained as in the present study in that the morphometric mean values were generally higher and had much greater standard deviations (Wagner et al. 1989; Mogensen et al. 1990). Although the differences in actual values obtained from the two techniques do not affect the conclusions reached here, it is apparent that caution should be exercised if direct comparisons are to be made between data derived from the two methods.

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References

Bendich AJ, Gauriloff LP (1984) Morphometric analysis of cucurbit mitochondria: the relationship between chondriome volume and DNA content. Protoplasma 119:1–7

Chiu W-L, Stubbe W, Sears BB (1988) Plastid inheritance in *Oenothera*: organelle genome modifies the exdtent of biparental plastid transmission. Curr Genet 13:181–189

Clauhs RP, Grun P (1977) Changes in plastid and mitochondrial content during maturation of generative cells of *Solanum* (Solanaceae). Am J Bot 64:377–383

Corriveau JL, Coleman AW (1988) Rapid method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am J Bot 75:1443–1458

Fairbanks DJ, Smith SE, Brown JK (1988) Inheritance of large mitochondrial RNAs in alfalfa. Theor Appl Genet 76:619–622

Hagemann R, Schröder MB (1989) The cytological basis of the plastid inheritance in angiosperms. Protoplasma 152:57–64

Kirk JTO, Tilney-Bassett RAE (1978) The plastids: their chemistry, structure, growth and inheritance, 2nd edn. Elsevier/North-Holland Biomedical Press, Amsterdam

Lee DJ, Blake TK, Smith SE (1988) Biparental inheritance of chloroplast DNA and the existence of heteroplasmic cells in alfalfa. Theor Appl Genet 76:545–549

Masoud SA, Johnson LB, Sorensen EL (1990) High transmission of paternal plastid DNA in alfalfa plants demonstrated by restriction fragment polymorphic analysis. Theor Appl Genet 79:49–55

Miyamura S, Kuraoiwa T, Nagata T (1987) Disappearance of plastid and mitochondrial nucleoids during the formation of generative cells of higher plants revealed by fluorescence microscopy. Protoplasma 141:149–159

Mogensen HL (1988) Exclusion of male mitochondria and plastids during syngamy in barley as a basis for maternal inheritance. Proc Natl Acad Sci USA 85:2594–2597

Mogensen HL, Rusche ML (1985) Quantitative ultrastructural analysis of barley sperm. I. Occurrence and mechanism of cytoplasm and organelle reduction and the question of sperm dimorphism. Protoplasma 128:1–13

Mogensen HL, Wagner VT, Dumas C (1990) Quantitative, three-dimensional ultrastructure of isolated corn (*Zea mays*) sperm cells. Protoplasma 153:136–140

Russell SD (1984) Ultrastructure of the sperm of *Plumbago zeylanica*. II. Quantitative cytology and three-dimensional organization. Planta 162:385–391

Russell SD (1985) Preferential fertilization in *Plumbago*: ultrastructural evidence for gamete-level recognition in an angiosperm. Proc Natl Acad Sci USA 82:6129–6132

Russell SD, Strout GW, Thompson RA, Mislan TW, Schoemann LM (1988) Generative cell polarization in *Plumbago zeylanica*. In: Wilms HJ, Keijzer CJ (eds) Plant sperm cells as tools for biotechnology. Pudoc, Wageningen pp. 17–26

Sager R (1972) Cytoplasmic genes and organelles. Academic Press, New York

Sager R, Kitchin R (1975) Selective silencing of eukaryotic DNA. Science 189:426–433

Schumann CM, Hancock JF (1989) Paternal inheritance of plastids in *Medicago sativa*. Theor Appl Genet 78:862–866

Sears BB (1980) Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4:233–255

Smith SE (1989a) Biparental inheritance of organelles and its implications in crop improvement. Plant Breed Rev 9:361–393

Smith SE (1989b) Evidence for genetic control of plastid inheritance in *Medicago sativa*. J Hered 80:214–217

Smith SE, Bingham ET, Fulton RW (1986) Transmission of chlorophyll deficiences in *Medicago sativa*, evidence for biparental inheritance of plastids. J Hered 77:35–38

Steer MW (1981) Understanding cell structure. Cambridge University Press, Cambridge

Tilney-Bassett RAE, Birky CW Jr (1981) The mechanism of the mixed inheritance of chloroplast genes in *Pelargonium*. Theor Appl Genet 60:43–53

Wagner VT, Dumas C, Mogensen HL (1989) Morphometric analysis of isolated *Zea mays* sperm. J Cell Sci 93:179–184

Weibel ER (1979) Stereological method, vol 1. Practical methods for biological morphometry. Academic Press, London

Young SJ, Royer SM, Goves PM, Kinnamon JC (1987) Three-dimensional reconstructions from serial micrographs using the IBM PC. J Electron Microsc Technol 6:207–218

Zhu T, Mogensen HL, Smith SE (1990) Generative cell composition and its relation to male plastid inheritance patterns in *Medicago sativa*. Protoplasma (in press)