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Inactivation of pollen and other effects of genome-plastome incompatibility in *Oenothera*

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Abstract. A series of strains of the homozygous species *Oenothera grandiflora* (characterized by the genome BB and plastome III) were combined with plastome IV from *O. parviflora* (BC-IV) by means of appropriate crosses. An incompatibility between genome B and plastome IV is expressed in the haplo- and diplophase: (1) B-IV pollen, though normally developed, is largely inactive. The extent of the inactivation varies between different strains and shows a seasonal fluctuation as determined by seed set in outcrossing and selfing experiments. (2) In most of the strains lethality of BB-IV embryos is the rule, leading to empty seeds. This can be ameliorated by including another plastome in the zygotes and developing embryos on account of the biparental plastid transmission in *Oenothera*. It can best be demonstrated in crosses with a seed parent having normal green plastids of plastome IV and mutated chlorophyll deficient plastids from a different plastome in the pollen parent, leading to variegated progeny as well as a remainder of empty seeds. (3) In about one-half of the strains the BB-IV plants exhibit a temporary bleaching of the *virescens* type. The incompatibily between genome B and plastome IV does not support the earlier assumption that plastome IV is the ancestor of plastomes II, III, and V. Instead, a precursor plastome is postulated from which plastomes II, III, and IV are descended. While plastome I can be derived from II, only plastome V can be descended from plastome IV.

Key words: *Onagraceae*, *Oenothera grandiflora*. Incompatibility between genome and plastome, pollen inactivation, embryo lethality, chlorophyll deficiency, pedigree of plastome evolution.

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Investigations of the interaction of genome and plastome (Stubbe 1959) have shown that subsection *Oenothera* of the section *Oenothera* comprises three basic genomes (A, B, C) and five plastomes (I–V), the former being arranged in 13 species in either a homozygous or complex-heterozygous state (Raven et al. 1979, Stubbe and Raven 1979a, Dietrich et al. 1997).

In crossing experiments in which the plastomes are combined with genomes with which they do not occur naturally, the progenies frequently show a disturbed development. Such incompatibility between genome and plastome is most noticeably expressed as different types of chlorophyll deficiencies. Due to the biparental transmission of plastids in *Oenothera*, interspecific hybrids are often variegated when one of the parental plastid types becomes green while the other turns pale. These phenomena are called hybrid paleness and hybrid variegation. Variegated plants may also be obtained by crossing if one of the partners carries mutated chlorophyll deficient plastids.

The number of plastids derived from the female parent is generally greater than that from the male parent, but the proportion of both varies greatly from cross to cross. Schötz (1954) concluded that the degree of variegation (variegation value or "Scheckungswert") is based on differences in the multiplication rate of the five plastid types. As a result, a competition takes place within the zygote and the "mixed" cells derived from it, leading to a deviation from the original proportion of maternal and paternal plastids during the development of the embryo and young plant. In apparently compatible genome-

plastome combinations the extent of plastid transmission to the progeny led to recognition of the following series: I > III > II > V > IV (Schötz 1954, 1974, 1975). Recently, the influence of the genome and plastome on plastid transmission has been reinvestigated in greater detail by Chiu and Sears (1993).

Another expression of the incompatibility between genome and plastome concerns pollen inactivation. This can be observed when genome B is combined with plastome IV (Stubbe 1959, 1960; Göpel 1967, 1970). (Other combinations showing the same effect will not be considered here.) The B genome is found in the homozygous species *O. grandiflora* (BB) and in the complex-heterozygous *O. nutans* Atkinson (B_1B_2) (syn. *O. austromontana* Munz) studied in detail by Wasmund (1984). In both cases it normally occurs with plastome III. It is also found in the complex-heterozygous species *O. biennis* (AB) (Raven et al. 1979).

Plastome IV occurs in the complex-heterozygous species *O. parviflora* and *O. oakesiana* (AC). These two species represent a refuge for plastome IV; they cannot be invaded by the other plastomes via crosses since the latter are not compatible with the C genome. Otherwise, because of its low multiplication rate, plastome IV would be displaced by the faster multiplying plastomes. Because of the low multiplication rate and its relatively greater tolerance with respect to the normal functioning of the chloroplasts when associated with the three genomes A, B, and C, plastome IV is regarded as the most primitive of the five plastomes in subsection *Oenothera* (e.g. Stubbe 1959).

The inactivation of one of the two complexes in a complex-heterozygous species of *Oenothera* is a familiar phenomenon. In the heterogamous species *O. parviflora* and *O. oakesiana* only the C complex is transmitted by the pollen; the inactivation of the partner complexes B and A is achieved by pollen lethals. In *O. biennis* subsp. *centralis* self-incompatibility (Si) genes have been demonstrated in the B complex which prevent the formation of BB homozygotes in the normally self-pollinating species (Steiner 1956, 1957). [More recently Si genes were also demonstrated in *O. grandiflora* (Stubbe and Raven 1979b).] In strains of *O. biennis* subsp. *caeciarum*, however, the B complex lacks Si genes and functions as the pollen complex, while the partner complex A carries a pollen lethal. In addition, the B complex frequently carries a sporophytic lethal by which the homozygotes are eliminated as proembryos, leading to empty seeds (Renner 1916).

In the context of the present investigation it should be noted that Renner (1919) first observed that the *gaudens* (B) complex of *O. lamarckiana* de Vries becomes inactivated in the pollen when it is transferred into the cytoplasm of *O. muricata* (AC–IV). The extra-chromosomal component of this incompatibility was later shown to be localized in the plastids (Stubbe 1959, 1960). The phenomenon of plastome-dependent pollen inactivation was studied in detail by Göpel (1967, 1970). He utilized different strains of *O. suaveolens* (a species which can be assigned to *O. biennis* subsp. *caeciarum*) in which the B complex *flavens* is free of pollen lethals and Si genes. It was found that the inactivation of the B-IV pollen was not always absolute. Thus, after selfing of the AB-IV combination (*O. suaveolens* with plastome IV) occasional fruits with a few seeds occur which give rise to progeny of the parental phenotype. That these rare active pollen grains were genetically altered by mutation or crossing-over could be excluded. Through breeding experiments it could be demonstrated that the genetic constitution of the sporophyte also influences the pollen activity. This was further confirmed by physiological investigations (Göpel 1976). However, *O. suaveolens* presented two disadvantages in these investigations: First, the pollen is not homogenous, since the *albicans* complex forms 50% empty grains due to its pollen lethals; further, an additional percentage of empty grains results from meiotic failures. Second, even if by crossing different strains of *O. suaveolens, flavens-flavens* homozygotes (BB) were obtained free of sporophytic lethals (cf. Stubbe 1953), these were weak because of their limited compatibility with plastome II which had to be replaced by plastome III. At that time plastome III was only available from *O. lamarckiana* and the crosses led to the *falcifolia* syndrome (Stubbe 1970, 1989a, b).

With the more recent availability of a number of strains of *O. grandiflora* which possess a homozygous B genotype combined with plastome III, it became feasible to reinvestigate plastome-dependent pollen inactivation utilizing these vigorous homozygous forms. More than 20 strains of these large flowered forms in our collection were outfitted with plastome IV through appropriate crossings. The expectation was that among these phenotypically diverse BB-IV combinations, we would find some with inactive as well as active pollen. The phenomenon of plastome-dependent pollen inactivation could then be studied in depth with physiological and molecular biological techniques. Moreover, the results should shed light on the evolution of the five plastomes in the subsection *Oenothera* and may provide a test of the hypothesis that plastome IV is primitive and the one from which the others evolved.

Materials and methods

The strains of *Oenothera grandiflora* L.'Her. here investigated are listed in Table 1. A description of their characteristics is given in the publications of Steiner and Stubbe (1984, 1986), Schumacher et al. (1992), and Schumacher and Steiner (1993).

Most of the strains when grown in the experimental garden at the University of Düsseldorf, flower late in the season and therefore need short-day treatment in a special greenhouse. Such treatment may be necessary in order to make crosses with those plants flowering under long-day conditions.

The compatibility relations between different genomes and plastomes with regard to plastid development are described by Stubbe (most recently in Stubbe 1989c, also reproduced in Schumacher et al. 1992 and Harte 1994).

Plastome III of *O. grandiflora* can be exchanged with plastome IV of a strain of *O. parviflora* without difficulty, provided certain complex-heterozygous hybrids with suitable chromosome arrangements which have already been combined with plastome IV and mutated chlorophyll-deficient plastids from the other plastomes, are utilized. In the present investigation the complex combinations of *albicans* Grado-*undans* (AA) or *albicans* Grado-*percurvans* (AC) were used as seed parents. The *albicans* complex is transmitted exclusively by the egg cell; (*undans* and *percurvans* are pollen complexes). The exchange of plastomes is accomplished by a crossing scheme, an example of which is described in Stubbe (1989c):

First, a complex-heterozygous variegated hybrid with the genetic constitution of *albicans*-^{h}*grandiflora* (AB) is produced. It bears mutated plastids (IIγ) from the seed parent and plastid type III from the pollen parent. (The diakinesis configuration is generally a circle of 14; in some cases a circle of 8 and a circle of 6 is observed, but due to the lethals in *albicans* the only segregants are the parental complexes A and B). White tissues of this variegated hybrid AB-IIγ/III, supply pollen which transmits mutated plastids with the ^{h}*grandiflora* complex (B-IIγ). If a complex-heterozygous hybrid which previously was combined with plastome IV (e.g. AC-IV), is pollinated with B-IIγ, variegated progeny of idiotype AB-IV/IIγ is obtained. This hybrid produces two types of egg cells in its green parts, namely *albicans* (A-IV) and ^{h}*grandiflora* (B-IV), but only B-IV pollen. Its white parts give rise to corresponding gametes but with mutated plastids. To obtain a *grandiflora* with plastome IV (BB-IV), the hybrid is self-pollinated. As will be explained later, it may be necessary to pollinate flowers of green sectors with pollen from white tissues. Using this procedure all the strains listed in Table 1 were combined with plastome IV. (One cannot always rely on the green color of the tissue. In some cases plastome III was obtained instead of plastome IV. The tissues which should have possessed mutated plastids exclusively may have also carried some proplastids of type III which could not be detected macroscopically. There is at present no indication of gene exchange by recombination between different plastomes in *Oenothera*, although theoretically possible (Medgyesy et al.1985). In any case, a test for the presence of

Table 1. *Oenothera grandiflora* strains used in the present investigation (for details see Steiner and Stubbe 1984 and 1986)

Collection	Collector	Date	Remarks
Avalon, FL	R. K. Godfrey	Oct. 1975	several lines
Bay Minette, AL	E. Steiner	Oct. 1983	two lines
Bellamy, AL	Sam B. Jones & Jud K. Arrington	Aug. 1974	several lines
Bigbee, AL	E. Steiner	Oct. 1983	
Bolinger, AL	E. Steiner	Oct. 1983	
Brewton, AL	E. Steiner	Sept. 1981	several lines
Cantonment, FL	E. Steiner	Oct. 1983	
Castleberry, AL	E. Steiner	Oct. 1983	two lines
Chastang, AL	E. Steiner	Oct. 1983	three lines
County Road 6, AL	E. Steiner	Oct. 1983	
Flomaton, AL	E. Steiner	Oct. 1983	
Frankville, AL	E. Steiner	Oct. 1983	
Monteagle, TN	R. Kral	Sept. 1979	
Seaburry Creek, AL	P. Biebel	Oct. 1962	two lines
Sims Chapel, AL	E. Steiner	Oct. 1983	
Stockton, AL	P. Biebel	Oct. 1962	two lines
Stockton derived[1]			
Tuscaloosa, AL	J. S. Lloyd	Aug. 1944	
York, AL	Sam B. Jones	unknown	several lines

[1] The strain "Stockton derived" is probably a hybrid between strains from Seabury Creek and Stockton, used in experiments by H. Kutzelnigg (1968).

plastome IV among the plastids should be carried out. This is easily done by determining the shape of the starch grains in the pollen, see below.)

After having replaced plastome III with plastome IV the effects of the exchange can be investigated. Both the diplo- and haplophases are affected:

1. For determination of the vitality and activity of the B-IV pollen either the hybrid *albicans*-^{h}*grandiflora* (AB-IV) or BB-IV homozygotes can serve as pollen sources. Microscopic examination of a pollen sample is critical in order to estimate the proportion of empty pollen grains and to determine the shape of the starch grains (stained with IKI). In contrast to B-II or B-III pollen which contain pointed angular starch grains, the grains of B-IV pollen are round. In this way the presence of plastome IV can be established.

   For the present it was not possible to test germination of pollen on the stigma or on stigma-secreted slime, nor were physiological measurements (e.g. Göpel 1976) carried out. The activity of the B-IV pollen was assessed exclusively by means of seed formation following surplus pollination. On the one hand, this was achieved through self-pollination of the AB-IV hybrids and the BB-IV homozygotes, and, on the other, by pollinating a large-flowered AA-I species, for example *O. elata*, with B-IV pollen. In the latter case the hybrids produced should be of the idiotype AB-I which is phenotypically a *lutescens* pale, expressed particularly in the cotyledons.

   The activity or inactivity of the B-IV pollen is indicated by the fruit development. Within certain limits the size of the capsule depends upon the number of seeds produced (Göpel 1967: 29; Chiu and Sears 1993: Fig. 3). It is worth pointing out that fruits of pollinated flowers which do not produce seeds do not fall off in contrast to those which have not been pollinated. This has also been observed after self pollination of self-incompatible plants.
2. If the B-IV pollen is inactive, BB-IV homozygotes can only be obtained by using pollen carrying a different plastid type, e.g. B-IIγ pollen. This implies that a variegated progeny (BB-IV/IIγ) must be taken into consideration. For entirely different reasons such a procedure proved necessary, as will be reported under "Results".
3. Although this investigation began with examination of plastome-dependent pollen inactivation, it seemed pertinent to also compare the BB-IV plants with the BB-III plants of the original strains.

Results

As previously pointed out by Steiner and Stubbe (1984, 1986), the various strains of *O. grandiflora* collected in Alabama, Florida and Tennessee show a considerable morphological diversity when grown in the experimental field. The differences relate to the contour of the leaves, the leaf colour, the branching of the stem, the size of flowers and fruits, the distribution and intensity of anthocyanin pigmentation as well as the pubescence of different parts of the plant body. Important differences also exist with regard to the initiation of flower formation in response to the day length. Late blooming can be overcome by short-day treatment after the plants have broken the rosette stage in long-day conditions. In spite of the wide variation of strains selected from natural populations (and now maintained as pure lines), their phenotypes still conform to the basic genotype B which is most compatible with plastome III.

It seems appropriate in this context to recapitulate the general appearance of the *grandiflora* phenotype: When sown in the greenhouse during winter, the normally biennial plants complete their life cycle in the current growing season. They are transferred to the experimental field in April or May. After having broken the rosette stage under long-day conditions the plants develop an erect main stem with side branches arising from its base. The broad rosette leaves may have a deeply scalloped contour. Leaf pigmentation is generally a light yellowish green which is characteristic for most BB-III genotypes, whereas the combination of a B genome with an A or C complex shows a deeper green. Usually the leaves are spotted with red flecks (resulting from the presence of the dominant *Maculans* gene, Renner 1942). The anthocyanin pigmentation of stems and leaves varies widely. The large flowers appear on numerous small branches near the tip of the stem. While in Düsseldorf most species of *Oenothera* start flowering under long-day conditions in June/July, the strains of *O. grandiflora* usually come into bloom in the short days between September and November. However, earlier flowering was observed in July and August with lines selected from the strains Bellamy, Bigbee, Brewton, County Road 6, Flomaton, Frankville, Monteagle, Tuscaloosa and York.

It may be mentioned that as far as possible self-compatible lines were used, though Si genes are present in many strains. In the strain from Monteagle, however, a self-compatible line is not available, thus, selfing of BB-IV must be replaced by crossing of lines with different Si genes. Furthermore, we do not consider the limited chromosomal variability between pure lines as significant, since it is without influence on the phenotype.

When plastome III was replaced by plastome IV, we found that the different strains did not react alike. Differences occurred not only with regard to pollen activity (which was the initial thrust of this investigation), but also with regard to the viability of embryos,

the greening of the leaves and the vigour, growth and branching of the plants.

The phenomenon of pollen inactivation when a B complex is combined with plastome IV, was studied in detail by Göpel (1967) using the *flavens* complex of *O. suaveolens*. The present investigation of more than 20 *grandiflora* complexes largely confirms Göpel's observations: B-IV pollen grains generally contain a healthy protoplast which is plasmolysable. B-I, B-II, and B-III pollen contain pointed angular or spindle shaped starch grains; in contrast, B-IV pollen has round or nearly round starch grains (Fig. 1). Pollen tube formation is usually inhibited, but the inactivation of B-IV pollen is not always absolute; on occasion several grains function normally and fertilize egg cells, leading to seed development. In so far our results are in good agreement with the rigorous investigations of Göpel (1967). Beyond that employing different homozygous strains of *O. grandiflora* led to new findings.

Our experiments were mainly extended after the acquisition of additional strains in 1984. Initially the method of choice to determine pollen activity was selfing. The majority of AB-IV and BB-IV failed to set seed (records not shown), but could be maintained by means of variegated progeny when B-IV pollen was replaced by pollen with mutated plastids of plastome II or III. A further test of pollen activity was carried out by pollinating AA-I plants with B-IV pollen from AB-IV and BB-IV plants (Table 2). These experiments generally showed that some seeds were produced, the size of the fruit corresponding to the amount of seeds in it, but a not predictable fluctuation of B-IV pollen efficiency persisted. However, in 1992 the fluctuation in most of the strains showed a remarkable pattern, namely, when AA-I species were pollinated between July 16 and 22, seed production was scarce or lacking, but when the experiments were repeated with the same plants a month later, seed set was normal or nearly so (Table 2A). A partial repetition of the test in 1993 gave, with only a few exceptions, similar results. These experiments were taken up again in 1996 to determine if the result of 1992 could be reproduced. For practical reasons the experiments were carried out a week later between July 22 and 31 and some of the tests were done with plants in the greenhouse. The results, shown in Table 2B, do not confirm those of 1992 in which a better seed set was obtained in August than in July; rather the trend was the reverse. Nevertheless, completely inactive B-IV pollen due to incompatibility between genome and plastome is no longer to be expected in either of the different strains. Whether external conditions directly or the developmental stage of the plants in relation to their age is responsible for this previously unobserved seasonal fluctuation is not known. Further, in some races the B-IV pollen is active enough to yield normal seed production at all times. This applies mainly to the strains Bellamy, Cantonment, Flomaton, Sims Chapel, Tuscaloosa and York. We assume that in these cases the B-IV pollen contains a relatively high percentage of active grains, but is not as vigorous as the B-III Pollen.

The results of the selfings of AB-IV and BB-IV plants (Tables 3 and 5) demonstrate by the frequently imperfect fruit development and reduced seed set also the unsatisfactory function of B-IV pollen. In addition to that, imperfect seed development points to a diminished vitality and vigour of the containing embryos.

If seeds from selfed complex-heterozygous AB-IV plants are grown (Table 4), two types of offspring are expected (AB and BB), but in the majority of the strains only AB-IV plants appear. A great part of the normally developed seeds is empty. Obviously, embryo development ceased early on; together with the imperfectly developed seeds the empty are regarded as mainly representing BB-IV homozygotes. (The proportion may deviate widely from a 1:1 ratio, presumably due to embryo-sac competition.) In other words, the BB-IV embryos are lethal.

In some strains BB-IV embroys from selfed AB-IV plants developed normally yielding a certain portion of seeds capable of germination. If such BB-IV plants are selfed, a great deal of the seeds is empty (Table 6). Viable BB-IV embryos have been observed with strains Avalon, Bay Minette, Bellamy, Cantonment, Castleberry B, Chastang 2, Flomaton, Frankville, Seabury Creek 2, Sims Chapel, Stockton 2, Stockton derived, Tuscaloosa and York.

Of particular interest is the fact that the failure of embryo development can be overcome, if a second plastid type is contributed to the zygote by the pollen. Usually a chlorophyll-deficient mutant of plastome II or III suceeds well in combination with plastome IV in the resulting variegated progeny. Since some empty seeds are still obtained, a sufficient number of plastids does not always seem to be distributed to the part of the zygote which gives rise to the embryo proper, and which is necessary to overcome the lethal effect of plastome IV. The only case in which the lethality of BB-IV embryos could not yet be overcome by using pollen with plastome II or III, was with AB-IV of County Road 6 as seed parent. Generally, using this procedure the BB-IV homozygotes can be brought to full development. Actually the mutated plastome II or III is no longer necessary for further development of BB-IV tissue immediately after germination. Thus, it

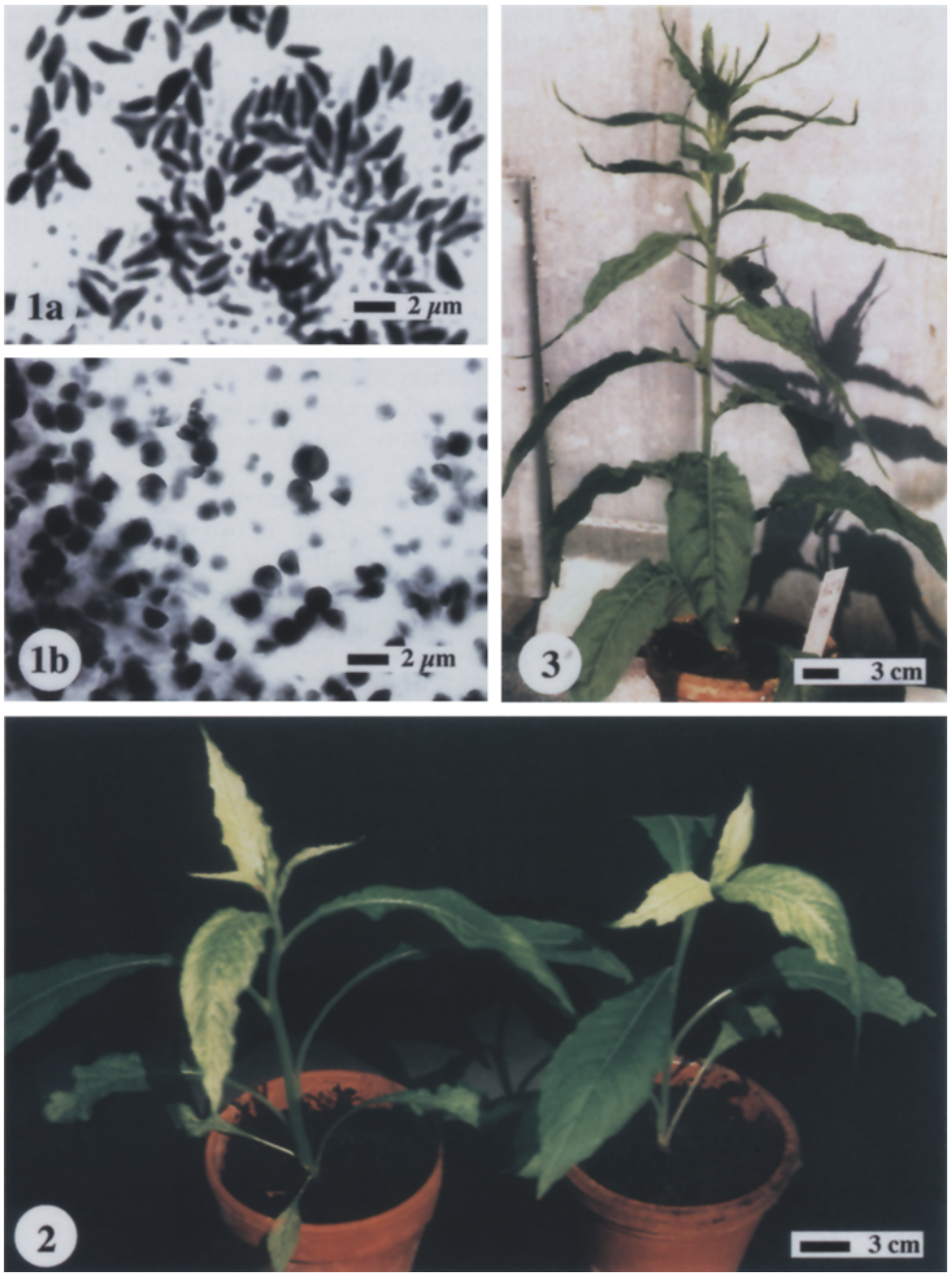

Fig. 1. *Oenothera* starch grains. *a* B-III pollen, *b* B-IV pollen. – **Fig. 2.** *Oenothera* BB-IV plants showing the *virescens* type of chloroplast deficiency. – **Fig. 3.** *Oenothera* BB-IV plant of strain Stockton 1 with an irregular contour of the upper leaves

becomes possible to compare the BB-IV plants with the original *O. grandiflora* strain with regard to phenotypic differences. This proved worthwhile especially for chloroplast development. Namely, in several of the strains a bleaching called *virescens* (Fig. 2) occurs similar to that known in the combination AA-III (Stubbe 1995, 1959). The expression of this character is not always strong. We observed the *virescens* phenotype in the following strains: Avalon, Bigbee, Cantonment (only slightly), Frankville (young plants), Monteagle, Stockton 2, Stockton derived, Tuscaloosa and York. In addition to this, the *virescens* character

appeared in special lines which were obtained by crossing a self-compatible with a self-incompatible complex of the strains Bellamy, Castleberry B and Seabury Creek 2.

As already observed in the case of AA-III, the *virescens* character of the BB-IV tissue can be influenced by tissues with mutated plastids (e.g. IIγ) in such a way that the bleaching is suppressed. Thus, a plant may contain three types of colored tissues, virescent BB-IV, green BB-IV and mutated BB-IIγ. This phenomenon was described earlier with the idiotype *albicans-velans* (AA) with plastome III and IIβ (Stubbe 1958; see also Harte 1994: plate 2e).

Another phenotypic expression of incompatibility between the B genome and plastome IV appeared only in the strain Stockton 1 as an irregular contour of the upper cauline leaves (Fig. 3).

The incompatibility reactions described above serve to supplement the earlier versions of Stubbe's scheme (Stubbe 1959) by adding to the BB-IV square the symbols for *virescens* and lethality (Stubbe 1989c).

Discussion

As known from earlier experiments (Renner 1919; Stubbe 1959; Göpel 1967, 1970) genomes of the B type derived from complex-heterozygous species are incompatible with plastome IV of *O. parviflora* because of the inactivation of the B-IV pollen. In order to determine if this behavior is also true of different strains of *O. grandiflora* (BB-III), the ancestors of which must have been ancestors of all B genomes, more than 20 strains of *O. grandiflora* were combined with plastome IV (Table 1). At least some of these new combinations were expected to show a complete inactivation of the B-IV pollen.

As indicated by seed set (Tables 2, 3 and 5), no strain shows an absolute inactivation of B-IV pollen, although distinct differences occur among the strains of various geographic origin. This is not too surprising, since the strains also differ greatly in phenotype. Further, the 1992 tests show differences between most strains in the seed set obtained in July and that obtained in August. These may be the result of developmental or environmental influences on the pollen parent, the sporophyte, during pollen grain development, or on the male gametophyte itself when it germinates and forms the pollen tube. At the present it has not been possible to establish a causal connection with climatic conditions. Sometimes a difference exists between plants of the same origin, when grown in the field and in the greenhouse, which suggests environmental influence (e.g. Table 2, Castleberry B 93-396).

The fluctuating nature of the inactivation of B-IV pollen is an incompatibility between genome and plastome that can be compared to disturbances of chloroplast development which lead to an incomplete bleaching of the leaves and the occasional recovery of their normal green colour. One can assume that differences in the activity of B-IV pollen occur in different parts of the anther.

The above results confirm those of Göpel (1967, 1970, 1976) who used mainly the *flavens* complex of different strains of *O. suaveolens*. His genetic and physiological findings are presented in great detail and his approach could well be profitably applied to further investigations on the phenomenon of pollen inactivation of *O. grandiflora*.

Beyond that, several hitherto unknown aspects of the vitality of BB-IV sporophytes were observed: The vitality and vigour of BB-IV homozygotes of the different strains varies. Two developmental stages are critical in this respect. The first is early embryogenesis and formation of viable seeds. The second concerns the plastid development in the cotyledons and the leaves of the rosette and shoot.

That the BB-IV homozygotes are lethal in most of the strains during the early development of the embryo was an unexpected finding. In some strains in which selfing of BB-IV plants give viable progeny (Table 6), a portion of seeds is empty, pointing to a diminished vigor of the embryos in an early stage of development.

Fortunately the lethality of BB-IV embryos can be overcome by introducing a different plastid type into the zygote from the pollen parent. Since the partner plastome is only necessary to overcome the critical embryonic stage, mutated chlorophyll-deficient plastids can be used. This neutralization of the incompatibility during the embryonic stage by a second plastid type allows us to grow all of the strains with plastome IV to maturity. Thus it is possible not only to test the pollen quality but also to compare the BB-IV phenotype with that of the original BB-III plants.

In about half of the strains a *virescens* paleness of the BB-IV is observed. This is new, since BB-IV combinations from complex-heterozygous species always showed normal greening. *Virescens* paleness was known earlier in the combination AA-III and occasionally in AB-III.

In general, the *virescens* character reduces the vigor of the shoot system, but this effect may be neutralized in variegated plants by the influence of the mutated plastids. Frequently, the *virescens* bleaching is overridden when tissues with mutated plastids are superimposed on those with plastome IV. Thus, tissues with green plastids occur along with those with

Table 2. A, B. Determination of the activity of *Oenothera* B-IV pollen from AB-IV and BB-IV plants by pollinating AA-I plants and counting the seeds produced, carried out with plants grown in the field (F) or in the greenhouse (G). Part *A* refers to 1992 and 1993, part *B* to 1996. Optimum seed production of the chiefly used seed parent *O. elata* strain Chapultepec is 550–600 seeds per capsule

A

Strain	Culture number	Genotype	F or G	Date of pollination	Seeds obtained	Date of pollination	Seeds obtained
Avalon	92-109	AB	G	16.7.92	65	14.8.92	280
	92-116	AB	F	14.7.92	57	14.8.92	121
	92-124	AB	F	20.7.92	195	14.8.92	215
Bay Minette A	92-127	AB	F	20.7.92	80		
	92-128	AB	F	16.7.92	26	14.8.92	115
Bellamy	92-131	BB	G	22.7.92	430		
	92-133	AB	F	20.7.92	472		
	92-135	AB	G	22.7.92	200		
	92-137	AB	F	20.7.92	444		
	92-139	AB	F	21.7.92	442		
	92-139	BB	G	22.7.92	324		
	92-140	BB	G	22.7.92	260		
	93-336	BB	G			21.8.93	505
	93-337	BB	F			21.8.93	551
	93-340	BB	G			21.8.93	517
	93-242	BB	F			21.8.93	>500
Bolinger	92-145	AB	F	21.7.92	164		
	92-146	AB	F	21.7.92	202		
	91-306a	AB	F	21.7.92	86		
	93-353	AB	F			21.8.93	220
	93-353	BB	F			6.10.93	270
Brewton	92-150	AB	F	22.7.92	138		
	92-150	BB	F	22.7.92	130	14.8.93	342
	93-368-1	BB	F			21.8.93	233
	93-368-2	BB	F			21.8.93	147
Cantonment	92-158	AB	F	21.7.92	45	14.8.92	359
						18.8.92	448
	92-160	AB	F	21.7.92	150		
Castleberry B	92-170	AB	F	21.7.92	98	14.8.92	187
	93-396-1	BB	G			21.8.93	374
	93-396-2	BB	F			20.8.93	40
County Road 6	91-350	AB	F	22.7.92	0	22.8.92	256
Flomaton	92-192	BB	G	22.7.92	22	14.8.92	190
	93-427	BB	G			21.8.93	306
	93-428	BB	G			21.8.93	448
	93-430	BB	G			21.8.93	236
Frankville	92-206	AB	F			19.8.92	66
	92-209b	BB	G	22.7.92	90	14.8.92	280
Seaburry Creek 1	92-239	AB	F			17.8.92	450
	92-240	AB	G	22.7.92	26	14.8.92	207
Seaburry Creek 2	92-260-1	BB	G	22.7.92	22	14.8.92	244
Sims Chapel	92-272	AB	F	22.7.92	137		
	92-273a	AB	F			14.8.92	674
	93-479	BB	F			21.8.93	577
Stockton 1	92-274	BB	G	22.7.92	0	14.8.92	204
Stockton 2	92-284	BB	G	22.7.92	0	14.8.92	288
Stockton derived	92-216	BB	G	22.7.92	40	17.8.92	380
Tuscaloosa	92-290	BB	G	22.7.92	464		
York	92-299	AB	F	19.7.92	106		

B

Strain	Culture number	Genotype	F or G	Date of pollination	Seeds obtained	Date of pollination	Seeds obtained
Bay Minette A	96-325	AB	F	26.7.96	148	2.9.96	48
	96-328	BB	G	29.7.96	87	23.8.96	137
	96-329a	AB	F	26.7.96	75	2.9.96	0
Bellamy	96-335-1	BB	G			1.9.96	185
	96-335-2	BB	G			2.9.96	156
	96-343a	BB	G	31.7.96	271	24.9.96	38
	96-346-1	BB	G	31.7.96	320	23.8.96	304
	96-346-2	BB	G	22.7.96	434		
Bigbee	96-349b	BB	G	22.7.96	27	22.8.96	122
	96-351	AB	F	26.7.96	54	2.9.96	0
	96-353a	AB	F	26.7.96	71	2.9.96	0
	96-353a	BB	G	25.7.96	67	23.8.96	214
	96-353b	BB	G	22.7.96	100		
	96-354-1	BB	G			23.8.96	274
	96-354-2	BB	G			28.8.96	275
	96-355a	BB	G	25.7.96	130	23.8.96	210
Bolinger	96-357a	AB	F	26.7.96	320	2.9.96	328
	96-357b	BB	G	25.7.96	287	23.8.96	169
Brewton	96-361b	BB	G			23.8.96	84
	96-362	AB^A	F	26.7.96	348	2.9.96	128
	96-364	BB	G	31.7.96	370	23.8.96	228
						28.8.96	288
Cantonment	96-366a	BB	G			23.8.96	163
	96-366b	AB	F	26.7.96	228	2.9.96	263
	96-368a	BB	G	25.7.96	176	23.8.96	178
	96-368b	BB	G	31.7.96	252	22.8.96	431
	96-369	BB	G			1.9.96	484
Castleberry B	96-371	AB	F	26.7.96	371	2.9.96	227
	96-372	BB	G	30.7.96	41	23.8.96	0
	96-372	BB	G	1.8.96	0	4.9.96	5
Chastang 7	96-378a	AB^A	G	29.7.96	21	1.9.96	17
	96-378c	AB^A	G	31.7.96	217	23.8.96	133
County Road 6	96-382	AB	G			23.8.96	0
	96-383a	AB	F	26.7.96	350	2.9.96	0
Flomaton	96-385	BB	G			1.9.96	49
	96-386b	BB	G	27.7.96	169	23.8.96	161
	96-389a	AB	F	26.7.96	386	2.9.96	466
	96-389b	AB	F			2.9.96	78
Frankville	96-391a	AB	F	26.7.96	131	2.9.96	21
Seabury Creek 1	96-409	AB	G			28.8.96	334
	96-410a	BB	G	31.7.96	182	26.8.96	245
	96-410a	AB	F			2.9.96	134
Seabury Creek 2	96-413	AB	F			3.9.96	315
	96-415b	AB	G			26.8.96	540
	96-417a	BB	G			26.8.96	155
Sims Chapel	96-425	AB	F	30.7.96	446		
	96-425	BB	G			26.8.96	186
	96-427	BB	G			26.8.96	331
	96-429a	BB	G			5.9.96	249
Stockton 1	96-430a	BB	G	22.7.96	332	26.8.96	272
	96-430a	AB	F			3.9.96	320
	96-432	AB	F			3.9.96	439
	96-434	BB	G	27.7.96	268	25.8.96	170
	96-434	BB	G			29.8.96	308

Table 2 (continued)
B

Strain	Culture number	Genotype	F or G	Date of pollination	Seeds obtained	Date of pollination	Seeds obtained
Stockton 2	96-436a	BB	G			26.8.96	127
	96-439	BB	G			26.8.96	127
Stockton derived	96-395a	BB	G			1.9.96	334
	96-399	AB	F	26.7.96	0	2.9.96	199
	96-400a	BB	G			25.8.96	368
						29.8.96	252
Tuscaloosa	96–441	AB	F	31.7.96	416	2.9.96	204
	96–441	BB	G			27.8.96	394
	96–443	BB	G	27.7.96	386		
	96–444a	BB	G			1.9.96	473
York	96–448	BB	G	28.7.96	396	27.8.96	358
	96–449	BB	F	30.7.96	394	2.9.96	523
	96–451	AB	F	30.7.96	358		

virescens paleness and others with pure chlorophyll-deficient mutated plastids. This phenomenon was previously observed in AA-III/IIβ combinations (Stubbe 1958).

Since until now a B genome which is fully compatible with plastome IV has not been observed, the question of the evolutionary significance of these results should be considered in the context of Cleland's hypothesis of the evolution of the subsection *Oenothera* (Cleland 1972: 225): His view was that the different populations evolved in the center of origin, probably Mexico and Central America, then spread in successive waves across the North American continent. The succession of genotypes is expressed in our terminology (Stubbe 1959) as first CC (*O. argillicola*), then BB (*O. grandiflora*) and finally AA (*O. elata*). According to Cleland (1972: 299–302), this happened as an adaptation to the changing climatic conditions of the Pleistocene. Cleland's original hypothesis regarding the evolution of the subsection *Oenothera* did not take into account the plastome. However, with the work of Stubbe (1959, 1964) on the compatibility relationships of genome and plastome and that of Schötz (1954, 1958) on the relative strength of plastid types in competition experiments, it became clear that the evolution of the plastome had to be considered as a significant element in the evolutionary history of the group. The crucial assumption is that plastome IV is primitive. This is based on the low multiplication rate of plastid type IV and its relatively wide compatibility during development with the diploid genomes as described in the scheme of Stubbe (1959, 1989c).

None of the extant homozygous species, however, carries plastome IV. Nevertheless, one of the parents of the hybrid species *O. parviflora* (BC-IV) and *O. oakesiana* (AC-IV) must have contributed plastome IV; this was most probably the precursor of *O. argillicola* which later acquired plastome V. Of the three basic genotypes, the *argillicola* genotype is still the most compatible with plastome IV. In contrast to AA and BB, CC shows no restriction of compatibility with plastome IV. The other parents of the hybrid species had probably already acquired more advanced plastomes at the time crossing occurred. Further, the current concept of the origin of the hybrid species requires that at least one of the parents was already a complex-heterozygote, thus insuring that the hybrid would be a constant complex-heterozygote and probably heterogamous from its inception (Stubbe 1980, Wasmund and Stubbe 1986).

Our concept of the evolution of the hybrid species still presents some unsolved problems. If we consider the basic genotype B, which is also present in *O. biennis* (AB-II, BA-III), one may ask whether the genomic constitution of the extant *O. grandiflora* strains correspond to that of the B complexes in *O. parviflora* and *O. biennis* or have possibly been conserved to some degree in an earlier state. Further, what plastids did the A and B genomes carry at that time? It seems most probable that AA was combined with plastome II and BB with plastome III, since *O. biennis* subsp. *caeciarum* contains plastome II and subsp. *centralis* plastome III at present. In the presence of BC the plastids of plastome II and III

Table 3. Examples of seed set obtained by selfing of *Oenothera* AB-IV plants. The A complex is always *albicans* Grado, the B complex corresponds to the strain in the first column. Abbreviations: *n* normal seeds, *i* imperfectly developed seeds, quota in parenthesis, *em* empty fruits, *sm* small fruits, *me* medium-sized fruits, *nm* normal fruits

Strain	Culture number	Date of first selfing	Number of fruits obtained, assigned to the categories				Seed content in the best fruit	Date of second selfing	Number of fruits obtained, assigned to the categories:				Seed content in the best fruit
			em	sm	me	nm			em	sm	me	nm	
Avalon	92-109	10.06.92	–	2	3	2	40(14 i)						
	92-116	20.07.92	3	–	–	–		14.08.92	3	–	–	–	
	92-124	15.07.92	4	1	1	1	115 (43 i)	16.08.92	–	–	1	1	150 (75 i)
	95-13-2	27.07.96	1	3	–	–	175 (145 i)						
	96-309a	24.07.96	–	–	–	4	272 (213 i)						
Bay Minette A	92-127							16.08.92	–	–	–	4	80 n
	96-325	26.07.96	–	–	–	2	167 (24 i)						
Bellamy	92-131-2	20.07.92	–	–	–	4	162 (14 i)						
	92-131-1	20.07.92	–	–	–	6	206 (24 i)						
	92-137	15.07.92	–	1	1	–	61 (53 i)						
	93-335	27.07.94	–	–	–	2	196 (8 i)						
Bigbee	90-69-1	4.08.90	1	5	–	–	157 (150 i)						
	94-909	29.07.94	5	1	–	–	81 (54 i)						
	96-351	26.07.96	–	–	–	3	212 (46 i)						
Bolinger	90-70-11	9.07.90	–	–	4	–	186 (170 i)	7.08.90	–	–	1	4	226 (27 i)
	91-306a	2.07.92	–	–	2	2	210 (182 i)						
	92-145	21.07.92	6	–	2	1	212 (180 i)						
	92-148b	15.07.92	–	3	–	3	129 (124 i)						
	94-637	28.07.94	–	–	–	4	132 (48 i)						
	96-357a	22.07.96	–	–	–	8	257 (195 i)						
Brewton	91-314	24.07.91	–	3	1	–	66 n+i						
	92-150	15.07.92	–	3	1	1	128 (40 i)						
Brewton AB[A]	96-362	23.07.96	–	5	1	–	100 (99 i)						
Cantonment	92-158							16.08.92	–	–	–	5	180 n
	92-160							20.08.92	–	3	3	4	204 n
	96-366b	23.07.96	–	–	1	2	149 (112 i)						
Castleberry B	92-166	18.07.92	–	1	1	–	160 (132 i)	13.08.92	3	–	–	–	
	92-170	21.07.92	3	–	–	–							
	94-643	28.07.94	–	–	–	5	213 (61 i)						
	96-371	26.07.96	–	–	–	5	144 i	2.09.96	1	1	–	–	26 i
Chastang 2	94-926-3	2.08.94	–	–	–	4	184 (26 i)						
	95-77	19.07.95	–	–	–	3	276 (4 i)	25.08.95	–	–	–	2	134 (24 i)
	96-374	23.07.96	–	–	–	4	250 (18 i)						
Chastang 7 AB[A]	92-174-10							16.08.92	–	–	–	5	114 n.
	92-177-1	14.07.92	–	1	–	3	84 n						
	92-177-2	14.07.92	–	2	3	–	68 n						
	92-179-7							16.08.92	13	2	–	–	8 n
	96-378a	25.07.96	6	2	–	–	25 (11 i)	25.08.96	–	3	3	–	21 n
	96-378c	25.07.96	–	1	–	2	191 n						
County Road 6	90-135	6.08.90	–	–	–	5	208 (14 i)						
	91-350	17.07.92	–	4	–	–	36 (32 i)						
	92-183	18.07.92	–	–	–	7	256 n						
	92-185b							16.08.92	–	–	–	6	142 n
	96-383a	23.07.96	–	–	–	4	269 (7 i)	2.09.96	3	–	–	–	
Flomaton	90-126	5.08.90	–	–	3	4	258 n						
	92-190b	22.07.92	3	–	–	–		16.08.92	–	1	–	3	230 n
	92-191a-1	21.07.92	–	–	–	2	93 n	14.08.92	–	–		4	112 n
	92-198-6	23.07.92	3	–	–	–		16.08.92	5	–	–	2	65 (40 i)
	96-389a	26.07.96	–	–	–	4	138 (11 i)						
	96-389b	26.07.96	–	–	–	4	107 (15 i)						
Frankville	90-138	6.08.90	–	–	–	6	182 (64 i)						
	91-358	23.07.91	3	1	–	–	40 (20 i)						

(contd.)

Table 3 (continued)

Strain	Culture number	Date of first selfing	Number of fruits obtained, assigned to the categories				Seed content in the best fruit	Date of second selfing	Number of fruits obtained, assigned to the categories:				Seed content in the best fruit
			em	sm	me	nm			em	sm	me	nm	
	92-206							17.08.92	–	–	5	–	157 (50 i)
	92-209b	18.07.92	–	2	–	4	181 (120 i)						
	96-391a	23.07.96	–	–	5	–	89 (71 i)						
Seabury Creek 1	90-102	5.08.90	–	–	–	8	204 (40 i)						
	91-398							23.08.91	1	1	5	–	102 n
	92-239	18.07.92	–	–	–	3	158 (27 i)						
	94-951-2	2.07.94	–	–	–	2	188 (32 i)	2.08.94	?	–	1	–	51 (22 i)
	96-409							26.08.96	–	–	1	3	229 (22 i)
Seabury Creek 2	91-401							23.08.91	–	–	3	3	184 (8 i)
	92-251	18.07.92	–	–	–	5	174 n						
	96-413	30.07.96	–	–	–	2	181 n						
	96-415b	26.07.96	–	–	3	3	208 (3 i)						
Sims Chapel	90-134	10.08.90	–	–	–	3	194 (120 i)						
	91-420							23.08.91	–	–	3	2	170 (17 i)
	92-269	22.07.92	–	–	5	–	88 n+i						
	92-272-3	22.07.92	–	–	1	1	117 (11 i)						
	92-272-9							18.08.92	–	2	3	–	64 n+i
	92-273a	15.07.92	3	–	–	–		18.08.92	–	3	–	–	194 n+i
	96-425							26.08.96	–	–	1	2	158 (4 i)
Stockton 1	91-426-5	23.07.91	–	–	1	3	304 (2 i)						
	92-275b	19.07.62	–	–	–	6	278 (2 i)						
	92-278	22.07.92	–	5	–	–	10 n						
	96-430a	27.07.96	–	–	3	–	156 (38 i)						
	96-432							3.09.96	–	–	–	3	165 (154 i)
Stockton 2	89-667	27.07.89	–	–	–	6	12 n						
	90-143	8.08.90	1	–	1	3	250 (168 i)						
	92-283							18.08.92	–	3	–	–	35 n
	93-489							24.08.93	6	1	–	–	11 (6 i)
Stockton derived	90-111-5	5.08.90	–	–	2	2	208 (138 i)						
	90-111-6	5.08.90	–	2	3	2	167 n+i						
	91-363-17	25.07.91	10	–	–	–		22.08.91	5	–	–	3	57 (2 i)
	91-364-8	18.07.91	–	2	–	–	130 n+i	22.08.91	–	–	3	–	134 (123 i)
	92-211b-1	18.07.92	–	3	1	–	183 n+i						
	92-211b-4	20.07.92	–	–	1	3	173 (95 i)						
	92-214	18.07.92	–	2	–	–	20 n	17.08.92	–	–	–	4	224 n+i
	92-215	18.07.92	3	–	–	–		17.08.92	2	–	2	–	153 (90 i)
	96-399	24.07.96	–	–	–	7	245 (50 i)						
Tuscaloosa	90-108	31.07.90	–	–	–	5	268 (66 i)	5.08.90	–	2	2	3	198 (80 i)
	91-440-1	20.07.91	1	3	2	–	74 n						
	92-287	19.07.92	–	–	1	3	160 n	18.08.92	–	–	–	6	not counted
	92-291							18.08.92	–	–	–	3	107 n
	96-441-1	28.07.96	–	–	–	3	320 (20 i)						
York	89-662	4.08.89	–	3	4	9	160 n						
	91-447	19.07.92	–	–	–	5	202 (10 i)	24.08.91	3	–	–	–	
	91-448							24.08.91	–	–	–	4	246 (6 i)
	93-502							26.08.93	–	–	3	2	322 (104 i)

are fully incompatible, but this would not have been an obstacle to the formation of BC-IV (*O. parviflora*), since the CC-IV parent could act as the female as well as the male parent. Assuming that the B complex had already lost its ability to harmonize with plastome IV in the male gametophyte, the hybrid would have been immediately semi-heterogamous. This remains to be supported by experimental evidence.

An alternative to the view that plastome IV is the ancestral type for the subsection is that a more

Table 4. Progeny obtained from selfed *Oenothera* AB-IV plants

Strain	Culture number of parent	Date of selfing	Seeds sown	Progeny identified as		Germlings died unidentified	Seeds not germinated with embryo	Empty seeds	Culture number (grown)
				AB	BB				
Avalon	90-120b	5.08.	64	5	–	5	1	53	92-115
	90-121-1	5.08.	66	6	–	–	–	60	92-124
	92-109	10.06	26	1	–	–	–	25	93-318
	92-124-3	15.07.	30	5	–	–	–	25	94-623
Bay Minette A	91-282	21.07.	31	1	–	–	–	30	92-128
	92-127	16.08.	80	–	–	–	–	80	94-625
Bellamy	91-290-4	20.07.	60	18	19	1	–	22	92-131
	91-293-1	19.07.	40	3	3	–	–	34	92-133
	89-635	1.08.	109	6	–	6	5	92	93-335
Bigbee	90-69-1	20.07.	7	–	–	–	–	7	91-301
	91-303-1	19.08.	27	–	–	–	–	27	92-147b
	93-347	5.07.	48	1	–	–	–	47	94-909
Bolinger	91-307	20.07.	66	2	–	–	–	64	92-148b
	92-145	21.07.	42	–	–	–	–	42	94-634
	92-148b	15.07.	20	–	–	–	–	20	94-636
	91-306a	2.07.	19	5	–	1	–	13	94-637
Brewton	91-310-2	21.08.	80	3	–	–	6	71	92-151b
	91-314-4	24.07.	78	–	–	1	–	77	92-154b
Cantonment	92-160	20.08.	60	2	–	–	1	57	93-379
	92-158	16.08.	75	2	–	–	2	71	94-641
	92-160	20.08.	30	7	–	1	–	22	94-642
Castleberry B	92-166-1	18.07.	50	1	–	1	–	48	94-643
Chastang 2	94-926-3	2.08.	60	1	1	5	2	51	95-76
Chastang 7*	92-174-10	16.08.	28	5	2	1	2	18	94–647
	92-179-7	16.08.	13	1	–	–	2	10	94-651
County Road 6	90-135-4	6.08.	54	1	–	–	1	52	92-187
	91-350-1	17.07.	30	3	–	–	–	27	94-652
	92-183-4	18.07.	30	1	–	4	–	25	94-653
	92-185b	16.08.	90	1	–	1	–	88	94-654
	92-187	18.07.	73	1	–	2	–	70	94-655
Flomaton	90-126-1	5.08.	43	10	1	–	–	32	91-353
	89-648	26.07.	30	15	–	–	–	15	92-191a
	92-190b	22.07.	30	3	–	2	–	25	94-656
	92-191a-0	21.07.	30	1	6	–	1	22	94-658
	92-191a-1	21.07.	30	7	5	–	–	18	94-659
	92-191a-1	14.08.	30	5	3	–	–	22	94-660
	92-191a-3	14.08.	34	7	–	–	–	27	94-661
	92-194-1	16.08.	32	14	–	–	–	18	94-662
	92-194-2	18.07.	35	4	–	–	1	30	94-663
	92-195-4	18.07.	30	5	–	–	–	25	94-664
	92-198-6	16.08.	34	1	–	–	–	33	94-665
	92-202-1	2.07.	35	–	–	1	1	33	94-666
	92-191a-1	14.08.	60	10	5	1	–	44	95-94
Frankville	90-138-8	6.08.	80	2	–	–	–	78	92-206
	91-358	23.07.	42	7	1	–	–	34	93-434
	92-206	17.08.	39	1	–	1	–	37	94-667
	92-209b	18.07.	30	2	–	–	–	28	94-669
Seabury Creek 1	91-393-1	23.08	25	4	–	1	1	19	92-240
	91-398	23.08.	60	–	–	–	1	59	92-243
	90-103-2	5.08.	40	4	–	–	3	33	92-247b
	91-393-3	23.08.	94	2	–	5	–	83	93-464

(contd.)

Table 4 (continued)

Strain	Culture number of parent	Date of selfing	Seeds sown	Progeny identified as		Germlings died unid-entified	Seeds not germinated with embryo	Empty seeds	Culture number (grown)
				AB	BB				
	92-239	18.07.	144	1	–	–	–	143	94-682
Seabury Creek 2	91-401	23.08.	40	–	–	–	4	36	92-250b
	92-251-1	18.07.	29	1	–	–	–	27	94-686
Sims Chapel	90-134-1	10.08.	50	18	–	4	–	28	91-420
	92-269	22.07.	80	3	1	17	–	21	94-695
	92-271a	17.08.	28	3	–	–	–	25	94-696
	92-272-3	22.07.	28	4	–	1	–	23	94-697
	92-272-9	18.08.	28	2	–	–	1	25	94-698
	92-273a	18.08.	40	9	1	1	2	27	94-699
Stockton 1	91-426-5	23.07.	60	3	–	2	–	55	92-275b
	92-275b-1	19.07.	50	6	–	–	–	44	93-484
	92-278	23.07.	31	–	–	–	–	31	94-700
Stockton 2	89-667	27.07.	32	–	–	–	–	32	91-433
	90-143-1	8.08.	60	4	–	–	–	56	91-434
	92-283	18.08.	44	–	–	–	–	44	94-702
Stockton derived	91-364-4	20.07	50	8	–	1	4	37	92-2111b
	90-111–5	5.08.	80	4	–	1	3	72	92-212
	91-363-17	22.08.	77	16	8	5	10	38	92-214
	91-364-8	22.08.	37	2	–	–	1	34	92-215
	90-111-0	10.08.	54	11	–	–	5	38	92-219b
	90-111-6	5.08.	68	3	1	4	35	25	92-442
	92-211b-1	18.07.	36	3	–	2	–	31	94-670
	92-211b-4	20.07.	30	4	–	3	–	23	94-671
	92-212-4	22.07.	124	–	–	5	3	116	94-672
	92-214-12	18.07.	26	–	15	–	7	4	94-676
	92-214-12	17.08.	30	–	26	–	–	4	94-677
	92-215	17.08.	30	7	–	4	–	19	94-679
Tuscaloosa	90-108-1	31.07.	61	7	4	–	–	50	91-440
	90-108	5.08.	58	9	12	3	–	34	92-287
	92-287-4	19.07.	30	–	12	–	–	18	94-704
	92-287	18.08.	30	3	1	–	–	26	94-705
	92-291	18.08.	30	4	5	1	–	20	94-710
	91-440	20.07.	40	11	10	1	–	18	94-792
York	91-448	24.08.	40	7	–	1	1	31	92-297
	91-447	19.07.	90	5	1	5	–	79	94-976

* The genotype of this strain is AB^A

primitive precursor of plastome IV existed which is now extinct. Such a plastome type would have exhibited a slow multiplication rate which was later replaced by more rapidly multiplying ones.

The results of the current investigation make it doubtful that the precursor of the extant *O. grandiflora* possessed plastome IV because of the now established incompatibility between the B genotype and plastome IV. Moreover, similar findings exist with the A genotype although to a limited degree (Stubbe 1963, Stubbe et al. 1978). Therefore, we favor the hypothesis that a precursor existed which tolerated the differentiation of the ancestral genome into three different genomes A, B, and C, and from which the three plastomes II, III, IV were derived later. A diagram of this evolutionary scheme is shown in Fig. 4. More detailed information is to be expected from molecular investigations on the plastome. A recent paper (Sears et al. 1996) describes comparisons of repetitive sequences of one of the putative cpDNA replication

Table 5. Examples of seed set obtained by selfing of *Oenothera* BB-IV plants. Abbreviations: *n* normal seeds, *i* imperfectly developed seeds, quota in parenthesis, *em* empty fruits, *sm* small fruits, *me* medium-sized fruits, *nm* normal fruits

Strain	Culture number	Date of first selfing	Number of fruits obtained, assigned to the categories:				Seed content in the best fruit	Date of second selfing	Number of fruits obtained, assigned to the categories:				Seed content in the best fruit
			em	sm	me	nm			em	sm	me	nm	
Avalon	96-460	28.07.96	–	–	–	5	215 i	4.09.96	2	2	–	–	104 (80 i)
Bay Minette A	96-328	28.07.96	–	–	1	1	181 i	23.08.96	3	1	1	–	61 n
Bellamy	93-337	6.07.96	–	–	1	4	172 (75 i)	1.08.93	–	–	–	6	101 (50 i)
	94-907	28.07.94	–	–	–	3	143 (78 i)	10.08.93	–	–	–	4	172 (18 i)
	96-343 a	31.07.96	–	–	–	2	127 (109 i)	23.08.96	–	1	1	–	140 (136 i)
	96-346 a	29.06.96	3	1	–	–	16 (13 i)	24.07.96	–	–	–	3	122 (37 i)
Bigbee	93-347	5.07.93	1	–	2	–	97 i						
	93-350-1	5.07.93	–	–	–	5	205 (48 i)	1.08.93	–	–	–	5	352 (210 i)
	93-350-2	7.07.93	–	–	–	4	167 (80 i)	2.08.93	–	–	–	3	330 (42 i)
	94-909-4							10.08.94	–	–	–	1	206 i
	95-49	17.07.95	–	–	1	–	236 i	22.08.95	–	–	3	–	104 i
	96-349b-1	22.07.96	3	–	–	–		23.08.96	1	2	–	–	40 i
	96-349b-2	25.07.96	–	3	2	–	65 i						
	96-353a-4	25.07.96	–	3	2	–	19 (8 i)	23.08.96	–	–	1	–	83 i
	96-354a	25.07.96	–	2	3	–	25 (15 i)	25.08.96	–	–	2	–	177 i
	96-355a	25.07.96	–	–	–	9	263 i	25.09.96	–	3	–	–	42 i
Bolinger	90-70	4.08.90	–	–	–	8	242 (239 i)						
	92-145							2.10.92	–	3	–		95 i
	95-56-2	19.07.95	–	–	–	4	284 (262 i)						
	96-357b	25.07.96	–	1	4	1	188 (176 i)						
Brewton	92-150	2.07.92	–	–	–	4	242 (239 i)	16.08.92	–	1	2	–	107 (42 i)
	96-361b							23.08.96	–	2	–	–	96 (52 i)
	96-364	29.07.96	3	1	–	–	34 i	28.08.96	–	–	5	–	68 i
Cantonment	96-366a	25.07.96	–	–	–	5	282 i	28.08.96	–	–	–	4	252 (12 i)
	96-368a-1	25.07.96	–	–	–	4	235 (185 i)						
	96-368a-2	25.07.96	–	–	–	3	117 (834 i)						
	96-368b-1	25.07.96	–	–	–	4	264 i						
	96-369							23.08.96	–	–	–	3	300 (34 i)
Castleberry B	93-396	1.08.93	–	3	–	–	27 n+i						
	94-922							2.09.94	–	2	–	–	102 (50 i)
	96-372	25.07.96	3	–	–	–		4.09.96	3	–	–	–	
Chastang 2	96-375b							25.08.96	–	1	1	4	112 (4 i)
Chastang 7*	92-171b							4.10.92	–	–	5	–	114 (20 i)
	96-387c	25.07.96	–	–	3	4	146 (9 i)	24.08.96	–	–	4	–	72 (35 i)
Flomaton	93-427-1	16.09.93	6	1	–	–	62 i	5.10.93	3	–	1	1	99 (25 i)
	93-427-3	22.08.93	–	–	–	6	310 (96 i)	18.09.93	–	–	–	1	292 (264 i)
	93-429-1	23.08.93	–	–	3	–	142 (130 i)	18.09.93	9	–	–	–	
	93-429-2							17.09.93	1	2	2	–	75 (26 i)
	94-935	3.08.94	–	–	3	–	60 i	5.09.93	–	–	3	–	70 i
	96-385							25.08.96	–	–	1	2	180 (30 i)
	96-386b	25.07.96	2	–	1	–	51 (19 i)						
Frankville	92-209b	2.07.92	–	3	–	–	75 (23 i)	22.07.92	–	–	7	–	182 n+i
	93-437	6.07.93	1	–	1	3	100 (19 i)	2.08.93	–	–	–	5	320 (86 i)
	94-939							10.08.94	–	–	2	6	207 (204 i)
Seabury Creek 1	94-951	2.07.94	–	–	–	2	188 (40 i)						
	96-410a	29.07.96	–	–	–	3	194 (11 i)	25.08.96	–	–	4	–	161 (7 i)
Seabury Creek 2	92-260-1	14.07.92	–	–	–	5	202 i						
	93-469	7.07.93	–	–	–	3	179 (124 i)	1.08.93	–	–	–	5	308(60 i)
	94-958							5.09.94	–	–	3	–	67 (5 i)
	95-115	18.07.95	–	–	–	2	173 (159 i)	25.08.95	–	–	3	–	46 i
	96-114	26.07.96	–	–	–	4	181 (47 i)						
	96-417a							26.08.96	–	4	–	–	7 (1 i)
Sims Chapel	92-270							20.08.92	–	–	–	2	186 (152 i)
	92-272	14.07.92	–	–	–	3	192 n						

(contd.)

Table 5 (continued)

Strain	Culture number	Date of first selfing	Number of fruits obtained, assigned to the categories:				Seed content in the best fruit	Date of second selfing	Number of fruits obtained, assigned to the categories:				Seed content in the best fruit
			em	sm	me	nm			em	sm	me	nm	
	93-478	17.09.93	–	–	–	3	236 (6i)	27.10.93	–	–	3	–	105 (37i)
	95-120	27.08.95	4	–	1	–	43 (13i)	23.09.95	2	–	1	–	36 (14i)
	95-126							22.08.95	–	–	–	2	382 (370i)
	96-425							26.08.96	2	–	3	–	85 (8i)
	96-427							26.08.96	–	–	5	–	137 (13i)
	96-429a							5.09.96	–	2	3	–	73n
Stockton 1	92-277	1.07.92	–	–	–	4	275 (263i)						
	95-130	28.08.95	–	–	–	2	309 (300i)	14.10.95	–	1	2	1	245i
Stockton 2	92-285b	15.07.92	2	2	–	–	58 (57i)						
	96-436a							26.08.96	–	1	4	–	83i
	96-439	28.07.96	4	–	–	–		26.08.96	–	–	3	–	120i
Stockton derived	91–360							22.08.91	–	–	6	–	128n+i
	92-213-2							18.08.92	–	–	–	5	163 (20i)
	92-213-3							18.08.92	–	–	1	1	182n
	93-440	1.08.93	–	–	–	4	118 (6i)	23.08.93	–	2	3	–	89 (61i)
	93-440	3.08.93	–	–	–	4	306 (136i)	26.08.93	–	–	–	6	146 (13i)
	94-677							5.09.94	–	–	2	3	112 (58i)
	96-395a							25.08.96	–	–	3	3	261 (37i)
	96-398	27.07.96	1	–	1	–	13 (2i)	25.08.96	3	1	8	–	232 (40i)
	96-400a	26.07.96	4	–	–	–		28.08.96	–	–	6	–	259 (57i)
Tuscaloosa	92-287							19.08.92	3	–	1	2	206n
	92-290-1							19.08.92	–	–	–	2	230n+i
	92-290-2							19.08.92	–	–	–	4	140n+i
	93-496-1	2.08.93	–	–	–	3	276 (114i)	20.08.93	–	–	–	5	208 (94i)
	93-496-3	2.08.93	–	–	–	4	186 (32i)	20.08.93	–	–	–	5	212 (90i)
	94-709	2.07.94	–	–	–	1	73n	28.07.94	–	–	–	4	114 (5i)
	95-134	18.07.95	–	–	–	4	252 (146i)	28.08.95	–	–	1	4	189 (165i)
	96-441-3	28.07.96	–	–	–	3	186 (77i)	27.08.96	1	–	3	–	164i
	96-441-4							27.08.96	–	–	1	5	240 (119i)
York	92-297							18.08.92	–	–	–	7	198 (14i)
	93-499-2	3.08.93	–	–	–	3	302 (228i)	27.08.93	–	–	–	3	362 (336i)
	93-501-1	6.07.93	–	–	–	7	167n						
	93-501-2	2.08.93	–	–	–	3	300n	27.08.93	–	–	–	5	240 (148i)
	96-445a							4.09.96	–	–	–	6	195 (109i)
	96-448	28.07.96	–	–	–	3	381 (114i)						
	96-452	28.07.96	–	–	–	2	373 (350i)	4.09.96	–	–	3	–	185i

*The genotype of this strain is B^AB^A

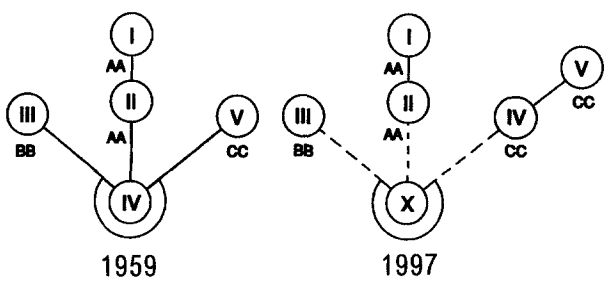

Fig. 4. Diagram of co-evolution of *Oenothera* genome and plastome from a common ancestor X, previous ("1959", after Stubbe 1959) and present ("1997") hypothesis

origins of plastomes I, II, III and IV of subsection *Oenothera*. It is concluded that plastome IV must have diverged quite early from the other types and cannot be considered to be ancestral to them. However, the same sequence data can be interpreted in a different way leading to conflicting conclusions (Hornung et al. 1996). Therefore, additional non-repetitive parts of the cpDNA have to be considered for molecular analysis in order to support the classical genetic studies. To this end, earlier work by Wolfson et al. 1991 already provides some evidence for the relatedness of plastomes I and II and predicts a common progenitor for plastomes III and IV. But a more comprehensive

Table 6. Progeny obtained from selfed *Oenothera* BB-IV plants

Strain	Culture number of parent	Date of selfing	Seeds sown	Viable BB-IV	Non viable	Seeds not germinated with embro	Empty seeds	Culture number (grown)
Avalon	96-460	28.07.	25	–	–	–	25	97-54 a
	96-460	4.09.	30	16	–	–	14	97-54 b
Bay Minette A	96-328-2	28.07.	30	24	–	4	2	97-53 a
	96-328-2	23.08	30	14	–	4	12	97-53 b
Bellamy	92-139-1	1.07.	30	3	7	–	20	95-40
	92-139-2	21.07.	30	2	–	–	28	94-630
	93-337-4	6.07.	80	25	12	–	43	94-905, 95-41
	93-337-4	1.08.	30	4	11	–	15	95-42
	93-340-2	1.08.	60	27	7	–	26	95-36
Bigbee	91-300	19.07.	62	–	–	–	62	94-633 a
	91-300	3.10.	65	–	–	–	65	94-633 c
	94-909	27.09.	40	–	–	–	40	95-48
Bolinger	90-70	4.08.	95	–	–	–	95	91-306
	96-357 b	25.07.	30	–	–	–	30	97-55
Brewton	92-150	2.07.	138	–	–	–	138	94-638
Cantonment	92-158	2.10.	96	–	4	2	90	94-640
	95-68	18.07.	30	23	–	–	7	96-338 a
	95-69	20.07.	30	30	–	–	0	96-368 c
Castleberry B	93-396	1.08.	27	9	6	–	12	94-922
Chastang 2	96-375 b	25.08.	30	27	–	–	3	97-56
Chastang 7*	92-171 b	4.10.	50	–	–	–	50	94-645
Flomaton	92-191 a	1.07.	30	15	2	–	13	94-657
	94-935	3.08.	33	–	–	–	33	96-388 a
Frankville	92-209 b	2.07.	38	17	–	–	21	93-435
	94-939	10.08	80	2	9	–	69	95-193
Seabury Creek 1	94-951-3	2.07.	40	–	–	–	40	96-408 a
Seabury Creek 2	93-469-1	1.08.	60	24	7	29	0	96-414
	94-958	5.09.	60	2	13	1	44	95-115
Sims Chapel	92-270	20.08.	113	–	–	–	113	93-475
	92-272	20.08.	67	51	–	–	16	93-478
	93-478	17.09.	60	13	6	–	41	95-126
	95-126	22.08.	30	1	1	–	28	96-429 a
Stockton 1	92-277	1.07.	80	1	–	–	79	93-485
Stockton 2	91-435	19.07.	50	3	1	2	44	92-285 b
	92-285 b-1	15.07.	40	9	–	–	31	93-490
	92-285 b-2	15.07.	30	–	1	–	29	94-703
Stockton derived	91-360-1	22.08.	34	26	–	–	12	93-440
	92-213-2	18.08.	40	32	1	–	7	93-444
	92-213-3	18.08.	55	37	1	–	17	94-675
	94-677	5.09.	60	38	3	–	19	95-102
Tuscaloosa	91-442	18.08.	40	27	2	–	11	92-290
	92-287	19.08.	35	17	1	–	17	94-706
	92-290-1	19.08.	72	11	3	2	56	94-708
	92-290-2	19.08.	60	24	1	–	35	94-709
	94-709	28.07.	40	39	–	–	1	96-444 b
York	92-297	18.08.	60	15	1	–	44	93-501
	93-501	6.07.	140	76	2	–	62	94-978, 96-445 b

*The genotype of this strain in B^A B^A.

comparative analysis regarding all five plastomes and several different sets of repetitive as well as non-repetitive DNA sequences is needed to further clarify the evolutionary history within the subsection *Oenothera*.

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