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GENETICS AND EVOLUTION OF THE CHLOROPLAST

(cytoplasmic inheritance, nucleus-cytoplasm interaction, plastome recombination, plastome mutator, Oenothera*)*

BARBARA B. SEARS

Department of Botany and Plant Pathology
Michigan State University
East Lansing, Michigan 48824-1312

SUMMARY

As a photosynthetic organelle and as the site of several important biosynthetic pathways, the chloroplast is essential to the survival of the plant cell. In evolutionary terms, the chloroplast genetic material can be considered conservative: from the green algae to higher plants, chloroplast DNAs have similar sizes, gene contents and arrangements, although some modifications of the chloroplast DNA have occurred as it has coevolved with the nucleus. Mutation and recombination are traditionally considered responsible for evolutionary change, but these may be quite limited in the chloroplast genetic system. Gene conversion may result in the elimination of new mutations, while the widespread occurrence of uniparental inheritance of chloroplasts reduces the likelihood of two different plastids meeting and their DNAs recombining. In other systems, transposons contribute to genetic change. One possible explanation for plastid mutator genes is that they activate transposable elements in the chloroplast DNA.

Among diverse genera in primitive and higher plants, both uniparental and biparental transmission of chloroplasts occur in sexual crosses. When maternal inheritance of plastids occurs, the paternal plastids may be excluded or eliminated from the male gamete in a variety of ways. Thus maternal inheritance has probably appeared in the algae, mosses, ferns, gymnosperms, and angiosperms through parallel evolution.

ROLE OF THE PLASTID

The plastid has a multi-faceted physiological role in the plant cell. The most obvious of its functions is photosynthesis, but it is by no means the only function. Since this has been thoroughly reviewed by Kirk and Tilney-Bassett (1978) and in several volumes edited by Stumpf and Conn (1980) only a brief

summary will be presented here. Proplastids can differentiate into many forms, each having a different function. Chromoplasts in floral petals contain the pigments which attract certain pollinators; the chromoplast coloration in fruits attracts animals who will eat the fruit and aid in dissemination of seeds. The leucoplast serves as a starch storage compartment; elaioplasts store oil.

A number of secondary metabolites such as plastoquinone, carotenoids, and chlorophylls are synthesized at least partially within the chloroplast (BICKEL & SCHULTZ 1976). Since these pigments are directly involved in photosynthesis their production within the chloroplast is not too surprising. Plastids are also involved in other biosynthetic pathways including the production of fatty acids (YAMADA & NAKAMURA 1975; MURPHY & LEECH 1977), particularly the C_{16} and C_{18} fatty acids (OHLROGGE & STUMPF 1979). Many of the enzymes for nitrogen assimilation are found in the chloroplast (LEA & MIFLIN 1974; RATHNAM & EDWARDS 1976), as are enzymes involved in amino acid biosynthesis (KIRK & LEECH 1972). The two processes are integrally linked (LEA & MIFLIN 1974; MIFLIN & LEA 198): nitrate from the cytoplasm is transported into the chloroplast and is reduced to nitrite. Within the chloroplast, the nitrite is converted to ammonia by a ferredoxin-dependent nitrite reductase. Glutamine synthetase catalyzes the reaction of ammonia + glutamate which results in the amino acid glutamine. Subsequent transaminations can eventually produce almost all of the other amino acids (reviewed by GIVAN 1980).

Although nitrogen assimilation and fatty acid synthesis are light-dependent in isolated chloroplasts, one would expect them to occur when another energy source is provided. Enzymes of nitrogen assimilation and amino acid biosynthesis are clearly present in nonphotosynthetic tissues such as roots (HIREL & GADAL 1980; MIFLIN & LEA, 1980). Furthermore, vigorous plants containing photosynthetically-defective chloroplasts may be propagated on sucrose-supplemented medium. In this case, the amino acid and fatty acid biosynthetic pathways must be independent of photosynthesis since cessation of these functions would surely be lethal. That must mean that these pathways are duplicated elsewhere in the plant cell and are independent of photosynthesis, or they occur in the plastid but are not obligatorily coupled to photosynthesis when other tissues of the plant are able to supply the photosynthate. Some enzymes seem to be duplicated in the mitochondria and cytoplasm (RATHNAM & EDWARDS;1976, STEWART et al., 1980), but an entire complement has not been identified. If the plastids are the only site in the plant cell for one or more of these biosynthetic pathways, then the plastid compartment is absolutely essential to the plant cell, even if it is photosynthetically defective. This may explain why it has not been possible to "cure" plant cells of their plastids.

Why do the photosynthetic functions occur together in a single compartment with various biosynthetic pathways? As mentioned previously, the biosyntheses of quinones, carotenoids, and chlorophyll logically occur in the plastid since that is their intracellular destination. Many of the other end products

are of course utilized by the plastids, but lipids and amino acids are also needed in other parts of the cell. Perhaps the most efficient utilization of the reducing power and energy generated by photosynthesis occurs when it is directly used without being transported out of the organelle. On the other hand, the compartmentalization of the pathways in the chloroplast may indicate something about the evolutionary origin of the organelle (Dr. Roger P. Hangarter, personal communication): the plastid may have arisen from an endosymbiotic association of an autotrophic prokaryote inside a cell which was no longer self-sufficient. The biosyntheses of amino acids, C_{16} and C_{18} fatty acids, and the secondary metabolites required for photosynthesis have simply been maintained in their original compartment, although the genes may have been transferred to the nucleus.

ORIGIN OF THE PLASTID

An endosymbiotic origin of the chloroplast has been proposed to explain the predominance of prokaryotic features within this photosynthetic organelle (reviewed by MARGULIS 1981; GRAY & DOOLITTLE 1982). As in bacteria, transcription and translation occur within the same compartment. The chloroplast ribosomes are 70S in size and are sensitive to inhibitors of prokaryotic translation (BOTTOMLEY AND BOHNERT 1982). All of the genetic information is contained on a circular DNA molecule rather than being scattered across many chromosomes. Strong sequence homologies exist between bacterial and chloroplast genes for tRNAs, rRNAs, and the elongation factor EF-tu (reviewed by BOHNERT et al. 1982). Other genes are also extremely similar, allowing cloned bacterial probes to be utilized to identify and locate the comparable chloroplast genes (e.g. WATSON & SURZYCKI 1982). Even stronger sequence homologies are apparent when chloroplast genes are compared with genes of the photosynthetic cyanobacteria (e.g. HECKER et al. 1982; CURTIS & HASELKORN 1983). In contrast to extant prokaryotes, the chloroplasts contain several functional genes which have intervening sequences (e.g. ROCHAIX & MALONE 1978, KOCH et al. 1981).

Two subcellular entities have seemed to cross biosystematic boundaries: the prokaryotic green alga *Prochloron* which lives symbiotically within colonial ascidians, and the cyanelles of *Cyanophora paradoxa*, which were thought to be symbiotic cyanobacteria, but now seem to be more appropriately classified as organelles. Physical characterizations of the DNA from the cyanelles show that the DNA has the same basic size, segmental structure and gene organization as green chloroplasts of higher plants (BOHNERT et al. 1983). DNA sequence characterizations of the ribosomal RNA genes from *Prochloron* have shown that it most closely resembles the Cyanobacteria (MacKAY et al. 1982; SEEWALDT & STACKEBRANDT 1982). These studies also show that the ribosomal RNA genes are more similar to those of the plastid than to those of other eubacteria. Thus *Prochloron* may be viewed as a chloroplast precursor, while the cyanelles may be derived from endosymbiosis of a cyanobacterium, followed by progressive loss of genetic information to the host nucleus.

COOPERATION AND COEVOLUTION OF PLASTOME AND GENOME

The photosynthetic functions of carbon fixation, electron transport, and ATP synthesis clearly require a coordination of both nuclear and plastome genes. Essential enzyme subunits are encoded by both genetic systems (reviewed by PARTHIER 1982). Regulatory controls must exist between genome and plastome to assure the appropriate stoichiometries of the enzyme subunits. The control mechanism must be particularly sophisticated since the diploid plant genome probably has only a few copies of the relevant genes, while each chloroplast may contain 20-50 copies of the plastome, and 20-400 chloroplasts may be found within a plant cell (HERRMANN et al. 1983).

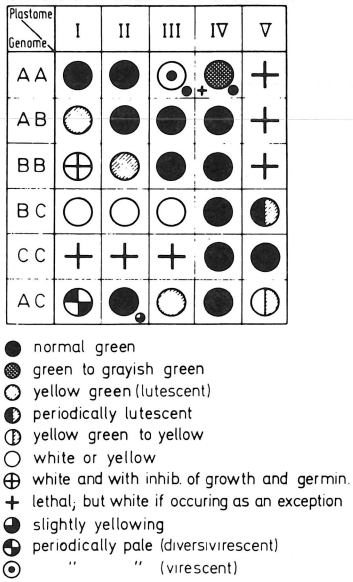

FIGURE 1. Compatibility relations between different diploid genotypes and plastid types (from KUTZELNIGG & STUBBE 1974, after STUBBE 1964). A smaller symbol in a square indicates a less frequently occurring compatibility reaction observed with one of the many different A genomes.

Proper development and functioning of the chloroplast have been shown genetically to require the cooperation of nuclear and plastid genes. Although chloroplasts can often survive in the nuclear background of a closely related species, various degrees of hybrid bleaching may occur (STUBBE 1964; METZLAFF et al. 1982). This dysfunction of the chloroplast indicates that these genetic systems have coevolved to produce a finely-tuned

cooperative relationship, termed *plastome-genome compatibility*. Genetic aspects of this phenomenon have been most thoroughly characterized in the evening primrose *Oenothera* (STUBBE 1964). Due to the existence of extensive reciprocal translocations between chromosomes and/or recessive lethal factors, the haploid genomes of many *Oenothera* species act as single linkage groups during meiosis. Thus, the plastome of one species can be introduced into the nuclear background of another species without a long series of backcrosses. Furthermore, since *Oenothera* shows biparental transmission of plastids, the offspring inherit chloroplasts from both parents. Within the subsection *Euoenothera*, Stubbe (1960, 1964) has classified plastomes into five types (I-V), based on their vigor in particular nuclear backgrounds (Fig. 1). Minor alterations in chloroplast DNA endonuclease fragments have been correlated with the five basic plastome types (GORDON et al. 1982). A few differences in molecular weights of plastome encoded polypeptides have also been noted (HERRMANN et al. 1980), but the cause of chloroplast dysfunction remains elusive. Incompatibility between the chloroplast and nucleus is expressed physiologically through hybrid bleaching, yet ultimate responsibility for this dysfunction must lie with the gene products of the two genetic compartments.

Among the five basic Euoenothera plastome types, plastome IV is thought to be the most primitive (STUBBE 1959, 1960, 1964). Plastome IV functions well in almost all nuclear backgrounds which have been tested (Fig. 1). This wide compatibility range suggests that plastome IV has not undergone as much evolutionary divergence as the other plastomes. Although plastome IV has a compatibility range advantage, it appears to have a slower replication rate than the other plastome types (SCHÖTZ 1954). Relative replication rates have been determined by observing the competitive success of two plastome types when they are placed (by crossing) into the same egg cell. Two factors are assessed in the offspring: the frequency of biparental versus maternal transmission of plastids and the ratios of the two plastid types in the variegated tissue of the biparental progeny. Since plastome IV is at a replicative disadvantage, probably only its broad compatibility range has saved it from extinction (STUBBE 1959).

EVOLUTION OF THE PLASTOME

The plastome is a very conservative genetic system. In general terms, gene content, arrangement, and even DNA sequences have undergone only minor changes when one compares the chloroplasts of some of the most primitive eukaryotic plants, the green algae, with the chloroplasts of higher plants (BEDBROOK & KOLODNER 1979; BARTLETT et al. 1980; BOHNERT et al. 1982).

Why has evolutionary change been comparatively slower for the chloroplast genetic system than for the nucleus? Perhaps this question is best answered by examining the factors which contribute to evolutionary change, and asking whether they are less influential for the chloroplast.

NATURAL SELECTION AND THE CHLOROPLAST

Since chloroplast function is essential for the survival and competitive success of the plant, selective pressures might be expected to be extremely great towards the evolution of improved photosynthetic efficiency. In fact this has occurred with the development of C_4 and CAM plants and the association of a broad range of light-trapping pigments. But in general these changes have been effected through differential expression of chloroplast genes or modification in nuclearly-coded components of the chloroplast, rather than through changes in the plastome genes.

Sager (1965) has suggested that since the chloroplasts contain essential gene complexes and combinations, any change in the genetic information could severely affect the fitness of the plant cell. Thus, packaging of essential photosynthetic genes into the chloroplast may have occurred to preserve optimal transcriptional arrangements and gene sequences.

One could argue that the chloroplast lives in the sheltered environment of the cell and is thereby insulated from direct selective pressures. However, this same argument should apply to the other DNA-containing organelles, the mitochondria. Yet mitochondria are extremely divergent when one compares the mitochondrial genetic systems in those same plant types mentioned previously, ranging in size from 9.7 Md in *Chlamydomonas* (GRANT & CHIANG 1980) to the DNA molecules of muskmelon which are 1600 Md (WARD et al. 1981).

MUTATION OF THE PLASTOME

Several inherent traits of the chloroplast genetic system make the establishment of mutations difficult. Perhaps the high degree of polyploidy of the chloroplast is the most important factor. Not only do most plant cells contain more than 20 chloroplasts, but each chloroplast contains many copies of chloroplast DNA. Expression and establishment of mutations must, therefore, require more time than for a haploid or diploid genome. Sorting out of chloroplasts results in pure cell lines, at least eventually. But if these cell lines are neither part of the germ line nor part of the plant which will vegetatively reproduce, the mutations will not be inherited, even if they have a selective advantage.

Another factor may influence the establishment of a mutation: the occurrence of gene conversion (non-reciprocal recombination). In *Chlamydomonas* (the only plant in which recombination of chloroplast markers has been studied), all recombination observed can be accounted for by gene conversion (VAN WINKLE-SWIFT & BIRKY 1978; SEARS 1980a). Birky (1978) has discussed how successive rounds of gene conversion among organelle DNA molecules could result in subcellular genetic drift for the alleles in which they differ, with fixation of one allele being the eventual result. Thus, even though many copies of chloroplast DNA exist within each chloroplast, any mutation has a chance of expression and establishment. Conversely, when gene conversion occurs in the other direction, the new mutations may

be lost rather than fixed. Since any new mutation would start at a large numerical disadvantage, genetic drift due to gene conversion would usually result in elimination of the mutation.

RECOMBINATION OF PLASTOME DNAs

Recombination generates new gene arrangements and combinations, and probably gene duplications which can provide the raw material for subsequent divergence. In order for recombination of plastome DNAs to occur, several conditions must be met: genetically different plastids must be introduced into the same cell, plastid fusion must occur, and chloroplast DNAs must subsequently recombine.

INTRODUCTION OF DIFFERENT PLASTIDS INTO ONE CELL. The absence of reports on recombination between chloroplast markers of higher plants can be attributed in part to the widespread occurrence of purely maternal transmission of plastids in crosses. In about two-thirds of the plants thus far investigated (reviewed by KIRK & TILNEY-BASSETT 1978), chloroplasts are inherited solely from the maternal parent, thus precluding the opportunity for chloroplasts to interact or recombine.

Polyethylene glycol-induced fusion has been employed to construct somatic cell hybrids having two different plastid types, but no plastome recombinants have been observed. Only one parental chloroplast type or the other has been found in plants derived from interspecific fusion products of *Nicotiana* (CHEN et al. 1977; IWAI et al. 1980; DOUGLAS et al. 1981; FLICK and EVANS 1982; GALUN et al. 1982; MALIGA et al. 1982) and *Lycopersicon* (SCHILLER et al. 1982). Considering the number of plastids in each parental cell at the time of fusion and the number of cell divisions prior to formation of the plant meristem, pure cell lines should not yet have been established (CHEN et al. 1977). In the *Nicotiana* fusion products, only one plant contained both parental cpDNAs, but rather than having mixed cells, this plant was a chimera (UCHIMIYA & WILDMAN 1979). *Recombinant chloroplast DNAs have not been observed.* Cytoplasmic mixing may be very low in the fusion products (PAUL GRUN, personal communication), resulting in little contact between plastids derived from different cells. Furthermore, due to experimental limitations, all of these investigations have dealt with very limited numbers of products. If recombination is infrequent, the chances of observing a recombinant product are not very high when only 10-20 clones can be analyzed. In this respect, sexual crosses of a biparental plant from which thousands of offspring can be scored should increase the likelihood of recovery of recombinants.

THE OCCURRENCE OF PLASTID FUSION. Ultrastructural observations indicate that chloroplast fusion occurs in a number of plants. It clearly happens in *Chlamydomonas* following fusion of the gametes (CAVALIER-SMITH 1970) and probably occurs in *Euglena* (CALVAYRAC & LEFORT-TRAN 1976), *Acetabularia* (CRAWLEY 1966) and in several species of mosses (BURR 1969; JENSEN & HULBARY 1978). Among the higher plants, electron microscopic studies have indicated that chloroplasts may be capable of fusion in *Mimosa* (ESAU 1972) and *Hosta* (VAUGHN 1981). Such ultrastructural observations

are difficult to interpret since adjacent or abutting chloroplasts could represent plastid division, or could be fixation artifacts. Besides *Chlamydomonas* , no complementary genetic evidence exists to indicate that different plastomes have a chance to interact with each other or that chloroplast markers recombine, and *Chlamydomonas* may be an exceptional case. In this unicellular alga, each normal haploid cell contains only one chloroplast and one nucleus. When gamete fusion places two chloroplasts and two nuclei into the same cell, "normalization" of the cellular contents may call for fusion of the nuclei and fusion of the chloroplasts. As a result, recombination is able to occur in both genetic systems. In contrast, higher plant cells usually contain many plastids and they have no obvious reason to fuse, unless it is to promote recombination of chloroplast DNAs in order to increase genetic diversity. But due to the widespread occurrence of maternal inheritance and the vegetative segregation of plastids when biparental inheritance occurs, almost all plant cells contain only one type of plastid. If all plastids are alike, then their fusion to promote recombination and genetic variation would be rather pointless. Again, the most promising cases to examine for the occurrence of plastome recombination may be plants which show a high frequency of biparental transmission of chloroplasts.

THE OCCURRENCE OF INTERMOLECULAR RECOMBINATION. Chloroplast markers and chloroplast DNAs have been shown to recombine in the unicellular green alga, *Chlamydomonas* (eg., GILLHAM 1978; LEMIEUX et al. 1980). Similarities exist between the chloroplast genetic system of *Chlamydomonas* and higher plants, including general physical size of the chloroplast DNA, gene content and organization, and instability of the heteroplasmic state. Conceivably, higher plant chloroplast DNAs are also capable of intermolecular recombination.

Recent observations have shown that cyanelle and chloroplast DNAs exist in two forms due to *intra*molecular recombination between the inverted repeat segments (BOHNERT & LÖFFELHARDT 1982; PALMER 1983). Thus the enzymes and mechanisms for recombination between chloroplast DNAs clearly exist. Sequences homologous to at least part of the chloroplast 16S rRNA gene have been shown to be present in mitochondrial DNA of maize (STERN & LONSDALE 1982). Although this could indicate that a strong conservation of 16S rRNA sequences has occurred during evolution of the organelles, it could also be due to genetic exchange between plastids and mitochondria. If the latter is the case, surely it is even more likely that genetic exchange within an organelle genetic system occurs.

Kung and colleagues (1981) have interpreted differences in chloroplast DNA restriction fragments of cytoplasmic male sterile *Nicotiana* plants to indicate that recombination has occurred between chloroplast DNAs. However this interpretation is not the only conceivable explanation, particularly since *Nicotiana* is thought to have purely maternal transmission of plastids. Such uniparental transmission of chloroplasts would normally preclude the opportunity for genetic exchange between plastids from the two parents. Frankel and colleagues (1979) have suggested that

chloroplast DNA alterations may be directly attributed to independent alterations occuring in the male sterile line.

In summary, with the exception of *Chlamydomonas*, recombination between chloroplast markers has not been demonstrated. Barriers to plastome recombination include maternal transmission of chloroplasts in sexual crosses and an absence or low frequency of plastid fusion. Since *intra* molecular recombination seems to occur, the potential for intermolecular recombination probably exists. For *Oenothera* (HERRMANN et al. 1983) and several legumes (CHU et al. 1981; PALMER & THOMPSON 1981; CHU & TEWARI 1982; SPIELMANN et al. 1983), rearrangements of chloroplast DNA segments and genes have been reported. Palmer and Thompson (1982) have suggested that loss of one of the two rRNA regions destabilizes the chloroplast DNA molecule and results in subsequent rearrangements. However, *Oenothera* and soybean plastomes contain both rRNA regions, yet have also undergone rearrangements. One trait shared by these two plant families is the frequent occurrence of biparental inheritance of plastids, and I would suggest that this is not coincidental. The likelihood of interaction of different plastid types would certainly be greater than in other plants, and recombination of chloroplast DNAs would be more probable. Errors occurring during intermolecular recombination could produce DNA rearrangements, and could also have resulted in the loss of one rRNA region.

ALTERATION OF CHLOROPLAST DNA BY TRANSPOSABLE ELEMENTS. In many organisms, transposable elements exist and have had a large impact on the genetic information. At the DNA level, transposons have caused mutations, deletions and inversions (reviewed by CALOS & MILLER, 1980). In evolutionary terms, transposons appear to contribute to speciation by causing hybrid dysgenesis in *Drosophila* (BINGHAM et al. 1982). In maize, multi-colored kernel patterns caused by the Activator-Dissociator system have had an evolutionary advantage because of the human factor in the selection process: the Indians preferred the multi-colored grains. Likewise, the spotted floral coloration of the snapdragon and African violet have been selectively cultivated by gardeners and horticulturists. Thus far, transposons have not been identified in the plastome. Their absence may contribute to the conservative nature of the chloroplast DNA.

Although transposons have not yet been found in chloroplast DNA, their existence should be considered since plant strains have been found which show a high frequency of plastome mutation (KIRK & TILNEY-BASSETT 1978). These plants have nuclearly-located mutator genes which cause a variety of mutations in chloroplast DNA (EPP 1973a, b; REDEI & PLURAD 1973). Among other possible explanations, the nuclear gene could be an activator of an otherwise stationary transposable element in the chloroplast.

Using the plastome mutator (*pm*) gene of *Oenothera johansen* which was isolated and genetically characterized by Epp (1973), I have established shoot cultures of homozygous plastome mutator lines. Whereas control lines appear to be stable, plastome mutations are produced at a high frquency in the homozygous plastome mutator plants. When maintained in this background, these new

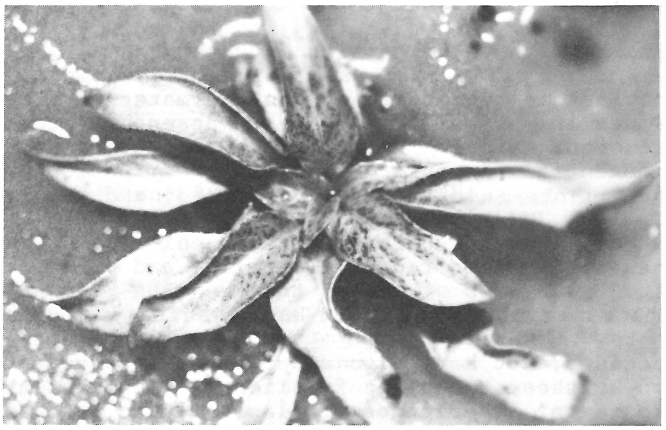

FIGURE 2. Shoot culture plant with a *pm*-induced plastome mutation. The plant is in the homozygous *pm* nuclear background; its leaves have many green flecks which are thought to be due to back mutations.

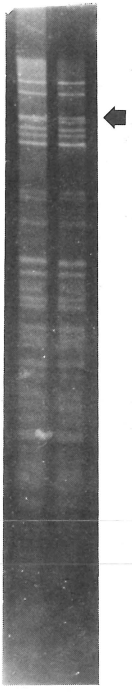

FIGURE 3. Bam Hl endonuclease restriction digests of chloroplast DNA from wild-type *Oenothera johansen* plants (left) and a mutant (right) induced by the plastome mutator. The arrow indicates a DNA fragment with altered mobility.

mutants show varying degrees of instability (Fig. 2). Since the reversions appear to occur at a much higher frequency than additional mutations, insertion and excision of a transposable element would be a more probable cause than reversion of point mutations. Furthermore, some mutants induced by the plastome mutator show changes in restriction enzyme patterns, which would not be expected if point mutations were occurring (Fig. 3). The observation that one of these DNA changes is similar to a chloroplast DNA difference in a closely related species *Oenothera hookeri* (unpublished results) is suggestive that a transposable element or some other inducible DNA rearrangement may have contributed to the evolutionary divergence of chloroplast DNAs.

EVOLUTION OF UNIPARENTAL INHERITANCE OF PLASTIDS

In crosses, the transmission of chloroplasts from only one parent to the offspring has been commonly observed. Yet frequently both gametes contain plastids. Why are the chloroplasts from one parent eliminated during spermatogenesis or fertilization? What factors have been influential in the evolution or maintenance of maternal versus biparental inheritance?

Only a few algae have been characterized in terms of the inheritance of chloroplast genes in crosses. Both the red alga *Gracilaria* (VAN DER MEER 1978) and several species of the green alga *Chlamydomonas* (GILLHAM 1978; METS 1980; VAN WINKLE-SWIFT & AUBERT 1983) show predominantly uniparental transmission of chloroplast genes. In another red alga, *Corallina*, degeneration of the plastids has been observed during spermatogenesis (PEEL & DUCKETT 1975). A similar mechanism in *Gracilaria* would explain why only the female parent transmits plastids to the progeny. For *Chlamydomonas* a more complicated explanation is required because the zygote is formed by the fusion of gametes of equal sizes, each containing one chloroplast. The gametes contain equal amounts of chloroplast DNA (CHIANG & SUEOKA 1967; SEARS et al. 1980) and the chloroplasts fuse shortly after gamete fusion (CAVALIER-SMITH 1970). Although the genetic contributions from the two parents would thus appear equal, 95-99% of the zygotes divide to produce colonies which contain chloroplast markers from only the "maternal" parent. This paradox shows the importance of having genetic data before conclusions are made from ultrastructural observations.

In most of the other green algae which have been examined, both gametes contain plastids (reviewed by SEARS 1980b). In *Ulva mutabilis*, several Zygnemataceae and some desmids, the chloroplasts from one parent have been reported to persist within the young zygote. Both the oocytes and spermatazoids of the brown algae, diatoms, Bacillariophyta, and Charophyta contain chloroplasts, but in many species, chloroplasts are eliminated from the male gametes before fertilization occurs.

Similarly, spermatazoids of the moss *Sphagnum* (MANTON 1957) and the liverwort *Sphaerocarpus donnellii* (DIERS 1967a) lose their plastids prior to contact with the archegonium. Other Bryophytina, as well as Lycophytina (*Lycopodium*, *Selaginella*, and

Isoetes) and Sphenophytina (*Equisetum*) clearly have male gametes which contain plastids, but the fate of the organelles after fertilization is unknown.

Among the ferns, elimination of plastids from the spermatazoids has been shown to occur in *Marsilea vestita* (MYLES 1975; MYLES & BELL 1975), *Thelypteris* (ATKINSON 1938) and *Pteridium aquilinum* (DUCKETT & BELL 1971; BELL & DUCKETT 1976). In contrast, biparental transmission and vegetative segregation of chloroplasts have been observed for *Phylitis scolopendrium* (ANDERSSON-KOTTO 1938). Thus ferns display the same variation in chloroplast inheritance as is seen in the algae.

In gymnosperms, chloroplasts seem to be inherited primarily from the father. This clearly occurs in the conifer *Cryptomeria japonica* in which the transmission of a color variant has been followed (OHBA et al. 1971). Ultrastructural observations indicate that the male gametes of the gymnosperms make a large cytoplasmic contribution to the fertilized egg (CHAMBERLAIN 1935; MAHESHWARI & KONAR 1971; ALLEN & OWENS 1972; CHOWDHURY 1974). Furthermore, plastids from the female parent may be preferentially eliminated during the early zygotic divisions (CAMEFORT 1966; CHESNOY & THOMAS 1971; GIANORDOLI 1974; WILLIAMS 1974). To complement the microscopic observations, additional genetic data from more genera are necessary to establish how frequently paternal inheritance of chloroplasts occurs among the Gymnospermae.

For the Angiosperms, both ultrastructural and genetic evidence abound (reviewed by KIRK & TILNEY-BASSETT 1978; HAGEMANN 1979; SEARS 1980b). The unequal cytoplasmic division of the microspore results in a generative cell (the male gamete) which often completely lacks plastids. In these cases, purely maternal transmission of chloroplasts in crosses is easily explained.

Both *Oenothera* (DIERS 1966) and *Pelargonium* (HAGEMANN 1979) have generative cells which contain numerous plastids and both have high frequencies of biparental plastid inheritance (reviewed by KIRK & TILNEY-BASSETT 1978). In many other cases, the generative cell contains plastids (e.g. LARSON 1965; VAZART 1970; JENSEN et al. 1974), but we lack the complementary genetic studies which would demonstrate whether the male plastids are transmitted to the offspring. Several plants which show maternal inheritance of chloroplasts (KIRK & TILNEY-BASSETT 1978) have pollen generative cells which initially contain plastids. The generative cells of *Solanum tuberosum* (CLAUHS & GRUN 1977) have plastids which degenerate during pollen maturation. From ultrastructural observations of pollen and albino plant cultivars derived from uninucleate pollen grains, Vaughn and colleagues (VAUGHN et al. 1980; VAUGHN 1981) propose that in the pollen of *Hosta* and *Oryza*, an "alteration" of the organelles occurs, debilitating them and thus precluding their successful transmission in crosses. Although this is a conceivable explanation for the albinism, it would be more credible if the authors had demonstrated that the plant cultivars were not albino due to the haploid nuclear condition. Other anther cultures have produced green and white plantlets (e.g. BULLOCK et al. 1982) in ratios which suggest that the albinism depends on two or more unlinked

nuclear loci.

Apparently both genome and plastome can affect the transmission of chloroplast genes in crosses. Schötz (1954) showed that different plstids had variable degrees of replication success when placed (by crossing) into the same cell as another plastid type. Although these crosses did not utilize isonuclear strains, subsequent investigations have confirmed the results at least partially (EPP 1972; KEMPER 1959). An example is shown in Table 1.

TABLE 1. Transmission and Replication Success of Wild-Type Plastome II in competition with Mutant Plastomes III or IV. Isonuclear strains of *Oenothera johansen* were emasculated and crossed with pollen from a single plant of *O. hookeri*. The female parent contained either mutant IVα or mutant IIIγ in the L2 (corpus and germ line) tissue layer. Seeds were germinated on Murashige and Skoog's medium. About 2 weeks after germination, the cotyledons were scored for amount of variegation.

Plastome	Genome	% Biparental Seedlings	Average % Green Tissue in Cotyledons of Biparental Offspring	Number of Seedlings Scored
IVα x II white green	johansen x hookeri (AA) (AA)	43.6	23.9	282
IIIγ x II white green	johansen x hookeri (AA) (AA)	26.1	7.8	291

In these isonuclear lines, the pollen contained wild-type plastome II, while the egg cell carried a mutant of either plastome III or IV. In the latter case, a higher frequency of variegated offspring was observed and the variegated plants had more green tissue. Thus plastome II is more successful when competing against plastome IV than against plastome III. According to Schotz' theory, this implies that plastome II replicates faster than plastome IV. The data of Table 1 also point out a paradox: when the female parent carried plastome IV, 44% biparental inheritance was obtained, but when the isonuclear maternal strain carried plastome III, only 26% biparental transmission was observed. Since the same pollen source was used, presumably at least 44% of the pollen generative cells were capable of transmitting chloroplasts to the offspring. If this is the case, why did only 26% of the seedlings from the second cross display green sectors? Perhaps the male plastids were destroyed within the zygotes or were preferentially segregated into the suspensor cell. Alternatively, the male plastids enter the zygote with a strong replicative disadvantage, which becomes more extreme when they cohabit the cell with certain other plastid types. If their replication rate is very slow, they may make an unnoticeable contribution to the progeny.

In *Pelargonium*, plastid content also affects the outcome of

crosses, with green plastids being more successful than white ones (TILNEY-BASSETT & ABDEL-WAHAB, 1979; ABDEL-WAHAB & TILNEY-BASSETT, 1981). Additionally, alleles at two nuclear loci of the female parent are able to alter significantly the balance between maternal and biparental transmission in this plant (TILNEY-BASSETT & ABDEL-WAHAB 1979, 1982). The paternal nuclear composition may also prove important: this could explain the absence of plastids from the pollen generative cells of *Castilleia foliosa* and their inclusion in the closely related species *C. wightii*. Thus, any angiosperm may have the genetic potential for either biparental or maternal transmission of chloroplasts (TILNEY-BASSETT & ABDEL-WAHAB 1979).

Although uniparental inheritance of plastids occurs in diverse species of the plant kingdom, the plastids of one parent may be eliminated by very different mechanisms: exclusion during spermatogenesis, loss from the motile sperm, elimination during fertilization, or degradation within the zygote. Since biparental plastid transmission also occurs in all divisions of the plant kingdom, this suggests that uniparental inheritance has evolved many times in response to different selective pressures. The spermatazoids of the mosses and ferns may discard excess cytoplasmic baggage because the more stream-lined spermatozoids are faster and thus have a higher chance of reaching the oocyte first. On the other hand, the gymnosperm male gametes are very large and contain hundreds of plastids as starch storage units. In the gymnosperms, months may elapse between pollination and fertilization and the spermatazoids must be able to maintain their cellular metabolism until gamete fusion is finally accomplished. Elimination of paternal chloroplast DNA may occur in *Chlamydomonas* to minimalize recombination which could alter advantageous gene arrangements. Another possibility is suggested from an examination of the *Chlamydomonas* life cycle. When the cells are starved for nitrogen, they will differentiate into gametes. Turnover of both cytoplasmic and chloroplastic ribosomes occurs at this time (MARTIN et al. 1976) and the chloroplast DNA content of the cell decreases (CHIANG & SUEOKA 1967; SEARS et al. 1980). The zygospores which result from gamete fusion "mature" under conditions of nitrogen starvation. Since they are photosynthetically dormant, perhaps excess plastid DNA is degraded and utilized as a nitrogen and carbon source.

It is more difficult to justify evolutionarily the existence of maternal versus biparental inheritance of plastids in angiosperms. When plastids are present in the generative cells, they are undifferentiated proplastids lacking starch, and therefore do not serve as food storage units. Closely related species are known to differ in chloroplast inheritance: in addition to the variation seen in *Pelargonium* and the diverse legume family. *Castilleia wightii* has generative cells which contain plastids, while *C. foliosa* lacks them (JENSEN et al. 1974). It seems unlikely that closely related species would be under such different selective pressures for this trait. In terms of minimizing recombination between chloroplast DNAs, if plastid fusion rarely occurs it would make little difference whether the plastids were inherited from only one or from both parents. Perhaps plastid exclusion from the generative cell occurs in some cases simply as

a consequence of an extremely unequal cytoplasmic division of the microspore.

To summarize, biparental and uniparental transmission of plastids probably occur for many different reasons in various divisions of the plant kingdom. These diverse plant species undoubtedly face very different selective pressures which may affect this trait.

CONCLUSIONS

Even though structural rearrangements of chloroplast DNA have occurred during the evolutionary divergence of plants, the plastome is extremely conservative in gene content, sequence, and arrangement. Considering these similarities, one is tempted to conclude that the genetic characterizations of the *Chlamydomonas* chloroplast will also apply to higher plants. In this regard, we should remember that *Chlamydomonas* and many higher plants are similar in showing predominantly uniparental transmission of chloroplasts in crosses, yet maternal inheritance occurs by very different mechanisms, and probably for very different reasons. Thus, the similarities in chloroplast inheritance are due to parallel evolution, rather than preservation of this trait. Although recombination and gene conversion have been demonstrated for *Chlamydomonas* they may be meaningless concepts for higher plants if biparental transmission of chloroplasts is infrequent or if chloroplasts rarely fuse.

Plastids perform many essential functions for the plant cell, yet much of the requisit genetic information is in the nucleus. Gene action in plastome and genome must be precisely regulated to coordinate the timing of transcription and translation of enzyme subunits and to assure their appropriate stoichiometries. Further characterization of this cooperative gene activity may help us understand how the plastome and genome have coevolved.

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