THE HOWS AND WHYS OF CYTOPLASMIC INHERITANCE
IN SEED PLANTS

H. LLOYD MOGENSEN

Department of Biological Sciences, Box 5640, Northern Arizona University, Flagstaff, Arizona 86011

Cytoplasmic organelles are inherited in a non-Mendelian fashion in all eukaryotic organisms investigated. Among the seed plants, plastids can be inherited strictly from the female parent, strictly from the male parent, or biparentally. Most flowering plants studied to date exhibit maternal plastid inheritance, but approximately one-third of the genera investigated display biparental plastid inheritance to some degree. Among the gymnosperms, paternal plastid inheritance is the rule in the conifers, whereas the other groups appear to have maternal plastid inheritance, although they have been less well studied. Mitochondrial inheritance is predominantly maternal in the seed plants, except for a few coniferous families where it is predominantly paternal. The advent of recombinant DNA technology has allowed restriction fragment length polymorphisms to be used as molecular markers, and has stimulated much research in organelle inheritance and its application to studies of population genetics and phylogenetic biology. This report emphasizes the various mechanisms by which organelles are, or are not, transmitted among the seed plants in order that researchers directly or indirectly involved with organelle inheritance may better understand the potential and the limitations of their investigations. A summary and discussion of the possible evolutionary significance of the various patterns of cytoplasmic inheritance among the seed plants are also included.

Key words: cytoplasmic inheritance; DAPI; mitochondrial inheritance; organelle inheritance; plastids; restriction fragment length polymorphisms.

Cytoplasmic inheritance has been of interest to plant biologists since it was first demonstrated early in the 1900s that chloroplasts of flowering plants are not transmitted according to the rules of Mendelian genetics (reviewed in Kirk and Tilney-Bassett, 1978; Birky, 1994). The vast majority of flowering plants studied to date transmit their plastids (general term usually referring to chloroplasts or their precursors) exclusively or predominantly from the female parent to sexual progeny. Except for the conifers, the same generalization can be made for the rest of the plant kingdom, although exceptions are known within all groups (Kirk and Tilney-Bassett, 1978; Sears, 1980; Whatley, 1982; Corriveau and Coleman, 1988; Hagemann and Schröder, 1989; Smith, 1989a). Much less is known about mitochondrial inheritance, but the existing evidence indicates that strictly maternal transmission of mitochondria is even more common among flowering plants than uniparental maternal inheritance of plastids (Smith, 1989a; Harrison and Doyle, 1990; Radetzky, 1990; Forsthoefer, Bohnert, and Smith, 1992; Rajora et al., 1992).

Among the gymnosperms, the conifers investigated inherit plastids exclusively or predominantly from the male parent, whereas Ephedra, Ginkgo, and the cycads most probably exhibit uniparental maternal inheritance of both plastids and mitochondria. Mitochondria are inherited predominantly from the female parent in the Pinaceae and probably the Taxaceae, but in four other coniferous families, mitochondrial inheritance appears to be predominantly paternal (Table 1).

Much information on plastid inheritance in angiosperms has been gained from genetic experiments involving plastome (plastid DNA) mutations that result in chimeric plants with chlorophyll-deficient and normal green sectors. These are easily recognized phenotypically and can be used in reciprocal hybridization studies (Kirk and Tilney-Bassett, 1978; Smith, Bingham, and Fulton, 1986). Other plastome mutations including resistance to antibiotics, herbicides, and phytotoxins, as well as differences in the chloroplast-encoded large subunit of ribulose-1,5 bisphosphate carboxylase (Rubisco), have been utilized as markers for studying plastid inheritance (reviewed in Sears, 1980; Smith, 1989a). Electron microscopy (Sears, 1980; Whatley, 1982; Connett, 1987) and the use of DAPI (4’-6-diamidino-2-phenylindole; Miyamura, Kuroiwa and Nagata, 1987; Corriveau and Coleman, 1988) to stain organelle (plastids and mitochondria) nucleoids (DNA aggregates) have also contributed greatly to our understanding of cytoplasmic inheritance.

Relatively recently, molecular techniques have allowed restriction fragment length polymorphisms (RFLPs) to be used as specific markers for organelle DNA. This technique is based on the digestion of organelle DNA with restriction endonucleases, which reveals genotype-specific patterns when cleavage products are separated electrophoretically. Typically, total cellular DNA is extracted, digested, separated on agarose gels, and blotted to nylon filters. Then organelle DNA is identified with specific, labeled probes for plastid or mitochondrial genes using the Southern hybridization technique. Once distinct organelle DNA genotypes are recognized by mapping the

1 Manuscript received 19 September 1994; revision accepted 6 July 1995.

The author thanks Steven E. Smith, Darlene A. DeMason, John N. Owens, Scott D. Russell, and Maxine L. Rusche for very helpful suggestions on the manuscript. Support for this work by the National Science Foundation under grant DCB-9103658 and by the Organized Research Fund of Northern Arizona University is gratefully acknowledged.
restriction fragment patterns, they can be utilized to identify the parental origin of the organelle DNA in hybrid plants by comparing the patterns of each parent with those of the progeny. PCR (polymerase chain reaction) amplification of organelle DNA, followed by RFLP analysis of the amplification products permits an increased detection rate of parental organelle DNA within progeny. This approach has recently been used by Cruzan et al. (1993) in studies of plastid DNA inheritance and evolution in Iris. The use of molecular markers often makes it possible to study organelle inheritance in crosses where no other markers are evident.

The development of molecular techniques has stimulated a new wave of research in the area of organelle inheritance and in its application to population genetics, systematics, and organismal evolution (e.g., Kumar and Cocking, 1987; Soltis and Soltis, 1989; Lavin, Mathews, and Hughes, 1991; Paige, Capman, and Jennetten, 1991; Schilling, Panero, and Eliasson, 1994; Pillay and Hilu, 1995). When organelles are inherited unparentally, organelle DNA will propagate clonally and genealogies can then be constructed on this basis (Wilch, Ward, and Castle, 1992), e.g., determination of the maternal parentage of allotetraploids (Soltis and Soltis, 1989). Because many such studies assume uniparental inheritance of cytoplasmic organelles, Smith (1989a) points out that it is important to realize that approximately one-third of all angiosperm genera studied to date inherit their plastids, at least occasionally, biparentally. Thus, it may not be correct to assume that plastids and mitochondria are routinely inherited unparentally, especially since the exact mode of organelle inheritance has been well established in relatively few species (Sears, 1980; Smith, 1989a; Smith, 1989b; Stine and Keathley, 1989; (26) Stine and Keathley, 1990; (27) Sutton et al., 1991; (28) Swamy, 1948; (29) Szmidt, Alden and Hallgren, 1987; (30) Wagner et al., 1987; (31) Wagner et al., 1989; (32) Wagner et al., 1991; (33) White, 1990; (34) Yamada, Miyamura and Hort, 1995.

Investigators directly or indirectly involved with organelle inheritance will better realize the potential as well as the limitations of their research through an increased understanding of the mechanisms by which organelles are transmitted. The primary goal of this paper is to examine such mechanisms in some detail, first in angiosperms and then in gymnosperms. The fate of organelles and/or their DNA is followed in generative and sperm cells, as well as during and after gametic fusion; and the relative efficiency with which organelles may be eliminated or preserved during the various steps of sexual reproduction is evaluated. Finally, the possible evolutionary selective advantages for the myriad of fascinating ways in which transmission of cytoplasmic organelles is suppressed or favored in seed plants are summarized and discussed.
Other reviews of cytoplasmic inheritance in seed plants as well as other plant groups include: Grun, 1976; Gillham, 1978; Kirk and Tilney-Bassett, 1978; Sears, 1980; Whately, 1982; Connett, 1987; Hagemann and Schröder, 1989; Reboud and Zeyl, 1994; Smith, 1989a; Gillham, Boynton, and Harris, 1991; Birky, 1994.

MECHANISMS OF CYTOPLASMIC INHERITANCE IN FLOWERING PLANTS

Exclusion of plastids from the generative cell—Diploid microsporocytes within the young anthers of angiosperms undergo meiosis to produce four haploid microspores each, thus initiating pollen development. Each microspore subsequently divides mitotically to produce two very unequal products: a smaller generative cell and a larger vegetative cell (Figs. 1, 5). The latter will eventually produce the pollen tube. It is during this first pollen mitosis (first division of the microspore) that the fate of paternal plastids is largely determined in the majority of species exhibiting maternal plastid inheritance. The generative cell, which is initially positioned laterally and only later becomes completely surrounded by the vegetative cell (Figs. 1–3), usually receives no plastids (Figs. 1, 5; Hagemann and Schröder, 1989). Since it is the generative cell that divides once more (second pollen mitosis) to produce two sperm cells, either within the pollen grain or the pollen tube depending on the species, the male sex cells typically lack plastids, even though they nearly always contain numerous mitochondria (Fig. 4; Corriveau and Coleman, 1988; Hagemann and Schröder,

Figs. 1–4. Diagrammatic representation of the later stages of pollen development in an angiosperm with tricellular pollen showing the mechanism of plastid exclusion from the generative cell. 1. The microspore has divided to produce a larger vegetative cell (VC), which contains numerous plastids (P) and mitochondria (M), and whose nucleus (VN) is shown centrally located. The smaller generative cell (GC) is located peripherally and contains many mitochondria (M) but no plastids, due to their being excluded at the time of microspore division. 2. The generative cell (GC) is moving away from the pollen wall and becoming enclosed by the vegetative cell. 3. The generative cell (GC) has become spindle shaped and is now positioned next to the vegetative nucleus (VN). 4. The generative cell has divided to produce two sperm cells (SC), which typically remain connected to each other and become intimately associated with the vegetative nucleus (VN) to form a male germ unit. Note that the sperm cells contain numerous mitochondria (M), but no plastids.
1989). Some members of the Orchidaceae have been reported to nearly or totally lack generative cell mitochondria as well as plastids (Yu and Russell, 1992).

Polarization of plastids prior to and during microspore division may be mediated by microtubules (Van Went, 1984; Tanaka, 1991), actin filaments (Schröder, van Lammeren, and Kieft, 1988; Pierson and Cresti, 1992), and/or a biochemical gradient (Schröder, 1985); however, more experimental studies are needed in this area. If some plastids are not included in whatever distributional mechanism is operating, they may be randomly distributed and, consequently, be included in the generative cell (Schröder and Oldenburg, 1990). Although several species possess generative cells containing plastids (Corriveau and Coleman, 1988; Hagemann and Schröder, 1989), this does not guarantee that these plastids will be inherited because several mechanisms can act to eliminate them, as well as the mitochondria, at later stages, as outlined in the next sections.

**Loss of cytoplasmic organelles from generative cells**—Modification and/or degeneration of plastids within generative cells has been described at the ultrastructural level in several species of angiosperms, occurring usually during pollen maturation (e.g., Clauhs and Grun, 1977; Vaughn, 1981, 1985; Schröder, 1986; Schröder and Hagemann, 1986; Bednarska, 1988; Yu and Russell, 1994a, b), but sometimes not until near the time of generative cell division within the pollen tube (Schmitz and Kowallik, 1987). The number of mitochondria within generative cells, however, appears to be maintained during pollen development, at least in *Solanum* (Clauhs and Grun, 1977) and *Nicotiana* (Yu and Russell, 1994a, b). Vaughn et al. (1980) purport that physical modification of both plastids and mitochondria occurs even before generative cell formation in rice and *Hosta*.

The exact process or processes by which organelles are degraded or otherwise structurally modified is not known, but a system involving the formation of autophagic vacuoles, as described by Yu and Russell (1994a), seems plausible. They envision that mitochondria and plastids are enclosed by endoplasmic reticulum-derived membranes, producing an autophagosome. Lyosomal vesicles fuse with the autophagosome, releasing hydrolytic enzymes that digest the enclosed organelles within what is now an autophagolysosome. Since this mechanism probably would not distinguish between plastids and mitochondria, Yu and Russell (1994a) presume that the mitochondrial population could be maintained by their preferential replication.

Cytoplasmic organelles may also be lost from generative cells by being included in enucleated cytoplasmic bodies that are reported to be produced and discarded from generative cells during pollen maturation in *Plumbago* (Russell and Yu, 1991) and *Cymbidium* (Yu and Russell, 1992), and during pollen tube growth in *Nicotiana* (Yu and Russell, 1994a). However, in *Cymbidium*, even the young generative cells usually contain no plastids or mitochondria (Yu and Russell, 1992), so the cytoplasmic diminution is most likely associated with the transformation of generative cell shape and size reduction, rather than with inheritance of cytoplasmic organelles.

**Loss of cytoplasmic organelles from sperm cells**—In barley (*Hordeum vulgare*), a quantitative, three-dimensional, ultrastructural study of sperm cell maturation within pollen indicates that the number of mitochondria per sperm cell is reduced by 50% (from a mean of 62 to a mean of 31) from the time the sperm cells are formed until pollen maturity at anthesis (Mogensen and Rusche, 1985). During the same period, sperm cell surface area and volume are reduced by 30% and 51%, respectively. No examples of sperm mitochondrial fusion or degeneration were observed. Based on these data, and observations of membrane apposition and vesiculation within cytoplasmic extensions containing mitochondria, these workers concluded that cytoplasm and organelle loss during sperm cell maturation result primarily from the formation of cytoplasmic projections that are subsequently discarded from the sperm cell body (Figs. 6–9). A similar mechanism of cytoplasm and organelle loss from sperm cells within the pollen tube has recently been reported in tobacco (Yu, Hu, and Russell, 1992); however, mitochondrial number per sperm cell apparently does not change significantly between 9 and 26 h postpollination (Yu and Russell, 1994a).
Sperm dimorphism—The two sperm cells of a pair in the pollen grain of Plumbago zeylanica are highly dimorphic. That is, they are very different from each other in cell size and shape, and also in the size of their nucleus and the number of cytoplasmic organelles (Figs. 10, 11). The smaller sperm cell contains an average of 24 plastids and 40 mitochondria, whereas the larger sperm cell usually contains no plastids and has an average of 256 mitochondria (Russell, 1984).

Differential packaging of organelles between the two sperm cells of a pair, which is apparently established at their inception in Plumbago (Russell et al., 1988), could obviously have a direct influence on organelle input into the zygote and, consequently, on patterns of organelle inheritance. In the case of Plumbago, the plastid-rich sperm cell preferentially fuses with the egg (Russell, 1985). Plumbago appears to be quite an unusual case in terms of the degree of differences between the two sperm cells. Other examples of sperm dimorphism are known, e.g., in spinach (Wilms, 1986), Brassica (McConchie, Hough, and Knox, 1987), and Euphorbia dulcis L. (Murgia and Wilms, 1988), but in these cases only mitochondria are present within the sperm cells (see Mogensen, 1992 for review).

Exclusion of male cytoplasm at gametic fusion—If the male organelles have survived possible degeneration within the microspore, exclusion from the generative cell, degeneration within the generative cell, and expulsion from the generative or sperm cells, it is still possible that they will not be transmitted into the egg. In barley, relatively large numbers of mitochondria are still present within sperm cells up to the time of fertilization. Two sperm cells, after being discharged from the pollen tube and positioned for gametic fusion within the degenerated synergid, have been shown to contain 38 mitochondria (sperm cell apposed to the egg cell) and 61 mitochondria (sperm cell apparently ready to fuse with the central cell) (Mogensen, 1990, unpublished data). Examination of embryo sacs of barley immediately after fertilization, when the sperm nuclei are within the egg and central cells, but still not completely fused with the female nuclei, reveals the presence of a cytoplasmic body, slightly smaller than a sperm cell, appressed to the egg cell at the point where the sperm nucleus likely entered the egg (Figs. 12–14). A detailed study of such a cytoplasmic body using serial ultrathin sections and three-dimensional, computer-generated reconstructions revealed that the body had no nucleus, but contained seven dictyosomes, 59 mitochondria, three plastids, and a large nonmembrane-bound vacuole (Mogensen, 1988, 1990). The interpretation is that essentially the entire sperm cytoplasm is excluded during the process of syngamy in barley (Figs. 15, 16). Since only a single enucleated cytoplasmic body has been found in a given embryo sac of barley, it may be that the other sperm cell, which fuses with the central cell, transmits its cytoplasm (Mogensen, 1988).

Other examples of enucleated cytoplasmic bodies within the synergid of recently fertilized embryo sacs have been reported. Jensen and Fisher (1968) found two such structures in the degenerated synergid of cotton and concluded that they were of sperm origin. In Populus deltoides, Russell, Rougier, and Dumas (1990) report the presence of an enucleated cytoplasmic body in the degenerated syngamy, which they propose resulted from a polarization of the sperm nucleus to one end of the cell, followed by a constriction that discards most of the male cytoplasm. Janson (1992) found two enucleated cytoplasmic bodies within the fertilized embryo sac of Lilium longiflorum, and Huang, Strout, and Russell (1993) reported “numerous” enucleated cytoplasmic bodies within the degenerated syngamy of tobacco, some apparently of sperm origin and others derived from the pollen tube cytoplasm. In the case of tobacco, however, there is still apparently a considerable amount of male cytoplasm transmitted into the egg (Yu, Huang, and Russell, 1994).

If the above mechanisms of male cytoplasmic organelle elimination are not enough, yet another process leading to strictly or predominantly maternal cytoplasmic inheritance recently has been shown to be commonplace, that of organelle DNA modification and/or disintegration, as described in the next section.

Degradation of organelle DNA within generative and/or sperm cells—In many species it is common to have high frequencies of albino among pollen-derived plantlets. The frequency of albino also increases as more mature pollen is cultured. Such plantlets have been shown to lack normal plastid genome products (the 23S and 16S ribosomal RNAs and the large subunit of Rubisco) in rice (Sun et al., 1979), and to have large deletions in chloroplast DNA in wheat, barley, and rice (Day and Ellis, 1984, 1985; Dunford and Walden, 1991; Harada et al., 1991). These results suggest that organelle DNA modification may take place during pollen maturation in vivo and, thus, may be related to the suppression of male cytoplasmic inheritance. Additional studies using a DNA-specific fluorochrome and molecular markers provide strong evidence that plastid and mitochondrial DNA modification/degradation during pollen maturation is a common occurrence among plants exhibiting maternal cytoplasmic inheritance.

Thus, although seemingly structurally intact plastids and mitochondria may survive and even be transmitted into the egg cell, these organelles may lack DNA, or possess DNA that is greatly modified such that they are not heritable.

Microscopic evidence—Miyamura, Kuroiwa, and Nagata (1987) used the DNA-specific fluorochrome DAPI (compare Figs. 17–19) to show that plastid and mitochondrial DNA disappear from the generative cells during their development within the anther. These workers monitored cytoplasmic DNA within developing pollen of 16 species of angiosperms representing four monocot and 11 dicot families. Organelle nucleoids disappeared from the generative or sperm cells by the time of pollen maturity in nine species (Lilium longiflorum, Nerium indicum, Nicotiana tobacum, Canna generalis, Commelina communis, Tradescantia reflexa, Triticum aestivum, Arabidopsis thaliana, and Cosmos bipinnatus), representing both bicellular and tricellular pollen types. In the remaining seven species (Trifolium repens, Cucumis sativus, Mirabilis jalapa, Vicia sativa, Oenothera sp., Rhododendron indicum, Pharbitis nil), organelle nucleoids were present within the generative cells of mature pollen.
A logical conclusion drawn from these results was that the loss or retention of extranuclear DNA from the male germ line during pollen maturation may explain the pattern for plastid and mitochondrial inheritance (Miyamura, Kuroiwa, and Nagata, 1987). With such mechanisms in place, even if these organelles were not excluded from the sperm cells or the zygote, without DNA they could not be perpetuated in the next generation. Moreover, cytoplasmic DNA was also degraded to some degree in the vegetative cell of all but two species (Nerium indicum and Canna generalis). But how good an indicator is DAPI staining of the mode of cytoplasmic inheritance? Genetic evidence for plastid inheritance correlates positively with the cytological data of Miyamura, Kuroiwa, and Nagata (1987) in the cases of Nicotiana tabacum and Triticum aestivum, both of which have strictly, or nearly so (Medgyes, Pay, and Marton, 1986), maternal plastid inheritance, and for Oenothera, which has biparental plastid inheritance. However, Mirabilis jalapa and Pharbitis nil have maternal plastid inheritance, yet their generative cells retain cytoplasmic nucleoids.

Also using DAPI staining techniques, Corriveau and Coleman (1988) made an extensive survey documenting the presence or absence of cytoplasmic DNA in generative and/or sperm cells of 235 angiosperm species representing 80 families. Their findings showed a high positive correlation between the cytological data and genetic studies on plastid inheritance. Of the 47 species for which genetic evidence for the mode of plastid inheritance is known, the cytological results agreed with the genetic data in 42 cases. In five species, the cytological and genetic data did not agree in that both Pisum sativum and Ipomea nil (synonym for Pharbitis nil) are reported to exhibit maternal plastid inheritance, yet plastid DNA was detected in their generative and/or sperm cells; and in Phaseolus vulgaris, Nepeta cataria, and Secale cereale, cytoplasmic DNA was not detected by DAPI staining, yet there is genetic evidence indicating biparental plastid inheritance in these species (Corriveau and Coleman, 1988). Interestingly, the results of Corriveau and Coleman (1988) also demonstrated a general lack of cytoplasmic DNA in the vegetative cell, regardless of the mode of cytoplasmic inheritance displayed.

There are several possible explanations for the conflicting cytological and genetic evidence presented above. Perhaps the genetic studies were flawed by the tracing of a phenotypic trait that did not result from a plastid DNA mutation but, rather, was due to nuclear-controlled chlorophyll deficiencies or a viral infection (Kirk and Tilney-Bassett, 1978; Corriveau and Coleman, 1988). In plants where maternal plastid transmission is very low (e.g., $<5\%$ of the progeny receive male plastids; Smith, 1989a), cytological analysis may not detect the male plastids unless many sexual progenies are evaluated. The presence of plastids within the generative or sperm cells of mature pollen or early pollen tubes could be eliminated at a later stage, i.e., during lateral pollen tube growth, during fertilization, or after fertilization (Sears, 1980; Vaughn, 1981, 1985; Whatley, 1982; Connott, 1987; Corriveau and Coleman, 1988; 1991; Mogensen, 1988; Hagemann and Schröder, 1989; Polans, Corriveau, and Coleman, 1990). DAPI-detectable plastid DNA may nevertheless be debilitated and not capable of further replication or expression (Vaughn, 1980; Day and Ellis, 1984).

A prominent question remaining after the works of Miyamura, Kuroiwa, and Nagata (1987) and Corriveau and Coleman (1988) was: how reliable is DAPI staining for the detection of cytoplasmic DNA, i.e., does the absence of DAPI-detectable cytoplasmic DNA really mean that, indeed, the DNA has been completely depleted? The answer to this question necessitated molecular studies.

**Molecular evidence**—In order to answer the above question, Corriveau, Goff, and Coleman, (1990) carried out a molecular study using snapdragon (Antirrhinum majus), which exhibits maternal plastid inheritance and shows no DAPI-stainable cytoplasmic DNA in the generative cells, and alfalfa (Medicago sativa), which has biparental plastid inheritance and contains numerous DAPI-stainable plastid nucleoids in its generative and sperm cells (Corriveau and Coleman, 1988; Zhu, Mogensen, and Smith, 1990, 1991, 1992; Shi et al., 1991). Using a specific probe for plastid DNA (a clone of part of the rbcL gene from the pea plastome, which encodes for the large subunit of Rubisco), these workers employed...
Figs. 12–16. The process of fertilization and the mechanism of male cytoplasm exclusion at the time of syngamy in barley. 12–14. Diagrammatic representation showing the embryo sac at the time of pollen tube (T) entry into the degenerated synergid (Sy; Fig. 12), the discharge of the two sperm cells (SC) from the pollen tube (Fig. 13), and the presence of an enucleated cytoplasmic body (CB) outside the recently fertilized egg (Z; Fig. 14). Note that one sperm nucleus (SN) is fusing with a polar nucleus (PN), while the other sperm nucleus is fusing with the egg nucleus. CC, central cell; E, egg. 15. Transmission electron micrograph showing a sperm cell (SC) within the intercellular space between the egg (E) and central cell (CC). The sperm cell has a prominent nucleus (SN) and several mitochondria (M) within its cytoplasm. Sy, degenerated synergid. ×9000. Bar = 1 μm. 16. Transmission electron micrograph of an enucleated cytoplasmic body (CB) tightly appressed to the recently fertilized egg cell (Z). The cytoplasmic body contains several mitochondria (M), and a plastid (P) is also seen. Sy, degenerated synergid. ×9000. Bar = 1 μm. Figs. 15, 16 reproduced with permission from Mogensens (1988).

Southern blot hybridization techniques with total DNA isolated from germinated pollen. They found that the plastid-specific probe hybridized with a restriction fragment from alfalfa pollen, but not with DNA from snapdragon pollen (Figs. 20, 21). These results indicate that the cytological method (Corriveau and Coleman, 1988) using DAPI staining to detect organelle DNA is reliable, i.e., that the presence of DAPI-stained nucleoids within
plastids demonstrates the presence of plastid DNA, and the absence of DAPI-stainable nucleoids reflects the absence of plastid DNA, at least in these species and at the levels detectable by the molecular techniques used (Corriveau, Goff, and Coleman, 1990).

In a subsequent study, Corriveau and Coleman (1991) used further molecular analyses to examine the correlations between DAPI staining and cytoplasmic DNA within pollen. Using additional plastid gene probes (tobacco plastid psaA, psbA, and psbC gene sequences), they verified the absence of detectable plastid DNA in mature snapdragon pollen, which corresponds to the lack of DAPI-stainable cytoplasm in the pollen of this species (Corriveau and Coleman, 1988, 1991). However, in DNA from tobacco pollen they detected a low level of plastid DNA with all four DNA probes; yet, no DAPI-detectable plastid DNA was found in either the vegetative cell or generative cell of this species (Corriveau and Coleman, 1988, 1991).

Regarding mitochondrial DNA, the same study (Corriveau and Coleman, 1991) revealed no DAPI-stainable mitochondrial nucleoids in the vegetative or generative cells in mature pollen of any of the six species studied. However, molecular detection of mature pollen mitochondrial DNA (using cytochrome oxidase I, cox1, and cytochrome oxidase II, coxII, mitochondrial gene sequences as probes) was successful in both tobacco and snapdragon, the only two species they analyzed at the molecular level. The above results demonstrate that, at least in the case of tobacco pollen, the absence of DAPI staining in the cytoplasm does not correlate with the total absence of organelle DNA, although this DNA may be reduced in amount and/or modified in some way.

Sodmergen et al. (1992) examined cytoplasmic DNA in the pollen of Lilium longiflorum and Pelargonium zonale (synonym for Pelargonium × Hortorum) using fluorescence microscopy (DAPI staining) and molecular analysis (Southern hybridization). They found that in the mature pollen of Lilium longiflorum, the generative cells were devoid of any DAPI-stainable organelle nucleoids (Figs. 17, 18), while the vegetative cell contained a few weakly stained cytoplasmic nucleoids. The latter were also observed in pollen tubes 12 h after germination. Their Southern hybridization analysis using plastid- and mitochondrial-specific probes (rbcL and cox1, respectively) with Hind-III-digested total DNA from immature and
mature pollen stages showed that plastid DNA is significantly reduced during pollen maturation in *Lilium longiflorum*, while mitochondrial DNA is reduced below the level of detectibility. Application of the same technique to pollen tubes 12 h after pollination showed that plastid DNA is still present, although at an even more reduced level than in the mature pollen. Mitochondrial DNA was also detected at low levels in the pollen tubes, suggesting that its amount had increased during pollen tube growth.

These data (Sodmergen et al., 1992) show a positive correlation between DAPI staining and results from molecular analysis, in that mature lily pollen with no DAPI-stainable organelle nucleoids in the generative cell and only a few poorly stainable organelle nucleoids in the vegetative cell show significant reduction in plastid DNA and complete loss of mitochondrial DNA during pollen maturation according to Southern hybridization analysis. However, this study was not able to address whether the lack of DAPI-stainable nucleoids in the generative cell is representative of the total absence of cytoplasmic DNA in generative cells because, in the material and at the stages of this study, there were always at least some DAPI-detectable cytoplasmic nucleoids in the vegetative cell.

In *Pelargonium zonale*, a species with tricellular pollen, abundant cytoplasmic nucleoids are detectable with DAPI staining in the generative cell and in the sperm cells (Fig. 19) throughout their maturation (Sodmergen et al., 1992). Cytoplasmic nucleoids are also present in the vegetative cell at pollen maturity. Southern hybridization analysis of mature *Pelargonium zonale* pollen DNA confirmed the epifluorescence microscopic observation that cytoplasmic DNA is present in the mature pollen of this species. Both plastid DNA and mitochondrial DNA showed strong signals, but with molecular techniques it is not yet feasible to determine within which cells of the pollen grain a particular type of cytoplasmic DNA is located. However, a subsequent study by Kuroiwa et al. (1993), which used fluorescence microscopy/DAPI staining along with colloidal gold immunoelectron microscopy has convincingly demonstrated that both plastids and mitochondria within the sperm cell of *Pelargonium zonale* contain DNA.

Enzymatic studies—If cytoplasmic DNA loss occurs during pollen maturation in plants displaying maternal cytoplasmic inheritance, perhaps the loss is due to specific nucleases within the pollen of these species. A survey of nuclease C (a unique Ca²⁺-dependent nuclease purified from *Chlamydomonas reinhardtii* that is believed to be responsible for male plastid DNA degradation within the zygote; Ogawa and Kuroiwa, 1985) activity within green plants from algae to angiosperms indicated that this enzyme is generally found in those plants that inherit plastids maternally (Nakamura, Ogawa, and Kuroiwa, 1987). Sodmergen et al. (1992) compared two species of flowering plants, one with maternal plastid inheritance (*Lilium longiflorum*) and one with biparental plastid inheritance (*Pelargonium zonale*) by extracting proteins from pollen and ovaries and assaying for four cation-dependent nucleases in situ on SDS-PAGE gels. For *Lilium longiflorum*, they found two nucleases (one Ca²⁺-dependent and the other Zn²⁺-dependent) within the pollen proteins. They also detected a Ca²⁺-dependent nuclease and a Mn²⁺-dependent nuclease in the ovary protein.
extracts. No nuclease were found in the pollen or ovary protein extracts of *Pelargonium zonale*.

In a similar study, Nakamura et al. (1992) compared one species having maternal plastid inheritance (*Mirabilis jalapa*) with four species known or suspected to exhibit biparental plastid inheritance (*Rhododendron kaempferi, Zygocactus truncatus, Oenothera laciniosa,* and *Oenothera speciosa*). They found that nuclease C activity was high in both the stamens and pistils of *Mirabilis jalapa*, but it was very low or lacking in these organs of the other four species.

The above studies provide evidence that specific nucleases produced within the anther result in male cytoplasmic DNA degradation during pollen maturation in those species displaying uniparental-maternal plastid inheritance.

**Leakage of male cytoplasm**—Despite the existence of an abundance of mechanisms resulting in the suppression of male cytoplasmic inheritance, many of which are operating at successive stages of male sex cell development and fertilization, there are increasing examples of “leakage,” i.e., cases where a small percentage of the progeny receive male cytoplasmic organelles, particularly plastids (e.g., Diers, 1967, 1971; Medgyesy, Pay, and Marton, 1986; Schmitz and Kowallik, 1986; Cornu and Dulieu, 1988; Horlow et al., 1990; Sewell et al., 1993). These cases point out that even though several effective mechanisms of male cytoplasmic organelle elimination may be in force, none alone, nor all acting together, are 100% effective all of the time.

**Biparental organelle inheritance**—The basic mechanism leading to a high frequency of biparental cytoplasmic inheritance obviously involves the delivery of intact, DNA-containing male organelles into the egg, presumably through the cellular fusion process of plasmogamy (Russell, 1992). The subsequent fate of cytoplasmic organelles in the sperm and egg can be quite variable, however, depending on the species involved, on the genotypes crossed, and on several possible mechanisms controlled by both nuclear and organelle genomes (Kirk and Tilney-Bassett, 1978; Chiu, Stubbie and Sears, 1988). Because of the scarcity of phenotypic markers for mitochondria, relatively little is known about biparental inheritance of these organelles in angiosperms (Smith, 1989a), even though the more recent use of RFLP analysis has increased our knowledge in this area. The existing evidence, however, indicates that paternal mitochondrial inheritance is very rare (Harrison and Doyle, 1990). Even in those species that exhibit biparental plastid inheritance, the mitochondria are still typically derived strictly from the female parent (Schumann and Hancock, 1989; Smith, 1989a; Forsthoefel, Bohnert and Smith, 1992). Exceptions to this rule have involved wide crosses such as between barley and rye (Soliman, Fedak, and Allard, 1987), or unique male and female genetic lines such as in rape seed (*Brassica napus*; Erickson and Kemble, 1990, 1993).

Well-known examples of biparental plastid inheritance include zonal pelargonium (*Pelargonium × Hortorum*), evening primrose (*Oenothera*), and alfalfa (*Medicago*). However, not all of the progeny from a given cross contain plastids from both parents in these species; in addition, some of the progeny may contain only female plastids, while others may possess only male plastids. In *Oenothera*, biparental plastid inheritance is strongly biased toward the maternal parent (Kirk and Tilney-Bassett, 1978), whereas in *Medicago* there is a strong paternal predominance with a high percentage of the progeny containing only male plastids (Smith, Bingham, and Fulton, 1986; Smith, 1989b; Lee, Blake, and Smith, 1988; Schumann and Hancock, 1989; Masoud, Johnson, and Sorenson, 1990). In *Pelargonium*, crosses between a normal green female parent and a white-margined, variegated male parent result in plastid inheritance patterns of two main types. In type I, most progeny contain maternal plastids, while the fewest contain paternal plastids, and progeny containing both plastid types (variegated) are intermediate in number. In type II, there is an approximately equal frequency of progeny containing maternal or paternal plastids, and mixed progeny are the least frequent (Tilney-Bassett and Almouslem, 1989).

What mechanisms result in these various plastid transmission patterns? In *Oenothera erythrosepala*, the number of female plastids within the newly formed zygoate was found to be much higher than the number of male plastids (25–29 maternal to 8–13 paternal plastids; Meyer and Stubbe, 1974). Since Schütz (1954) found the number of plastids per egg cell to be quite consistent among four strains of *Oenothera*, the high ratio of female to male plastids within the zygote could be an important factor leading to a predominance of maternal plastid inheritance. However, based on a recent genetic study comparing the transmission of four *Oenothera* plastome types in different nuclear backgrounds, Chiu and Sears (1993) concluded that, rather than variable plastid input frequencies, efficiency of plastid multiplication after fertilization, as influenced by plastome–genome interactions, affects plastid inheritance patterns.

In *Pelargonium*, Tilney-Bassett (1976) has suggested that “nuclear genes determine plastid inheritance by the selective control of plastid replication so that the output of plastids need have little resemblance to the input ratio.” More recently, Tilney-Bassett and Almouslem (1989) argue that “the possible spatial separation of the plastids in the zygoate coupled with the asymmetric division of the zygoate into a larger suspensor cell and a smaller terminal cell may be of overriding significance.” It is possible that the male and female plastids remain in their own separate groups to a greater or lesser degree, and that only those plastids transmitted to the apical cell will be inherited because the larger basal cell gives rise to the suspensor, which eventually degenerates.

In alfalfa, recent cytological studies lend strong support to a plastid distribution/apportionment mechanism (Figs. 22, 23) similar to that discussed by Tilney-Bassett and Almouslem (1989). The generative cells of alfalfa contain large numbers of plastids (mean of 62–265) and plastid nucleoids (mean of 45–60), which vary according to genotype. Yet, no consistent correlations were found between generative cell plastid load and the observed variation in paternal plastid inheritance patterns among genotypes (Smith, Bingham, and Fulton, 1986; Smith, 1989b; Zhu, Mogensen, and Smith, 1990, 1991;
Figs. 22–23. Computer-generated, three-dimensional reconstructions of alfalfa egg cells. 22. Egg cell (E) from genotype CUF-B, a weak female plastid transmitter, showing the plastids (P) distributed mostly below (toward the micropyle) the midregion of the nucleus (N), which is the future division plane of the zygote. 23. Egg cell (E) from genotype 6–4, a strong female plastid transmitter, showing the plastids (P) distributed essentially equally around the nucleus (N). Figs. 20, 21 ×2,500. Bar = 10 μm. Reproduced with permission from Zhu, Mogensen, and Smith (1993).

Shi et al., 1991). And both sperm cells of a pair contain equal numbers of plastids (Zhu, Mogensen, and Smith, 1992). A positive correlation was found, however, between egg cytology and plastid transmission frequencies between two genotypes. Genotype CUF-B is considered a weak female because when it is crossed with genotype 301 as the male parent, over 90% of the progeny contain only male plastids (Smith, 1989b). Genotype 6–4 is classified as a strong female because when crossed with the same male parent, 41% of the progeny possess only paternal plastids (Smith, 1989b). Quantitative, three-dimensional analysis of ten eggs from each genotype showed that plastid distribution is fundamentally different in the two types of eggs. In CUF-B (weak female) the egg plastids are located primarily below (toward the micropyle) the midsection of the nucleus (i.e., below the future division plane of the zygote; Fig. 22), whereas in 6–4 (strong female) the egg plastids are equally distributed around the nucleus (Fig. 23; Zhu, Mogensen, and Smith, 1993).

Analysis of zygotes and early embryos from a cross between CUF-B (weak female) and 301 (strong male plastid transmitter) demonstrates that the same distributional pattern of female plastids present in the egg cells is maintained within the zygote. Consequently, after zygotic division the basal cell, which forms a portion of the suspensor, contains considerably more female plastids than does the apical cell, which forms the functional embryo (Rusche et al., 1995). Interestingly, the same distributional pattern is seen for the male plastids, i.e., the majority are located below the division plane of the zygote. However, typically, many more male plastids are present in the apical portion of the zygote than are female plastids (Rusche et al., 1995).

Clearly, additional factors could influence biparental plastid inheritance frequencies, such as: (1) sorting out of plastid types during subsequent cell divisions; (2) differential plastid DNA degradation within the zygote, similar to what occurs in the unicellular green alga Chlamydomonas (Kuroiwa et al., 1985); and (3) degeneration of entire female plastids, as seems to occur in eggs of Daucus muricatus (Hause, 1991) and in two-celled embryos of alfalfa (Rusche et al., 1995). Undoubtedly, several mechanisms are operative, and their relative significance likely varies among species (Tilney-Bassett and Almouslem, 1989).
CYTOPLASMIC INHERITANCE IN GYMNOSPERMS

Conifers—Plastids—The vast majority of information on cytoplasmic inheritance in gymnosperms comes from studies on the conifers, the largest group of gymnosperms. In direct contrast to most flowering plants, plastid inheritance in the conifers appears to be predominantly paternal. Structural studies provide evidence for this conclusion in six of the seven families of conifers (Camefort, 1968; Chesnoy and Thomas, 1971; Kaur and Bhatnagar, 1984; Chesnoy, 1987; Owens and Morris, 1991), and genetic studies using plastid mutants or RFLP analysis have, in several cases, borne out predictions based on structural investigations (Table 1). Ohba et al. (1971), analyzed a plastid anomaly in Cryptomeria japonica (Taxodiaceae) and concluded that plastids are inherited paternally 90%–99% of the time. Recently, RFLP analysis has been used to demonstrate that plastid DNA is inherited predominantly or exclusively from the male parent in several conifer species included in the Pinaceae, e.g., Pseudotsuga menziesii (Neale, Wheeler, and Allard, 1986), Pinus banksiana (Wagner et al., 1989), hybrids of Pinus taeda and P. rigida (Neale and Sederoff, 1989), Pinus contorta and P. banksiana hybrids (Wagner et al., 1987), Larix hybrids (Szmidt, Alden, and Hallgren, 1987), hybrids of Picea pungens and P. glauca (Stine, Sears, and Keathley, 1989), hybrids of Picea engelmannii and P. pungens (Stine and Keathley, 1990), and Pinus monticola (White, 1990); the Cupressaceae: Calocedrus decurrens (Neale, Marshall, and Harry, 1991); and the Taxodiaceae: Sequoia sempervirens (Neale, Marshall, and Sederoff, 1989).

Mitochondria—Mitochondrial inheritance in the conifers can be either from the male or the female parent but, although the data are scarce at this point, it appears that the majority of coniferous families also transmit their mitochondria paternally (Table 1).

In the Pinaceae, however, the largest coniferous family, maternal inheritance of mitochondria is the rule so far, as evidenced by RFLP analysis, e.g., in Pinus taeda (Neale and Sederoff, 1989), Pinus banksiana and Pinus contorta (Wagner et al., 1991), Pseudotsuga menziesii (Marshall and Neale, 1991), spruce hybrids (Sutton et al., 1991) and Larix hybrids (DeVerno, Charest, and Bonen, 1993). These findings correlate positively with conclusions based on structural studies in this family (Owens and Morris, 1991). In the Taxaceae, maternal inheritance of mitochondria is also likely the case, according to an ultrastructural study in Taxus baccata (Pennell and Bell, 1988).

In the Taxodiaceae (coast redwood, Sequoia sempervirens; Neale, Marshall, and Sederoff, 1989) and the Cupressaceae (incense cedar; Calocedrus decurrens; Neale, Marshall, and Harry, 1991) mitochondrial DNA has been shown, by RFLP analysis, to be derived from the male parent. The RFLP data agree with conclusions from earlier structural studies in the oriental arborviteae (Biota orientalis) and Port-Orford cedar (Chamaecyparis lawsoniana) of the Cupressaceae (Chesnoy, 1969, 1973, 1975, 1977) and a recent electron microscopic/epifluorescence study on Cryptomeria japonica of the Taxodiaceae (Yamada, Miyamura, and Hori, 1993), that both mitochondria and plastids are inherited paternally in these species.

In the Cephalotaxaceae (Cephalotaxus drupacea; Singh, 1961; Gianordoli, 1974) and the Araucariaceae (Agathis robusta; Kaur and Bhatnagar, 1984), mitochondrial transmission is also likely paternal according to structural studies that show the same basic fertilization process for these species as occurs in Biota orientalis and Cryptomeria japonica, except that the sperms are free nuclei, and they both may enter the egg.

In the remaining coniferous family, the Podocarpaceae, there is not sufficient evidence at this time to predict the mode of inheritance for mitochondria or plastids (Owens and Morris, 1991). However, according to a light microscopic study by Looby and Doyle (1944), a considerable amount of male cytoplasm does enter the egg and becomes closely appressed to one side of the zygotic nucleus.

Other gymnosperms—In the cycads and Ginkgo, limited structural evidence indicates that plastid inheritance is most likely maternal (Whatley, 1982). No specific data are available for mitochondria, but presumably their pattern of inheritance follows that of the plastids. In the Gnetophyta, an ultrastructural study on fertilization in Ephedra distachya (Moussel, 1978) provides good evidence that in this group both plastids and mitochondria are inherited strictly maternally (Table 1).

Mechanisms of organelle transmission in gymnosperms—The various modes of organelle inheritance outlined above can be explained by the results of structural studies that have traced the fates of male and female organelles before and after fertilization.

Paternal plastids and maternal mitochondria—In the case of the Pinaceae where the plastids are inherited from the pollen parent and mitochondria are derived from the female parent, Douglas-fir is representative. This is illustrated in Figs. 24–26, which are based primarily on studies by Owens and Morris (1990, 1991). The body cell, which is a product of a mitotic division of the generative cell, is rich in cytoplasmic organelles at the time the pollen tube arrives at the female gametophyte after growing through the nucellar tissue (Fig. 24). At about the time the pollen tube penetrates the megaspore wall and expands to fill the archegonial chamber, the body cell nucleus divides mitotically, but no cytokinesis occurs (which is typical in the Pinaceae), resulting in two male gamete nuclei still surrounded by a cytoplasm rich in plastids and mitochondria (Fig. 25). After the pollen tube forms a narrow extension that grows between the neck cells and through the ventral canal cell, the two male nuclei are released into the egg. The male cytoplasm travels between the sperm nuclei as the leading sperm nucleus migrates toward the egg nucleus (Fig. 26), which is already tightly surrounded by a dense population of female mitochondria (the perinuclear zone; Figs. 24–26). Although the female plastids are numerous within the egg they are greatly modified and are not closely associated with the egg nucleus. Maternal plastid transformation actually begins at the central cell stage. As the gamete nuclei fuse, the male cytoplasm becomes incorporated into
Figs. 24–29. Diagrammatic representation of the mechanisms of organelle transmission in conifers. 24–26. Fertilization in Douglas-fir (Pseudotsuga menziesii). Based on Owens and Morris (1991). 24. The pollen tube (T) has arrived at the archegonial chamber (AC) after growing through the nucellus (Nuc). The body cell (BC) contains a prominent nucleus along with many plastids and mitochondria. Note that the modified female plastids (P) within the egg cell (E) are not closely associated with the egg nucleus (EN), whereas the female mitochondria (M) tightly surround the egg nucleus. NC, neck cells; V, ventral canal cell; FG, female gametophyte. 25. The pollen tube (T) tip has settled into the archegonial chamber and the body cell has divided to form two sperm nuclei (SN) unseparated by a cell wall. EN, egg nucleus; P, female plastids; M, female mitochondria. 26. The pollen tube (T) has grown between the neck cells, into the ventral canal cell, and discharged both sperm nuclei (SN) into the egg cell. One sperm nucleus has migrated, along with most of the male cytoplasm (MCy), to the female nucleus (EN) and karyogamy is taking place. The
the perinuclear zone (Fig. 26) and, after zygotic division, the two free proembryo nuclei are engulfed in “neocytoplasm” composed primarily of female mitochondria and male plastids. Male mitochondria are also included in the neocytoplasm, but are fewer in number. The paternal organelles remain in a rather distinct cluster while two additional free proembryo nuclei are formed and the group of four nuclei and neocytoplasm migrates to the chalazal end of the former egg. Thereafter the male cytoplasm becomes dispersed throughout the neocytoplasm as the four free nuclei divide. Next, cell walls form to produce an eight-celled then a 12-celled proembryo, which continues to develop and grows chalazally into the female gametophyte tissue. Thus, the embryonic cytoplasm is derived from a combination of both parents, but includes almost exclusively male plastids and female mitochondria.

It can be seen from the cytological data that this mechanism could lead to some “leakiness” of maternal plastids into the offspring if they were not totally excluded from the neocytoplasm. In Douglas-fir, Owens and Morris (1991) observed occasional large inclusions, which represent modified female plastids within the neocytoplasm, and maternal chloroplast DNA has been detected by RFLP analysis in some species, e.g., in seedlings of western white pine (Pinus monticola; White, 1990) and in apparent hybrid trees of Pinus banksiana × Pinus contorta (Govindaraju, Wagner, and Smith, 1988). Likewise, the male mitochondria included within the neocytoplasm could be perpetuated in the offspring. From controlled crosses within and between jack pine (Pinus banksiana) and lodgepole pine (Pinus contorta), Wagner et al. (1991) found only maternal mitochondrial restriction fragments in a majority of the seedlings. However, exclusively paternal mitochondrial DNA was detected in six of 58 seedlings. In the case of the Taxaceae, Pennell and Bell (1988) found that the eggs of Taxus baccata have no recognizable plastids, yet the newly formed zygote has conspicuous proplastids, indicating that plastid inheritance in this species is strictly paternal. However, the egg does contain numerous mitochondria, which would likely lead to maternal inheritance of these organelles.

Paternal plastids and paternal mitochondria—Figures 27–29, which are based primarily on Biota orientalis (Cupressaceae; Chesnay, 1977) illustrate a likely mechanism for the paternal inheritance of both plastids and mitochondria as demonstrated by RFLP analysis in the Taxodiaceae (Neale, Marshall, and Sederoff, 1989) and Cupressaceae (Neale, Marshall, and Harry, 1991), and implied by structural studies in the Cupressaceae (Chesnay, 1969, 1973, 1975, 1977), the Taxodiaceae (Yamada, Miyamura, and Hori, 1993), the Cephalotaxaceae (Singh, 1961; Gianordoli, 1974) and the Araucariaceae (Kaur and Bhatnagar, 1984). The spermatogenous cell (body cell) divides to form two sperm cells, rather than free nuclei as in the Pinaceae, but the contents of only one sperm cell enter the egg. In Biota orientalis, each sperm cell is \( \approx 50 \mu m \) in diameter (Chesnay, 1977), which is nearly the width of the archegonium containing the egg cell. In the egg the female plastids and mitochondria are distributed throughout the cytoplasm, which is primarily positioned at the two ends of the egg due to a large central vacuole. There is no perinuclear zone of female organelles (Fig. 27). Once in the egg, the sperm nucleus remains tightly encompassed by its cytoplasm, which contains numerous rod-shaped plastids and spherical mitochondria. During karyogamy (Fig. 28), the male cytoplasm surrounds the zygote nucleus, forming a compact zone consisting of only male plastids and mitochondria. Zygotic mitosis produces two free proembryo nuclei, which are also enclosed by male cytoplasm (Fig. 29) as they migrate to the chalazal end of the archegonium where additional free nuclei, then cell walls, are formed. Meanwhile, the female cytoplasm degenerates, particularly in the micropylar portion of the former egg cell (Fig. 29), resulting in proembryo cells typically containing only male cytoplasm.

As in the Pinaceae, the mechanism of cytoplasmic transmission described above could lead to some leakiness. In this case, inheritance of both plastids and mitochondria may not be strictly paternal if the female organelles do not degenerate and are included in the proembryo cells. Chesnay (1977) reported the presence of a few maternal mitochondria in the proembryonic cytoplasm of the oriental arborvitaes, and Yamada, Miyamura, and Hori (1993) found that some female plastids are occasionally included in the proembryo cells of Cryptomeria japonica. Neale, Marshall, and Sederoff (1989) state that they cannot unequivocally conclude that mitochondria and plastids are strictly paternally inherited in coast redwood because their data were based on only two crosses of 10–12 progeny each. In incense cedar, Neale, Marshall, and Harry (1991) found that all progeny had only the chloroplast DNA restriction fragments of the male parent, except for one example, which had only the fragments of the female parent. Mitochondrial DNA in this species was found to be strictly paternal.

Maternal plastids and maternal mitochondria—Figures 30–32 illustrate the mechanism of organelle transmission in Ephedra distachya, which is based primarily on an ultrastructural study by Moussel (1978). The sperm nuclei occur in pairs, unseparated by cell walls, as in the Pinaceae. However, compared to the conifers, the sperm nuclei have very few associated plastids and mitochondria.
Figs. 30–32. Diagrammatic representation of fertilization in Ephedra distachya, based primarily on Mousel (1978). 30. The binucleate sperm cell (SC), containing few cytoplasmic organelles, is positioned at the micropylar end of the egg cell (E). Note that the female cytoplasm (FCy), containing both plastids and mitochondria, is closely associated with the egg nucleus (EN). A ventral canal nucleus (unlabeled arrowhead) is located at the micropylar end of the egg. 31. Both sperm nuclei (SN) have entered the egg, but only one has migrated and is fusing with the egg nucleus. The male cytoplasm remains with the other sperm nucleus at the micropylar end of the zygote (Z). 32. The zygote nucleus has divided to produce two proembryo nuclei, which are completely surrounded by female cytoplasm (FCy). At the micropylar end of the former zygote, a sperm nucleus and its associated cytoplasm are degenerating, as is the ventral canal nucleus (unlabeled arrowhead). This type of fertilization results in maternal inheritance of both plastids and mitochondria.

dria (Fig. 30). Both sperm nuclei enter the egg cell, but only one migrates toward the female nucleus. The second sperm nucleus, along with the male cytoplasm, which is very distinct from that of the egg, remains at the micropylar apex of the egg near the ventral canal nucleus. As gametic karyogamy occurs, the fusing nuclei become surrounded by the dense perinuclear zone consisting of only female plastids and mitochondria (Fig. 31). Formation of the zygote nucleus is completed at the chalazal end of the zygote where eventually eight free proembryo nuclei are produced (Fig. 32), each of which, upon cellularization, produces a separate proembryo (Friedman, 1992).

The cycads and Ginkgo have some features in common with the mechanism of cytoplasmic transmission in Ephedra distachya, but they also have some unique features as well. Both of these groups have large, flagellated, free-swimming sperm cells that are deposited within the archegonial chamber. In all other groups of seed plants, the sperms are transferred directly from the pollen tube to the egg cell (Bold, Alexopoulos, and Delevoryas, 1987). In Ginkgo, only one sperm nucleus enters the egg and typically the rest of the sperm cell is excluded (Lee, 1955; Whatley, 1982), similar to what occurs in some angiosperms (Mogensen, 1988). The spermatogenous cell (the precursor of the two sperm cells) of Ginkgo has been studied ultrastructurally, and it does contain abundant mi-

thochondria and plastids (Gifford and Lin, 1975; Gifford and Larson, 1980). Camefort (1965) studied the egg cell of Ginkgo at the ultrastructural level and found that the female plastids do not undergo the profound modifications observed in the Pinaceae. However, apparently no ultrastructural studies are available on the details of fertilization in Ginkgo, and no genetic studies have been carried out, so the mode of cytoplasmic inheritance is still uncertain.

In the cycads, more than one sperm cell can enter the egg, but only one sperm nucleus migrates and fuses with the egg nucleus. The male cytoplasm of Zamia, which has abundant plastids and mitochondria (Norstog, 1974), appears to degenerate near the point of sperm entry into the egg (Chamberlain, 1935; Swamy, 1948). This mechanism basically resembles that of Ephedra (see Figs. 30–32), but no ultrastructural details are available on fertilization and, as Whatley (1982) points out, it is possible that some paternal cytoplasm accompanies the functional sperm nucleus. Unlike Ephedra, in the cycads and Ginkgo cellularization of the proembryo nuclei results in a single proembryo, rather than multiple proembryos (Friedman, 1992).

**Organelle DNA**—Cytoplasmic organelle DNA (presumably mitochondrial DNA) has been visualized within the perinuclear zone of the eggs of Douglas-fir (Chesney and Thomas, 1969; Thomas and Chesney, 1969) and Ephedra nevadensis (Friedman, 1990) using the Feulgen reaction and DAPI staining, respectively. Yamada, Miyamura, and Hori (1993) used the DAPI staining technique to show sperm cytoplasmic nucleoids before and after fertilization in Cryptomeria japonica. These workers also demonstrated that maternal cytoplasmic DNA is maintained in this species at least until after the second proembryonic mitosis.

Additional studies that track organelle DNA during gametogenesis and embryogenesis to determine the fate of such DNA as it relates to cytoplasmic inheritance are greatly needed in the gymnosperms. Many questions remain to be addressed. For instance, in Ephedra, Ginkgo, and the cycads, is the paternal cytoplasmic DNA degraded before fertilization, as is the case in many angiosperms exhibiting uniparental-maternal cytoplasmic inheritance?

**DISCUSSION**

The numerous and elaborate ways in which cytoplasmic organelle transmission is restricted or enhanced among the seed plants are curious and intriguing. Why are there so many mechanisms, several of which are often operative sequentially in the same species, leading to a predominance of uniparental organelle inheritance? What evolutionary selective advantages have resulted in widespread maternal plastid inheritance in the angiosperms, and a very strong paternal bias in plastid inheritance in conifers, while at the same time mitochondrial inheritance remains strictly maternal (with few exceptions) in angiosperms and the largest coniferous family, the Pinaceae? Is there a selective advantage, or are the patterns of organelle inheritance merely the indirect consequence of other directly selected processes? Many attempts to
answer these and other questions have been made, as outlined and discussed below.

Upon comparing the modes of cytoplasmic inheritance in seed plants, it is evident that the common theme is one of uniparental organelle inheritance. This is true for plastids in general, and to a very strict degree for mitochondria. Uniparental organelle inheritance is the general rule also for most other groups including algae, ferns, and animals (Sager, 1975; Kirk and Tilney-Bassett, 1978; Gillham, 1978; Sears, 1980; Whatley, 1982; Lambert and Battaglia, 1993; Birky, 1994). Even in those species exhibiting biparental plastid inheritance, sorting out typically results in the individual cells of the plant being homoplasmic, i.e., having only maternal or paternal plastids (Birky, 1983). This phenomenon is not restricted to individuals produced following sexual hybridization. In somatic hybridizations too, the individual progeny from protoplast fusion and plant regeneration normally contain only the plastids from one or the other of the parents, not both (e.g., Schiller, Herrmann, and Melchers, 1982; Kumar and Cocking, 1987; Li and Sink, 1992).

Some argue that uniparental inheritance guards against any potential incompatibility between organelle genomes and between organelle and nuclear genomes (Eberhard, 1980). Sager (1975) suggests that stability of organelle DNA from generation to generation is important because mutations and recombination of these genes are more likely to adversely affect the fitness of the organism than are similar modifications in nuclear genes, since some subunits of critical enzymes (e.g., Rubisco of chloroplasts, cytochromes a and b, and the F1-ATPase of mitochondria) are coded by organelle genes while other subunits of the same enzyme are coded by nuclear genes. Thus, if two types of organelle DNAs are present, heterogeneous subunits may combine and alter the expression of essential coadapted gene complexes (Sager, 1975; discussed in Sears, 1980). There is some experimental evidence for this in interspecific somatic hybridizations (Chinese hamster × mouse) where it was found that survival and growth of fusion products are enhanced when the mitochondria of one of the donor cells are eliminated by applying a mitochondrial poison before fusions are made (Ziegler and Davenport, 1981). The incompatibility model, at least with respect to recombination, would seem to be much more applicable to mitochondria than to plastids because mitochondrial DNA recombination occurs readily in somatic hybrids (Xu et al., 1993), whereas chloroplast DNA recombination is very rare or lacking in both somatic (Medgyes, Fejes, and Maliga, 1985; Maliga, Fejes and Svb, 1987) and sexual hybrids (Meltzaff, Borner, and Hagemann, 1981; Chiu and Sears, 1985; Masoud, Johnson and Sorensen, 1990). The apparent lack of a mechanism preventing mitochondrial DNA recombination may help to explain why there are, as yet, no examples of regular biparental mitochondrial inheritance in intraspecific sexual crosses of seed plants.

Why is uniparental organelle inheritance so prevalent in maternal? Why is the female cytoplasm not eliminated? Grun (1976) proposes that it would be more disadvantageous if aggressive, deleterious plastid types were transmitted through the pollen because they would be more widespread than similar mutations occurring in the host (female) plant. That is, it may be selectively advan-

tageous if a new cytoplasmic DNA mutation were tested in the female, to which debilitating effects would likely be confined (Kirk and Tilney-Bassett, 1978). However, Kirk and Tilney-Bassett (1978) note that there is no experimental evidence for a plastid mutation behaving in this way, and Sears (1980) counters Grun’s idea by suggesting that natural selection alone would eliminate such deleterious organelles from the population. Moreover, the conifers have adopted just the opposite strategy by transmitting their plastids almost exclusively through the pollen, and a male bias in plastid transmission also seems to work very well for alfalfa.

Maternal cytoplasmic inheritance may have evolved as a mechanism that prevents foreign or pathogenic DNA from entering the egg, and male organelles just happen to fall into this category. Because plastids apparently evolved from photosynthetic endosymbionts (Gray, 1993), the process of maternal plastid inheritance may act to eliminate “unwanted” or “alien” plastids from the egg (Coleman, 1982; Whatley, 1982; Law and Hutson, 1992). A similar argument could be made for mitochondria (Gray and Hutson, 1992). Of course, this hypothesis does not hold for the cases of biparental plastid inheritance in angiosperms, nor for paternal plastid inheritance in the conifers. There are also cases where virions can be transmitted through the pollen, e.g., in barley (Carroll and Mayhew, 1976), while in the same species plastids and mitochondria are not.

Perhaps it really does not matter which parental cytoplasm is eliminated, as long as only one is inherited. Reduction in sperm cytoplasm may have evolved as an adaptation that facilitates sperm movement and/or translocation within the pollen tube and may be only indirectly related to maternal cytoplasmic inheritance (Sears, 1980; Whatley, 1982; Tilney-Bassett, 1989). The smaller, streamlined morphology of a typical sperm cell would seem to result automatically in a decided inequity in organelle number between male and female gametes, e.g., the egg of maize has a volume ≈300 times larger than that of the sperm cell (Fautur, et al., 1992). Kirk and Tilney-Bassett (1978) suggest that it is of no consequence for the male gamete to eliminate all of its plastids because it is surrounded by the plastid-rich vegetative cell of the pollen grain or the pollen tube. Although it is true in general that the smaller sperm cell contains many fewer organelles than the egg (e.g., the egg cell of Plumbago contains ≈730 plastids and ≈40,000 mitochondria compared to an average of 24 plastids and 40 mitochondria in the sperm cell that fuses with the egg; Russell, 1984, 1987), this is not obligatory. For instance, in alfalfa, a plant exhibiting biparental plastid inheritance, the eggs contain an average of 28 and 41 plastids in two genotypes (Zhu, Mogensen, and Smith, 1993), whereas the sperm cell contains ≈70 plastids, although many of these may not contain DNA (Zhu, Mogensen, and Smith, 1992). In the cycads, the sperm cell may be up to 400 μm in diameter (a sperm cell of alfalfa is ≈3 μm × 9 μm) and may carry a large amount of both plastids and mitochondria, yet it appears that cytoplasmic inheritance is maternal in this group. In some species, maintenance of male plastids may be physiologically important and may contribute to pollen tube and germ cell nutrition and survival, e.g., in the pines where the pollen is dispersed great dis-
tances and the time between pollination and fertilization is 12–14 mo (Willson and Burley, 1983).

Other features of the highly specialized sperm cell may lead to maternal cytoplasmic inheritance. In maize, sperm mitochondria are highly modified, often occurring in large, complex shapes consisting of sheet-like, finger-like, and reticulate patterns. No mitochondria of this type are found in the surrounding vegetative cell cytoplasm (McConchie et al., 1987; Mogensen, Wagner, and Dueser, 1990). In many mammals, mitochondria in spermatozoa are also unique in morphology, as well as polypeptide composition (Hecht et al., 1984). Likewise, in most species of pterygota insects, mitochondrial structure is completely modified during spermatogenesis (Bao et al., 1992). Perhaps these modifications, along with the general degradation of cytoplasmic DNA known to occur in the pollen of many species displaying maternal cytoplasmic inheritance, render paternal organelles uninheritable.

Why does cytoplasmic DNA degradation occur within pollen? Is it a direct adaptation to effect maternal cytoplasmic inheritance? On the other hand, one might ask: why should organelle DNA be maintained within pollen? Perhaps there is a natural tendency for the process of organelle DNA repair and maintenance to shut down within cells that are approaching dormancy and/or impending unfavorable conditions such as dessication or ultraviolet radiation. Sears and VanWinkle-Swift (1994) propose that chloroplast DNA degradation within the zygote of *Chlamydomonas* (a cell that is subjected to considerable stress and is progressing into a dormant state) has evolved as a direct selective benefit to the organism, in that the degraded paternal chloroplast DNA may be utilized as sustenance, rather than being directed toward causing maternal chloroplast inheritance. The various patterns of cytoplasmic inheritance in some species of seed plants may be merely the consequence of the degree to which organelle DNA is degraded, restored, or maintained during pollen maturation and pollen tube growth.

Jinks (1964, reviewed in Kirk and Tilney-Bassett, 1978) proposed that elimination of male plastids is necessary in order to reduce the total number of plastids in the zygote, i.e., so that the number of plastids in the cells of the offspring will be maintained at more or less the same level as that of the parents. However, Kirk and Tilney-Bassett (1978) remind us that plastids do not necessarily replicate with each nuclear division. Therefore, the number of plastids per cell can be readily reduced by missing a division cycle or by an unequal cell division. Moreover, it does not appear to be important that there be a uniform number of plastids per cell, since this number varies greatly from tissue to tissue within the same plant.

Kirk and Tilney-Bassett (1978) consider biparental plastid inheritance to be most probably the primitive condition, and suggest that the extant biparental genera may be in an evolutionary transitional phase leading to purely maternal plastid inheritance. In support of this reasoning is a recent study in alfalfa that suggests that genotypes whose sperm cells contain fewer plastids may have a competitive advantage at fertilization (Keys, Smith, and Mogensen, 1995). However, there is as yet no clear evolutionary pattern to the modes of cytoplasmic inheritance, since both biparental and maternal plastid inheritance occur in primitive and advanced plant groups (Sears, 1980).

Clauhs and Grun (1977) theorize that biparental plastid inheritance could be advantageous by allowing recombination between plastomes and, thus, provide variability for selection. Yet, in practice there is apparently a mechanism that inhibits or prevents plastid fusion and, thus, plastome recombination. Biparental mitochondrial inheritance would lead more likely to mitochondrial DNA recombination but, based upon its apparent rarity in nature, it seems that any potential selective advantage for biparental mitochondrial inheritance is far outweighed by the potential disadvantages.

The “hows” of cytoplasmic inheritance in seed plants, in terms of the mechanisms by which organelles are inherited, are reasonably clear-cut, but much work remains to be done in greater detail and in additional species. We have seen that the mechanisms resulting in the prevalence of uniparental maternal organelle inheritance in angiosperms are varied and can take place at many different stages in the sexual process. Plastids may be excluded from the generative cell during the first microspore division, or they may be discarded or degenerated during generative cell or sperm cell maturation. Organelles may be distributed to only one sperm cell of a pair, or be excluded from the female gamete at the time of syngamy. Although the organelles may remain seemingly intact, their DNA may be degraded or grossly altered before fertilization occurs. Despite this redundancy in paternal organelle elimination, there are increasing examples of trace paternal organelle inheritance being discovered as the methods of detection of organelle DNA become more refined. Biparental inheritance of plastids is routine in some species, whereas maternal mitochondrial inheritance is apparently quite rare. In the gymnosperms, the mechanisms of cytoplasmic inheritance are more uniform within groups; there do not appear to be multiple mechanisms that may occur at different stages of the sexual process as in many angiosperms. The predominance of uniparental paternal inheritance of plastids in the Pinaceae is effected by a drastic transformation of the maternal plastids along with their distribution away from the egg/zygote nucleus. At the same time, the maternal mitochondria form a tightly packed perinuclear zone and are subsequently included along with the paternal plastids in the neocytoplasm, which is transmitted to the next generation (Figs. 24–26). In those conifers displaying paternal inheritance of both plastids and mitochondria (Table 1), the maternal organelles do not form a perinuclear zone, and they degenerate shortly after gametic fusion (Figs. 27–29). Maternal inheritance of plastids and mitochondria in *Ephedra* results from the retention and degeneration of paternal organelles in the micropylar end of the fertilized egg (Figs. 30–32). Although considerable work has been done on the specific mechanisms of organelle transmission in seed plants, the fact remains that only a small fraction of species has been studied so that the true picture may still be very incomplete.

Answers to the “whys” of cytoplasmic inheritance in seed plants are far from evident, especially if one is searching for a common explanation that will be all-inclusive. Why are there so many mechanisms, particularly in angiosperms, leading to a predominance of uniparental
maternal organelle inheritance? Why is uniparental organelle inheritance, whether maternal or paternal, the prevailing rule among the seed plants? What are the selective advantages of mostly maternal plastid inheritance in the flowering plants and a predominance of paternal plastid inheritance in the conifers, while mitochondrial inheritance has a very strong maternal bias in the angiosperms, the Pinaceae, and probably the Taxaceae? Are these patterns of organelle inheritance merely the indirect result of selection for other processes? Likely, no one explanation is applicable for all species, but various strategies have been adopted among different groups. Additional research in the field of organelle inheritance, particularly experimental studies where possible, will help to answer these questions in the future.

LITERATURE CITED


GIBBARD, E. M., AND J. LIN. 1975. Light microscope and ultrastructure


Moussel, B. 1978. Participation des elements cytoplasmatiques males et
Mogensen.—Cyttoplasmic inheritance in seed plants


