# Plastome-Genome Interactions Affect Plastid Transmission in Oenothera

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ABSTRACT

Plastids of Oenothera, the evening primrose, can be transmitted to the progeny from both parents. In a constant nuclear background, the frequency of biparental plastid transmission is determined by the types of plastid genomes (plastomes) involved in the crosses. In this study, the impact of nuclear genomes on plastid inheritance was analyzed. In general, the transmission efficiency of each plastome correlated strongly with its compatibility with the nuclear genome of the progeny, suggesting that plastome-genome interactions can influence plastid transmission by affecting the efficiency of plastid multiplication after fertilization. Lower frequencies of plastid transmission from the paternal side were observed when the pollen had poor vigor due to an incompatible plastome-genome combination, indicating that plastome-genome interactions may also affect the input of plastids at fertilization. Parental traits that affect the process of fertilization can also have an impact on plastid transmission. Crosses using maternal parents with long styles or pollen with relatively low growth capacity resulted in reduced frequencies of paternal plastid transmission. These observations suggest that degeneration of pollen plastids may occur as the time interval between pollination and fertilization is lengthened.

THE majority of angiosperms inherit their plastids only from the maternal parent. However, in some angiosperms, plastids are transmitted at a high frequency from both parents [reviewed by KIRK and TILNEY-BASSETT (1978) and GILLHAM, BOYNTON and HARRIS (1991)] or predominantly from the paternal parent [*e.g.*, LEE, BLAKE and SMITH (1988), SCHUMANN and HANCOCK (1989), MASOUD, JOHNSON and SORENSEN (1990) and BOBLENZ, NOTHNAGEL and METZLAFF (1990)]. Genetic studies of plastid transmission in different plant species indicate that plastid inheritance can be affected either by the parental nuclear genomes (CORNU and DULIEU 1988; TILNEY-BASSETT 1988; SMITH 1989; MASOUD, JOHNSON and SORENSEN 1990) or by the plastid genomes (plastomes) involved in the crosses (SCHÖTZ 1974, 1975; CHIU, STUBBE and SEARS 1988).

In Oenothera, plastids are transmitted from both parents. In some interspecific crosses of Oenothera, biparental inheritance of the plastids is observed as "hybrid variegation," with plastids from one parent failing to become fully pigmented in the hybrid nuclear background. This genetic dysfunction has been termed "plastome-genome incompatibility." The genetic studies of STUBBE (1959) on this subject led him to categorize the chloroplasts of the subsection *Oenothera* into five basic types (*I*, *II*, *III*, *IV* and *V*) according to their compatibilities with the six major diploid nuclear genotypes (*AA*, *BB*, *CC*, *AB*, *AC* and *BC*). In the more serious cases of incompatibility, not only plastid development, but also the development of the embryo sac and pollen, can be inhibited (STUBBE 1963).

The five plastome types of Oenothera also differ in their transmission efficiencies. SCHÖTZ (1954, 1974, 1975) studied the frequencies of biparental plastid transmission in crosses of a large number of wild-type Oenothera species with several strains carrying mutant plastids. He concluded that the five plastome types have differing abilities to compete with incoming plastomes in crosses and classified the five plastome types as strong (types *I* and *III*), intermediate (type *II*), or weak (types *IV* and *V*), according to their relative competitive abilities. The same relative transmission efficiencies were observed when plastome types *I* through *IV* were compared in reciprocal crosses in a constant nuclear background (CHIU, STUBBE and SEARS 1988). These studies, which span four decades, are the basis of the belief that the efficiency of plastid transmission in Oenothera is determined mainly by the plastids themselves (KIRK and TILNEY-BASSETT 1978; STUBBE 1989).

Although SCHÖTZ (1974) pointed out that the nuclear genome can also influence plastid inheritance, the actual role of the nuclear genome in plastid transmission has not been defined in Oenothera. Since both plastome- and nuclear-encoded gene products are required for transcription and translation within the plastid [reviewed by SUGIURA (1989) and MAYFIELD (1990)], it is conceivable that critical processes such as plastid multiplication and transmission may also rely on the interactions between plastome and

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**TABLE 1**

**Oenothera strains used in the crosses**

| Oenothera species | Genome complexes[a] | Genotype[b] | Plastome type[c] | Source of plastid |
|---|---|---|---|---|
| *O. hookeri* str. Joh | *$^h$Johansen·$^h$Johansen* | $A^jA^j$ | *I-ζ* | *O. hookeri* str. Joh |
| *O. hookeri* str. Joh | *$^h$Johansen·$^h$Johansen* | $A^jA^j$ | *II-ε* | *O. suaveolens* |
| *O. hookeri* str. Joh | *$^h$Johansen·$^h$Johansen* | $A^jA^j$ | *III-γ* | *O. grandiflora* |
| *O. hookeri* str. Joh | *$^h$Johansen·$^h$Johansen* | $A^jA^j$ | *IV-α* | *O. ammophila* |
| Hybrid | *albicans* ♀·*percurvans* ♂ | $A^aC^p$ | *II* | *O. suaveolens* |
| Hybrid | *albicans* ♀·*percurvans* ♂ | $A^aC^p$ | *III* | *O. grandiflora* |
| Hybrid | *albicans* ♀·*percurvans* ♂ | $A^aC^p$ | *IV* | *O. ammophila* |
| *O. ammophila* | *rigens* ♀·*percurvans* ♂ | $A^rC^p$ | *II* | *O. suaveolens* |
| *O. ammophila* | *rigens* ♀·*percurvans* ♂ | $A^rC^p$ | *IV* | *O. ammophila* |
| *O. parviflora* | *augens* ♀·*subcurvans* ♂ | $B^aC^s$ | *IV* | *O. parviflora* |
| *O. atrovirens* | *pingens* ♀·*flectens* ♂ | $B^pC^f$ | *IV* | *O. atrovirens* |
| *O. grandiflora* | *grandiflora·grandiflora* | $B^gB^g$ | *III* | *O. grandiflora* |

[a] The name of each chromosome complex follows CLELAND (1972). Haploid genome transmitted through only one of the gametes is indicated with male or female symbol.

[b] Each haploid genome is represented by *A*, *B* or *C* according to the compatibility group to which it belongs (STUBBE 1959). The superscript letter is an abbreviation of the name of the haploid genome.

[c] The Roman numerals indicate the plastome type. If followed by a Greek letter, a plastome mutant is indicated.

genome. In this study, crosses were performed to analyze the impact of both the plastid and nuclear genomes on plastid transmission.

## MATERIALS AND METHODS

**Plant material:** Oenothera strains used in this study are listed in Table 1. All mutant plastids were maintained as periclinal chimeras in *Oenothera hookeri* str. Johansen (genotype $A^jA^j$) that carried wild-type plastids of plastome type *IV*, in addition to the mutant plastids (CHIU, STUBBE and SEARS 1988). All the strains heterozygous for chromosome complexes were provided by W. STUBBE (University of Düsseldorf, Germany).

### Genetic crosses

**Group 1. Formation of hybrid genotype *AC*:** $A^jA^j$ plants with mutant plastomes of types *I*, *II*, *III* and *IV* were used as the maternal parent in this set of crosses. Pollen was obtained from the Oenothera hybrid albicans/percurvans ($A^aC^p$) with plastomes of type *II*, *III*, or *IV*, *Oenothera ammophila* ($A^rC^p$) with plastomes of type *II* or *IV*, *Oenothera parviflora* ($B^aC^s$-*IV*) or *Oenothera atrovirens* ($B^pC^f$-*IV*). Due to the pollen lethal factors carried by the $A^a$, $A^r$, $B^a$ and $B^p$ genome complexes, the pollen transmits only the type *C* chromosome complex (CLELAND 1972), and hence, offspring of these crosses have the genotype *AC*, with *A* contributed by the egg and *C* by the pollen.

**Group 2. Formation of hybrid genotype *AB*:** Crosses yielding progeny of *AB*-genotype were performed by pollinating Oenothera strains carrying a type *B* chromosome complex with pollen from an $A^jA^j$ plant carrying a mutant plastid. Due to the egg lethal factors carried by the $C^f$ and $C^s$ chromosome complexes (CLELAND 1972), crosses using either *O. parviflora* ($B^aC^s$) or *O. atrovirens* ($B^pC^f$) as the maternal parent result in progeny with nuclear backgrounds $A^jB^a$ or $A^jB^p$, respectively.

**Germination and scoring of the seedlings:** Seeds were placed in water in a beaker, and were shaken at 100 rpm under constant room light until they germinated. The seeds were surface-sterilized daily with a combination of 20% commercial bleach and 0.1% sodium dodecyl sulfate (SDS) until roots emerged (3–6 days). Due to the strong maternal bias in Oenothera plastid transmission, crosses in which mutant plastids were contributed by the maternal parents produced mainly white seedlings. For this reason, germinating seeds from White × Green crosses were placed on agar medium, as previously described (CHIU, STUBBE and SEARS 1988), while germinating seeds from Green × White crosses were transferred to soil. Progeny with plastids from both parents were recognized by the variegation of their cotyledons. In each cross, the number of progeny with plastids from both parents was recorded, as well as the fraction of both cotyledons with pigmentation characteristic of the plastids inherited from the paternal parent.

**Germination of Oenothera pollen *in vitro*:** Mature Oenothera pollen grains were harvested from flower buds that were ready to open on the same day (judging from the size and color of the flower bud) by aspirating the pollen into a pasteur pipet. These pollen were sprinkled onto germination solution (10% sucrose, 0.01% $H_3BO_3$, and 0.02% $CaCl_2$) (CORRIVEAU and COLEMAN 1988) in a Petri plate. After 4 hr incubation at room temperature, the samples were fixed in acetic acid and ethanol (1:3) for 2 hr at room temperature and stored in 70% ethanol at 4°. To measure the length of pollen tubes, the samples were allowed to air dry on the petri plate and the distance between the base and the tip of the pollen tube was estimated with a ruler under an inverted microscope.

**Statistical analysis of biparental transmission frequencies:** The frequencies of plastid transmission obtained from crosses mentioned above were first subjected to arcsin transformation (angles were expressed in radians) and then analyzed by two- or three-way analysis of variance.

## RESULTS

**Haploid chromosome complex *C* can facilitate but does not ensure the transmission of plastome type *IV* from pollen:** If the efficiency of plastid transmission is influenced by the relative compatibility between the plastome and the genome, an enhanced transmission of paternal plastids might be seen when the nuclear background of the progeny is more compatible with the paternal plastid than with the mater-

nal plastids. In order to test this hypothesis, the relative efficiencies of plastid transmission in crosses producing progeny with $A^jA^j$ or $A^jC^p$ genotypes were compared. Genotype *AA* is associated with plastome type *I* in nature (STUBBE 1964). In the $A^jA^j$ nuclear background, chloroplasts with plastomes *I*, *II*, and *IV* have normal pigmentation, while those with plastome *III* are periodically bleached (STUBBE 1959). Genotype *AC* is the natural nuclear background of plastome type *IV*. The relative compatibilities of the same plastome types in an *AC* nuclear background, according to the extent of greening, are in the order of *IV* > *II* > *III* > *I* (STUBBE 1959). Due to plastome-genome incompatibility, *AC-I* plants were not viable in the field and $A^aC^p$-*III* plants produced pollen with poor vigor. Thus, the analysis of paternal parents with an *AC* genotype could only include plastomes *II* and *IV*.

In the crosses labeled set 1 and 2 from two field seasons (Table 2), the maternal parent had the genotype $A^jA^j$ and contained mutant plastids, representing each of the four basic plastome types. Wild-type plastids of plastome type *II* or *IV* were contributed by the pollen, as was the *C* genome. The frequencies of biparental plastid transmission from crosses performed in the first field season appeared to be higher than those obtained from the second field season (Table 2). Hence, the relative contribution of maternal and paternal plastome types as well as seasonal variations on plastid inheritance were analyzed by three-way analysis of variance. As shown in Table 3, changing the paternal plastome types has the strongest effect ($P < 0.01$) on the frequency of biparental plastid transmission, and the environmental influences are as impactful ($P < 0.025$) as the influences from the maternal plastome types. However, none of the interactions between any two major factors is significant. Because of the environment effects, direct comparison of transmission frequencies can only be made among crosses from the same field season.

In crosses producing $A^jC^p$ progeny from both seasons, paternal plastome type *IV* was transmitted to the progeny in a higher frequency than was plastome type *II*. These relative transmission efficiencies of plastome types *II* and *IV* are reversed as compared to results obtained from earlier studies using the *AA* nuclear background (SCHÖTZ 1974, 1975; CHIU, STUBBE and SEARS 1988). As illustrated in Figure 1, the transmission efficiency of paternal plastome type *II* in crosses producing progeny with the genotype $A^jC^p$ (panel B and C) is only slightly higher than that obtained from crosses producing genotype $A^jA^j$ (panel A). But for plastome type *IV*, crosses producing progeny with genotype $A^jC^p$ have a much higher transmission efficiency of paternal type *IV* plastome than is the case for crosses producing progeny with the $A^jA^j$ genotype. Crosses involving paternal strain $A^rC^p$-*IV* (Table 2, season 2, set 3) also produced progeny with genotype $A^jC^p$. Transmission frequency of plastome IV from $A^rC^p$ pollen (set 3) is slightly lower ($P < 0.25$) than that of the same plastome carried by $A^aC^p$ pollen (set 2). An attempt to include $A^rC^p$-*II* in these comparisons was not successful because pollen from $A^rC^p$-*II* plants had very poor vigor probably due to plastome-genome incompatibility.

The relative success of different maternal plastids also varied with nuclear background of the progeny. In crosses producing genotype $A^jA^j$ (Figure 1A), the transmission success of the maternal plastome was in the order of $I$-$\zeta$ > $II$-$\epsilon$ > $III$-$\gamma$ > $IV$-$\alpha$, as seen by the increasing frequency of plastid transmission from the male parent (measured by the frequency of variegated progeny). However, in crosses producing genotype $A^jC^p$, the relative success of maternal plastid transmission was in the order of $II$-$\epsilon$ > $III$-$\gamma$ > $I$-$\zeta$ > $IV$-$\alpha$ (Figure 1, B and C).

Crosses with two other strains capable of contributing chromosome complex *C* from pollen, $B^aC^s$-*IV* and $B^pC^f$-*IV*, were also conducted to allow a comparison of the impact of different *C* genomes on plastid transmission. As shown in Table 2, the transmission frequency of plastids from $B^aC^s$-*IV* pollen (season 1, set 3) was lower ($P < 0.1$) than that from $A^aC^p$-*IV* pollen (season 1, set 2). Crosses between $A^jA^j$ strains and $B^pC^f$-*IV* produced very few seeds. As will be discussed later, this may have been due to limited pollen tube growth. No plastids from the paternal $B^pC^f$-*IV* parent were detected in a total of 50 progeny produced from these crosses (season 1, set 4). Despite the difficulty in obtaining seeds from some crosses, the results from crosses involving pollen carrying a *C* genome indicate that the presence of a *C* genome in the pollen or progeny does not ensure a high level of transmission of plastome type *IV* from pollen. Furthermore, the genotype of the paternal parent may modify the efficiency of plastid transmission, for example, by affecting the input of paternal plastids during fertilization.

**Relative efficiency of plastid transmission differs in reciprocal crosses that result in progeny with different hybrid genotypes:** In reciprocal crosses of those performed in season 2 of Table 2, the same $A^jA^j$ plants carrying mutant plastomes *I* through *IV* were used as paternal parents while $A^aC^p$-*II*, $A^aC^p$-*IV* and $A^rC^p$-*IV* plants were used as maternal parents. Due to the egg lethal factors carried by chromosome complex $C^p$, only the *A* complexes are transmitted by the maternal parents. As shown in Table 4, all of the crosses produced the hybrid nuclear background $A^aA^j$ and yielded extremely high frequencies of biparental plastid transmission. In these crosses, maternally contributed plastome type *II* (Table 4, set 1) was more successful ($P < 0.05$) than maternally contributed

**TABLE 2**

**Plastid transmission in crosses producing progeny with *AC* genotype**

| Season | Set | Crosses: Maternal | Crosses: Paternal | $F_1$ hybrid genotype | Transmission: *N* | Transmission: %BP[a] | Average area of green tissue in BP progeny |
|---|---|---|---|---|---|---|---|
| 1 | 1 | $A^jA^j$-*I*$\zeta$ | $A^aC^b$-*II* | $A^jC^b$ | 96 | 11.5 ± 3.3 | 0.18 |
| | | $A^jA^j$-*II*$\epsilon$ | $A^aC^b$-*II* | $A^jC^b$ | 84 | 3.6 ± 2.0 | 0.17 |
| | | $A^jA^j$-*III*$\gamma$ | $A^aC^b$-*II* | $A^jC^b$ | 138 | 8.7 ± 2.4 | 0.15 |
| | | $A^jA^j$-*IV*$\alpha$ | $A^aC^b$-*II* | $A^jC^b$ | 53 | 22.6 ± 5.7 | 0.31 |
| | 2 | $A^jA^j$-*I*$\zeta$ | $A^aC^b$-*IV* | $A^jC^b$ | 132 | 26.5 ± 3.8 | 0.29 |
| | | $A^jA^j$-*II*$\epsilon$ | $A^aC^b$-*IV* | $A^jC^b$ | 136 | 5.9 ± 2.0 | 0.13 |
| | | $A^jA^j$-*III*$\gamma$ | $A^aC^b$-*IV* | $A^jC^b$ | 67 | 22.4 ± 5.1 | 0.11 |
| | | $A^jA^j$-*IV*$\alpha$ | $A^aC^b$-*IV* | $A^jC^b$ | 133 | 31.1 ± 4.0 | 0.29 |
| | 3 | $A^jA^j$-*III*$\gamma$ | $B^aC^s$-*IV* | $A^jC^s$ | 131 | 6.9 ± 2.2 | 0.13 |
| | | $A^jA^j$-*IV*$\alpha$ | $B^aC^s$-*IV* | $A^jC^s$ | 30 | 10.0 ± 5.5 | 0.17 |
| | 4 | $A^jA^j$-*III*$\gamma$ | $B^bC^f$-*IV* | $A^jC^f$ | 21 | 0 | |
| | | $A^jA^j$-*IV*$\alpha$ | $B^bC^f$-*IV* | $A^jC^f$ | 29 | 0 | |
| 2 | 1 | $A^jA^j$-*I*$\zeta$ | $A^aC^b$-*II* | $A^jC^b$ | 162 | 3.1 ± 1.4 | 0.14 |
| | | $A^jA^j$-*II*$\epsilon$ | $A^aC^b$-*II* | $A^jC^b$ | 188 | 2.7 ± 1.2 | 0.14 |
| | | $A^jA^j$-*III*$\gamma$ | $A^aC^b$-*II* | $A^jC^b$ | 190 | 3.7 ± 1.4 | 0.13 |
| | | $A^jA^j$-*IV*$\alpha$ | $A^aC^b$-*II* | $A^jC^b$ | 27 | 7.4 ± 5.0 | 0.15 |
| | 2 | $A^jA^j$-*I*$\zeta$ | $A^aC^b$-*IV* | $A^jC^b$ | 176 | 10.8 ± 2.3 | 0.15 |
| | | $A^jA^j$-*II*$\epsilon$ | $A^aC^b$-*IV* | $A^jC^b$ | 99 | 4.0 ± 2.0 | 0.20 |
| | | $A^jA^j$-*III*$\gamma$ | $A^aC^b$-*IV* | $A^jC^b$ | 101 | 9.9 ± 3.0 | 0.18 |
| | | $A^jA^j$-*IV*$\alpha$ | $A^aC^b$-*IV* | $A^jC^b$ | 173 | 24.9 ± 3.3 | 0.24 |
| | 3 | $A^jA^j$-*I*$\zeta$ | $A^rC^b$-*IV* | $A^jC^b$ | 438 | 5.9 ± 1.1 | 0.19 |
| | | $A^jA^j$-*II*$\epsilon$ | $A^rC^b$-*IV* | $A^jC^b$ | 152 | 3.3 ± 1.4 | 0.20 |
| | | $A^jA^j$-*III*$\gamma$ | $A^rC^b$-*IV* | $A^jC^b$ | 205 | 6.8 ± 1.8 | 0.18 |
| | | $A^jA^j$-*IV*$\alpha$ | $A^rC^b$-*IV* | $A^jC^b$ | 125 | 8.0 ± 2.4 | 0.15 |

[a] The biparental (BP) transmission frequencies are presented with their standard deviations estimated by the normal approximation for the binomial population (STEEL and TORRIE 1980).

**TABLE 3**

**Analysis of variance of biparental transmission frequencies in $A^jA^j \times A^aC^b$ crosses**

| Analysis | d.f. | MS | *F* | Significance |
|---|---|---|---|---|
| Main effects | | | | |
| Seasonal (S) | 1 | 0.064 | 32.0 | $P < 0.025$ |
| Paternal (P) | 1 | 0.076 | 38.0 | $P < 0.010$ |
| Maternal (M) | 3 | 0.049 | 24.5 | $P < 0.025$ |
| Interactions | | | | |
| S × P | 1 | 0.001 | 0.25 | |
| S × M | 3 | 0.004 | 2.0 | |
| P × M | 3 | 0.004 | 2.0 | |
| Error | 3 | 0.002 | | |

plastome type *IV* (Table 4, set 2) in competing with incoming paternal plastids. No significant difference in the frequency of biparental plastid transmission was observed between sets 2 and 3, in which the maternal parents contributed different *A* genomes, but the same plastome type. In the three sets of crosses presented in Table 4, the relative efficiencies of paternal plastid transmission were in the order of *I*-$\zeta \geq$ *III*-$\gamma >$ *II*-$\epsilon >$ *IV*-$\alpha$. Hence, these relative transmission efficiencies are the same as those observed in crosses resulting in genotype $A^jA^j$ (CHIU, STUBBE and SEARS 1988).

**The relative transmission efficiencies of plastome types *I* and *III* from the pollen are reversed in progeny with *AB* vs. *AA* nuclear backgrounds:** Plastome type *I* is more compatible with an *AA* nuclear background than is plastome *III*, while the reverse is true for the *AB* nuclear background (STUBBE 1959). The crosses shown in Table 5 resulted in hybrid genotype *AB*. In all three sets of crosses, mutant plastome *III*-$\gamma$ was transmitted by the pollen to a higher proportion of the progeny than was mutant plastome *I*-$\zeta$. In crosses resulting in genotype $A^jB^a$ (set 1) or $A^jB^g$ (set 3), the relative efficiency of paternal plastid transmission was in the order of *III*-$\gamma >$ *II*-$\epsilon >$ *I*-$\zeta >$ *IV*-$\alpha$, while in crosses resulting in genotype $A^jB^b$ (set 2), the order of *I*-$\zeta$ and *II*-$\epsilon$ was reversed.

In variegated progeny, the abundance of tissue displaying the paternal plastid phenotype in individual progeny is an additional parameter that can be used to assess the competitive multiplication abilities of the

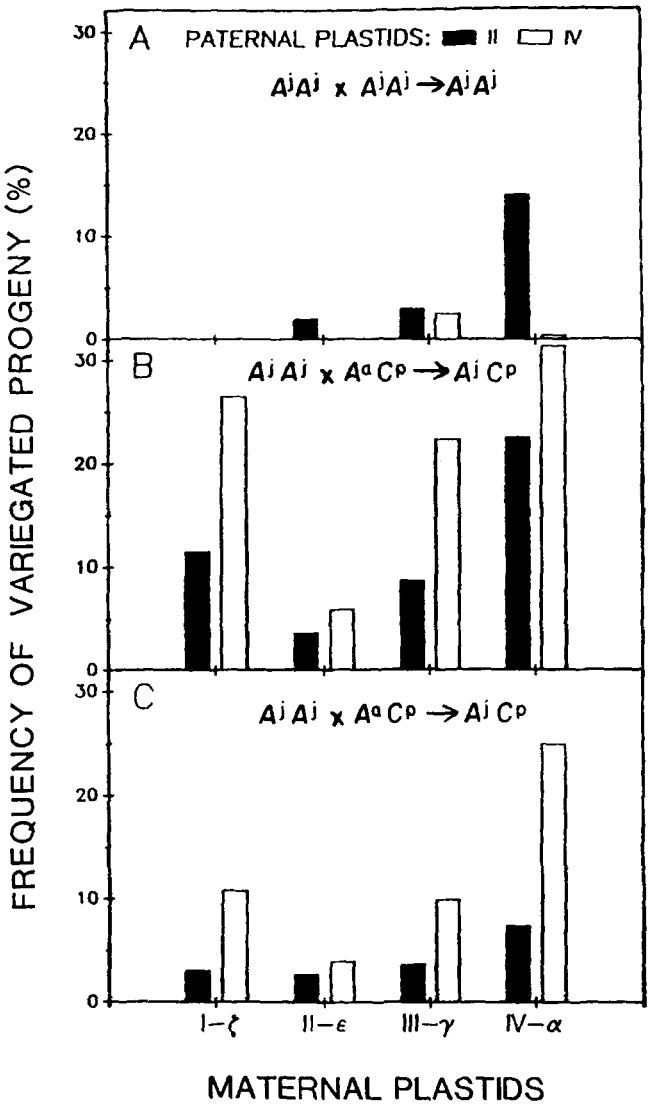


FIGURE 1.—Frequency of progeny carrying plastids from both parents in White × Green crosses of Oenothera. The maternal parents had the genotype $A^iA^i$ and the plastome type indicated on the x-axis. The pollen carried either plastome *II* (closed bars) or *IV* (open bars). (A) Both paternal and progeny genotypes were $A^iA^i$. (B) and (C) Paternal genotype was $A^aC^p$, and progeny genotype was $A^iC^p$. The data for (A) are taken from CHIU, STUBBE and SEARS (1988). The data for (B) and (C) are from Table 2 season 1 and season 2, respectively.

various plastome types (CHIU, STUBBE and SEARS 1988). In general, the abundance of paternal plastids in the cotyledons has a positive correlation with the frequency of biparental plastid transmission, especially when both values are high (see Tables 2, 4 and 5). In progeny with an $A^iB^p$ nuclear background, the fraction of white tissue was much higher when the pollen contributed mutant plastome *III*-γ, than when mutant plastome *I*-ζ was brought in through the pollen (Figure 2B). This was true even though the crosses producing these progeny had very similar frequencies of biparental transmission (91.8% *vs.* 85.7% in Table 5). The relative abundance of the two paternal plastids was reversed in progeny with an $A^iA^a$ nuclear background (Figure 2A), which is more compatible with plastome *I*.

**Plastome-genome interaction affects pollination:** To examine the impact of the genetic constitution of the male parent on the fertilization process, flowers of the $A^iA^i$ genotype having mutant *IV*-α as a periclinal chimera were emasculated and pollinated with pollen from $A^iA^i$-*IV*, $A^iC^p$-*IV* or $A^iC^p$-*II* plants. The extent of capsule development and seed maturation were scored after one month. As shown in Figure 3, the size, and to some extent, the shape of the capsule reflect the extent of seed development. In general, only ovules located in the upper part of the capsule were fertilized by the less vigorous pollen. As presented in Table 6, pollen from $A^iC^p$ plants was much less successful in accomplishing fertilization than was pollen from $A^iA^i$ carrying the same plastome type. According to the $\chi^2$ test, with the same plastome type, the effect of paternal genotype on capsule development is significant ($P < 0.025$). Within the $A^iC^p$ genotype, pollen from plants carrying plastome type *II* was less effective in producing seeds than pollen from plants carrying plastome type *IV*. The effect of paternal plastome type on the success of capsule development is also significant ($P < 0.005$) in a $\chi^2$ test of independence.

The differential capacity for pollen tube growth among Oenothera strains was examined *in vitro*. We observed that under constant germination conditions, pollen tubes from large flowered $A^iA^i$ strains carrying any of the four plastome types can grow at least twice as fast as pollen tubes from $A^aC^p$ strains. In order to see if differences in the relative compatibility affect pollen development in $A^aC^p$ strains, the growth capacity of pollen from $A^aC^p$-*II* and $A^aC^p$-*IV* strains were compared in a more careful fashion. As listed in Table 7, the average length of pollen tubes from $A^aC^p$-*IV* is slightly longer than that of pollen tubes from $A^aC^p$-*II* (6.3 *vs.* 5.2 mm). According to analysis of variance, the lengths of pollen tubes from these two strains are significantly different from each other at the $P < 0.01$ level. Overall, the *in vitro* results suggest that, beside other factors, the compatibility between plastome and genome can affect pollen growth and that differences in this aspect of pollen vigor can explain the observed differences in the efficiency of fertilization (Figure 3).

## DISCUSSION

The pattern of plastid inheritance in Oenothera has been thought to be determined primarily by the plastome, due to different intrinsic rates of plastid multiplication associated with each plastome type (SCHÖTZ 1954, 1974, 1975; CHIU, STUBBE and SEARS 1988). In this study, we have compared the transmission of the four Oenothera plastome types simultaneously in several different nuclear backgrounds. This made it

**TABLE 4**

**Plastid transmission in *AC* × *AA* crosses**

| Set | Crosses[a] | | $F_1$ hybrid genotype | Transmission | | Average area of green tissue in BP progeny |
|---|---|---|---|---|---|---|
| | Maternal | Paternal | | *N* | %BP[b] | |
| 1 | $A^aC^b$-*II* | $A^jA^j$-*I*$\zeta$ | $A^aA^j$ | 164 | 75.0 ± 3.4 | 0.34 |
| | $A^aC^b$-*II* | $A^jA^j$-*II*$\epsilon$ | $A^aA^j$ | 121 | 19.0 ± 3.6 | 0.14 |
| | $A^aC^b$-*II* | $A^jA^j$-*III*$\gamma$ | $A^aA^j$ | 93 | 61.3 ± 5.0 | 0.20 |
| | $A^aC^b$-*II* | $A^jA^j$-*IV*$\alpha$ | $A^aA^j$ | 195 | 6.2 ± 1.7 | 0.12 |
| 2 | $A^aC^b$-*IV* | $A^jA^j$-*I*$\zeta$ | $A^aA^j$ | 127 | 82.7 ± 3.4 | 0.45 |
| | $A^aC^b$-*IV* | $A^jA^j$-*II*$\epsilon$ | $A^aA^j$ | 184 | 69.0 ± 3.4 | 0.24 |
| | $A^aC^b$-*IV* | $A^jA^j$-*III*$\gamma$ | $A^aA^j$ | 201 | 85.6 ± 2.5 | 0.30 |
| | $A^aC^b$-*IV* | $A^jA^j$-*IV*$\alpha$ | $A^aA^j$ | 120 | 39.2 ± 4.5 | 0.14 |
| 3 | $A^rC^b$-*IV* | $A^jA^j$-*I*$\zeta$ | $A^rA^j$ | 235 | 89.4 ± 2.0 | 0.45 |
| | $A^rC^b$-*IV* | $A^jA^j$-*II*$\epsilon$ | $A^rA^j$ | 187 | 76.5 ± 3.1 | 0.29 |
| | $A^rC^b$-*IV* | $A^jA^j$-*III*$\gamma$ | $A^rA^j$ | 209 | 85.6 ± 2.4 | 0.35 |
| | $A^rC^b$-*IV* | $A^jA^j$-*IV*$\alpha$ | $A^rA^j$ | 196 | 31.6 ± 3.3 | 0.25 |

[a] Reciprocal crosses of those listed in Table 2, season 2.
[b] See Table 2.

**TABLE 5**

**Plastid transmission in crosses producing progeny with genotype *AB***

| Set | Crosses | | $F_1$ hybrid genotype | Transmission | | Average area of green tissue in BP progeny |
|---|---|---|---|---|---|---|
| | Maternal | Paternal | | *N* | %BP[a] | |
| 1 | $B^aC^c$-*IV* | $A^jA^j$-*I*$\zeta$ | $A^jB^a$ | 218 | 77.1 ± 2.8 | 0.14 |
| | $B^aC^c$-*IV* | $A^jA^j$-*II*$\epsilon$ | $A^jB^a$ | 129 | 82.2 ± 3.4 | 0.25 |
| | $B^aC^c$-*IV* | $A^jA^j$-*III*$\gamma$ | $A^jB^a$ | 108 | 87.0 ± 3.2 | 0.25 |
| | $B^aC^c$-*IV* | $A^jA^j$-*IV*$\alpha$ | $A^jB^a$ | 23 | 53.8 ± 10.4 | 0.16 |
| 2 | $B^bC^f$-*IV* | $A^jA^j$-*I*$\zeta$ | $A^jB^b$ | 196 | 85.7 ± 2.5 | 0.33 |
| | $B^bC^f$-*IV* | $A^jA^j$-*II*$\epsilon$ | $A^jB^b$ | 285 | 75.4 ± 2.6 | 0.37 |
| | $B^bC^f$-*IV* | $A^jA^j$-*III*$\gamma$ | $A^jB^b$ | 293 | 91.8 ± 1.6 | 0.47 |
| | $B^bC^f$-*IV* | $A^jA^j$-*IV*$\alpha$ | $A^jB^b$ | 162 | 35.2 ± 3.8 | 0.19 |
| 3 | $B^gB^g$-*III* | $A^jA^j$-*I*$\zeta$ | $A^jB^g$ | 220 | 16.4 ± 2.5 | 0.19 |
| | $B^gB^g$-*III* | $A^jA^j$-*II*$\epsilon$ | $A^jB^g$ | 50 | 24.0 ± 6.0 | 0.10 |
| | $B^gB^g$-*III* | $A^jA^j$-*III*$\gamma$ | $A^jB^g$ | 156 | 46.2 ± 4.0 | 0.17 |
| | $B^gB^g$-*III* | $A^jA^j$-*IV*$\alpha$ | $A^jB^g$ | 203 | 8.4 ± 2.0 | 0.11 |

[a] See Table 2.

possible to obtain a more complete view of the role of both plastid and nuclear genomes in plastid transmission.

In general, the efficiency with which plastids are transmitted in crosses correlates fairly well with the relative compatibilities of the plastomes with the nuclear background of the progeny. For example, crosses between maternal parents with the $A^jA^j$ genotype and paternal parents with $A^jA^j$ or $A^aC^b$ genotypes produce progeny with $A^jA^j$ or $A^jC^b$ respectively (Figure 1). Since the maternal parent is identical, so too is the input of maternal plastids. Yet, in crosses producing progeny with the $A^jA^j$ genotype, the relative success of the maternal plastome was in the order of *I*-$\zeta$ > *II*-$\epsilon \geq$ *III*-$\gamma$ > *IV*-$\alpha$, while in crosses producing progeny with the $A^jC^b$ genotype, the order was *II*-$\epsilon$ > *III*-$\gamma \geq$ *I*-$\zeta$ > *IV*-$\alpha$. With the exception of *IV*-$\alpha$, these relative efficiencies of plastome transmission coincide with the relative compatibilities between each plastome and the nuclear background of the progeny. Similarly, when the same pollen source was used, meaning that input of paternal plastids was identical, the relative transmission efficiencies of paternal plastomes *I*-$\zeta$ and *III*-$\gamma$ were reversed in crosses producing progeny with *AA* or *AB* genotypes (Figure 2). Again, these results mirror the relative compatibilities of plastomes *I* and *III* with the two nuclear backgrounds (STUBBE 1959). Since the input of plastids did not vary in these comparisons, the linkage between plastome-genome compatibility and the efficiency of plastid transmission can best be explained if the efficiency of plastid multiplication is affected by how well the plastid and the nuclear genome can interact with each other. An extreme example of the influence of plastome-genome

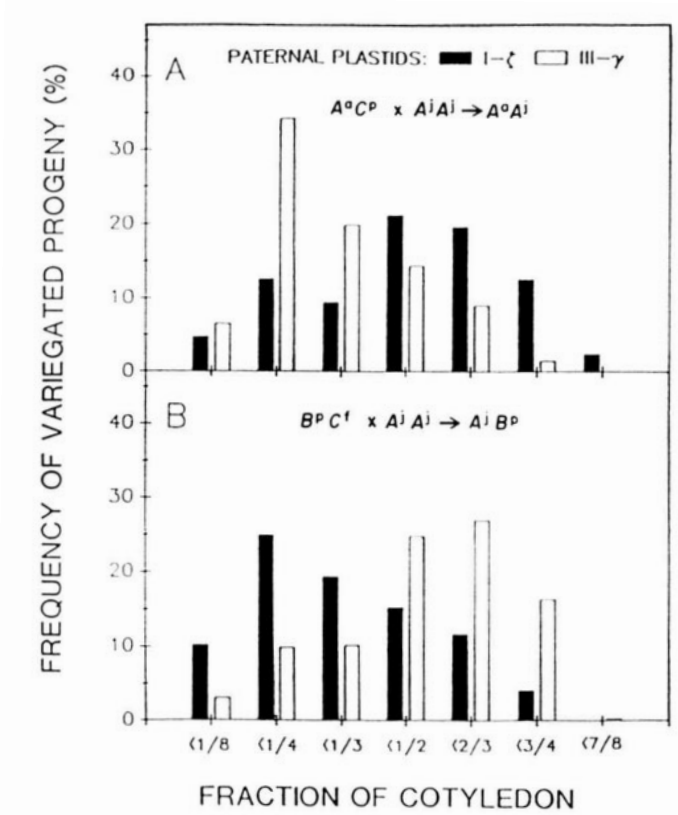


FIGURE 2.—Frequency distribution of the degree of variegation in biparental progeny. Crosses were performed between maternal parent (A) *A^aC^p-IV* or (B) *B^pC^f-IV* and paternal parent *A^jA^j-I-ζ* (closed bars) or *A^jA^j-III-ζ* (open bars). The *x*-axis indicates the fraction of cotyledon area occupied by the white plastids transmitted from the pollen parent, with the height of the bars showing the percentage of progeny in each group. The frequencies of biparental plastid transmission from these crosses are listed in Tables 4 and 5, respectively.

FIGURE 3.—Capsule development as an indicator of fertilization success and seed development. Capsules of approximately the same age (1 month after pollination), showing about 95% seed development (left), 10% seed development (center) and no seed development (right).

compatibility on plastid transmission in Oenothera was reported by STUBBE (1963), who recovered progeny carrying only paternal plastids from crosses producing a hybrid genotype that was severely incompatible with the maternal plastid. Although the plastome-genome compatibility is generally measured as the ability of plastids to become fully pigmented in a given nuclear background (STUBBE 1959), cooperation between the two genetic compartments is clearly important in a broader sense.

In addition to its influence on the relative efficiency of plastid multiplication, the compatibility between plastome and genome may also affect plastid transmission through its influence on other steps in the process of fertilization, such as pollen tube growth. According to our observations, the growth capacity of pollen from various Oenothera strains is correlated positively to the size of the flowers from the pollen donor. However, the growth of the pollen tubes also can be affected by the plastome type carried by the plant: pollen from an *A^aC^p* strain that carries plastome type *II* are less successful in fertilizing eggs, as compared to pollen from the same strain carrying the more compatible plastome type *IV* (Figure 3, Table 6). A growth differential was observed in comparison of *in vitro* germinated pollen from strains *A^aC^p-II* and *A^aC^p-IV* (Table 7). Poor pollen tube growth may have been responsible for the failure of seed production in crosses between *A^jA^j* and two even less compatible combinations, *A^rC^p-II* and *A^aC^p-III*. As discussed below, the vigor of pollen tube growth may have a significant effect on the input of paternal plastids to the zygote.

The impact of the maternal genotype on Oenothera

TABLE 6

**Efficiency of fertilization using pollen from *A^jA^j-IV*, *A^jC^p-II* or *A^jC^p-IV* plants**

| Pollen donor | Extent of seed maturation in individual capsule (%) 0 | 10–24 | 25–50 | 50–89 | 90–10 | *n* |
|---|---|---|---|---|---|---|
| *A^jC^p-II* | 19 | 3 | 1 | 0 | 0 | 23 |
| *A^jC^p-IV* | 13 | 8 | 10 | 0 | 0 | 31 |
| *A^jA^j-IV* | 1 | 0 | 0 | 1 | 13 | 15 |

The maternal parent in these crosses had genotype *A^jA^j* with mutant plastome. In each column, the number of capsules having a certain fraction of viable seed is indicated.

TABLE 7

**Pollen tube length distribution in Oenothera strains *A^aC^p-II* and *A^aC^p-IV***

| Strain | Tube length (mm) 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | *n* | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| *A^aC^p-II* | 2 | 5 | 8 | 6 | 1 | 2 | 0 | 0 | 24 | 5.2 |
| *A^aC^p-IV* | 2 | 6 | 6 | 6 | 6 | 1 | 2 | 1 | 24 | 6.3 |

Pollen were fixed after 4 hr of incubation in the germination medium. Over 90% of the pollen from both strains germinated during this time.

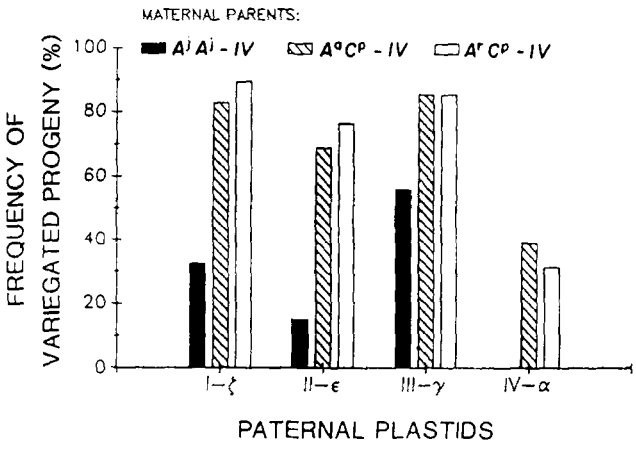


FIGURE 4.—Frequency of biparental plastid transmission in Green × White crosses of Oenothera. The paternal parents had the genotype $A^jA^j$ and the mutant plastome type indicated on the x-axis. The maternal parents carried plastome type *IV* with $A^jA^j$ (large flower), $A^aC^p$ (medium flower) or $A^rC^p$ (small flower). Data for crosses involving $A^jA^j$-*IV* (closed bars) are from Chiu, Stubbe and Sears (1988); data for crosses using $A^aC^p$-*IV* (hatched bars) and $A^rC^p$-*IV* (open bars) as maternal parents are from Table 4.

plastid transmission appears to correlate with the size of the flower. Among the species used in this study, *O. hookeri* str. Johanssen ($A^jA^j$) and *Oenothera grandiflora* ($B^gB^g$) are strains with large flowers. The length of the style in these large-flowered strains is 7–9 cm. *O. ammophila* ($A^rC^p$), *O. parviflora* ($B^aC^s$) and *O. atrovirens* ($B^pC^f$) have small flowers and their styles are only 2–3 cm long. The flower of hybrid strain $A^aC^p$ is of medium size with an average style length of 4.5 cm. In all cases, flower size is not significantly influenced by the plastome type of the plant. When a constant pollen source is used in crosses, a much higher frequency of paternal plastids is detected in the progeny when strains with small flowers are used as maternal parents, as compared to maternal parents with large flowers (Tables 4 and 5) (CHIU, STUBBE and SEARS 1988). The influence of maternal flower sizes on plastid transmission is illustrated by the three sets of crosses shown in Figure 4, where pollen was contributed from $A^jA^j$ lines carrying mutant plastids and the maternal parent had plastome type *IV* combined with genotype $A^jA^j$ (large flower), $A^aC^p$ (medium flower), or $A^rC^p$ (small flower). Progeny from these crosses all have an *AA* genotype. Although biparental plastid transmission occurred at similar frequencies with plants having small and medium flowers as the maternal parent, when crosses involved a large-flowered maternal parent, a significantly lower ($P < 0.001$) frequency of biparental plastid transmission was observed. A similar observation was made by SCHÖTZ (1974), who reported that a small flower variant of *O. hookeri* allowed the transmission of more paternal plastids than did the original strain. Conceivably, the differences in plastid heredity could be due to differences in the input of maternal plastids that correlate with the size of the flower. According to SCHÖTZ (1954), the strain with the smallest flowers has slightly fewer plastids in the egg (between 14 and 26), but all other strains examined have similar numbers (between 20 and 30). These results led Schötz to conclude that differences in the input of maternal plastids should not have a major impact on plastid transmission.

We agree with SCHÖTZ, and find it more likely that aspects of the pollination process that affect the input of paternal plastids have a major impact on the final result of plastid transmission. For all Oenothera strains examined, 90–100% of the mature pollen generative cells contain plastid DNA aggregates [CORRIVEAU and COLEMAN (1990) and personal communication]. However, high frequencies of paternal plastid transmission are achieved only when the maternal parent is a small-flowered strain or when the paternal parent produces pollen capable of vigorous growth. The influence of maternal flower size and the growth capacity of pollen tubes on plastid transmission can be best explained if plastids degenerate in the generative cell as the pollen tube extends toward the egg. According to this hypothesis, when a slow growing pollen tube progresses through a very long style, the time between pollination and fertilization is longer, and more plastids in the generative cell would be degraded before fertilization. Plastid degeneration during pollen development is one of the mechanisms through which maternal plastid inheritance is achieved in several plant species [reviewed by HAGEMANN and SCHRÖDER (1989)] including *Epilobium* (SCHMITZ and KOWALLIK 1987), a close relative of Oenothera. In *Epilobium*, plastids are inherited almost exclusively from the maternal side, but biparental transmission can occasionally be detected (SCHMITZ and KOWALLIK 1986).

At least 14% of the angiosperms surveyed contain plastid DNA in their pollen generative cells (CORRIVEAU and COLEMAN 1988) and should be able to transmit their plastids through the pollen. However, based on this study and earlier studies of plastid transmission in *Oenothera* (SCHÖTZ 1954, 1974, 1975; CHIU, STUBBE and SEARS 1988) and *Pelargonium* (TILNEY-BASSETT and ALMOUSLEM 1989), the final pattern of plastid inheritance depends not only on the input of the parental plastids but also on the plastome, the interaction between plastome and genome, and plastome-independent factors determined by the nuclear genome of the parental plants.

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