The Complex-Heterozygotes of *Oenothera grandiflora* L'Her.

E. Schumacher[1], *E. Steiner*[2], *and W. Stubbe*[1]

[1] Botanisches Institut, Heinrich-Heine-Universität Düsseldorf, FRG
[2] Department of Biology, The University of Michigan, Ann Arbor, USA

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Abstract

Complex-heterozygotes discovered in populations of *O. grandiflora* in Alabama are composed of a typical grandiflora (B) complex and an altered B complex which modifies the phenotype in the direction of *O. villosa* (A). A cytogenetic analysis of the new complex, designated B^A, is reported including results of its compatibility with different plastomes. The evidence suggests that the B^A complex resulted from hybridization between *O. grandiflora* and the pollen complex of the sympatric *O. biennis*.

Key words

Oenothera, evolution, complex-heterozygotes.

Introduction

In the two contributions of Steiner and Stubbe (1984, 1986) dealing with *Oenothera grandiflora* and its population structure, structurally heterozygous races indistinguishable from *O. grandiflora* were reported from 3 sites in Alabama. Through further analysis these proved to be complex-heterozygotes. At diakinesis at least 10 of the 14 chromosomes form a circle, the remaining chromosomes being present either as a circle of 4 or 2 pairs. Configurations of a circle of 12 and a pair and a circle of 14 have also been observed. This is in contrast to the typical *O. grandiflora* which is characterized by meiotic configurations of 7 pairs or a circle of 4,5 pairs; two circles of 4,3 pairs and a circle of 6, a circle of 4, and 2 pairs occur less frequently.

To better understand the evolution of species in the subsection *Oenothera* it is important to determine if the large-circled forms are true grandifloras, i.e. do they possess the same genomes as the homozygous races? Further, have their unusual meiotic configurations arisen through an accumulation of reciprocal translocations, e.g. as has apparently occurred in *O. wolfii* (Wasmund and Stubbe, 1986), or do the newly discovered large-circled strains contain elements introduced into *O. grandiflora* through hybridization with the sympatric *O. biennis*?

In the more recent report Steiner and Stubbe (1986) pointed out that the complex-heterozygotes were composed of a typical and an altered *grandiflora* complex; homozygotes of the latter occur occasionally upon the selfing of the heterozygotes, although such forms have not been found in samples of the natural populations.

The present paper reports the results of a detailed cytogenetic analysis of selected large-circled *grandiflora* races. The study, carried out from 1985 to 1991, had the objective of determining the segmental arrangements of the chromosomal complexes and identifying the genomic differences between the two complexes with the hope of establishing the origin of the modified *grandiflora* complex.

Materials and Methods

One of the large circled forms was collected in 1981 in the vicinity of Brewton, AL; the remaining were found in 1983 in Castleberry and Chastang, AL. These were grown in Ann Arbor, MI and Düsseldorf, Germany. The methods employed for cytogenetic analysis were presented in the previous publications. The interactions between genome and plastome were determined by utilizing crosses combining plastids of other species with complexes of the *grandiflora* strains. These are based on the study of Stubbe (1959) on plastid inheritance in *Oenothera*.

Results

The phenotype of *Oenothera grandiflora* on which the following comparison is based has been characterized by Steiner and Stubbe (1984); a further account of the systematics of *O. grandiflora* can be found in Dietrich, Wagner and Raven (in press). When the collections from various sites are grown in the experimental field and compared, they show a pronounced morphological diversity. Apart from their wide variation, the phenotypes of the homozygous races of *O. grandiflora* are unquestionably classified as genome type B by both geneticists and systematists familiar with the subsection *Oenothera*. Genome B is most compatible with plastome type III, as previously determined for all *grandiflora* strains. This genome is also found in *O. biennis* (Cleland's *biennis* groups 1, 2 and 3, the latter now known as *O. nutans*). Since the B genome is to

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varying degrees incompatible with the remaining plastome types (I, II, IV, V) of the subsection *Oenothera*, the genome-plastome interaction provides another criterion for identification.

The newly discovered large-circled forms differ only slightly from the homozygous strains of *O. grandiflora*; the differences are only detectable when plants are compared in the experimental plots. A somewhat narrower leaf and often an earlier flowering are the characters which generally distinguish them from the homozygous *O. grandiflora* races. When the large-circled strains are used as pollen parents in crosses with homozygous or heterogamous races, twin hybrids are produced which differ phenotypically as well as cytologically. Thus, both complexes are active in the pollen. The one produces the typical B phenotype, while the other is modified in the direction of the A phenotype (e.g., as found in *O. villosa*). However, it is far from identical with the beta complexes of the *O. biennis* group 1. To designate this altered B complex, we have selected the symbol B^A.

The large-circled forms generally carry an Si gene in the B complex; thus BB homozygotes are not obtained upon selfing. However, in the Castleberry A-9 culture one plant (309-1) lacked an Si gene, and in Castleberry A-7 the Si gene occurs in the B^A complex.

On the maternal side the behavior of the two complexes is strongly influenced by varying degrees of embryo sac competition among the different races. When the B^A complex lacks an Si gene and is not fully eliminated through embryo sac competition, B^AB^A homozygotes segregate upon the selfing of the heterozygote. Table 1 shows the ratios of homozygotes to heterozygotes; these allow deductions regarding embryo sac competition. If homozygotes are rare or absent, the possibility of the presence of sporophytic lethals may also have to be considered. The occurrence of B^AB^A homozygotes is of great importance in judging the origin of the large-circled *grandiflora*-like strains, since the B^A complex is completely expressed in the phenotype. The homozygotes are ordinarily sufficiently viable to flower and yield seed when grown in culture. Pure lines have been established for the races Brewton, Castleberry A-1, A-4, A-9 and Chastang 1 and 7. The self-incompatible B^A complex of Castleberry A-7 can only be obtained when combined with B^A complexes of other strains.

Table 1 Investigated complex-heterozygous races with their chromosome configurations at diakinesis and the segregations of B^AB^A homozygotes following self-pollination.[a]

Race	Chromosome configuration	Number of Plants evaluated	No. of B^AB^A homozygotes	% of B^AB^A homozygotes
Brewton	⊙ 10,2 pairs	112	19	15.0
Castleberry A-1/1	⊙ 10,2 pairs	56	3	5.7
Castleberry A-1/2	⊙ 10,2 pairs	80	17	21.3
Castleberry A-4	⊙ 12,1 pair	174	94	54.0
Castleberry A-7	⊙ 14	106	15	14.2
Castleberry A-9	⊙ 10,2 pairs	150	2	1.4
Chastang 1	⊙ 12,1 pair	–	–	–
Chastang 7	⊙ 8,3 pairs	–	–	–
Route 4	⊙ 14	–	–	–

[a] Through an oversight the number of homozygotes were not recorded for the Chastang 1, Chastang 7, and Route 4 strains.
B^AB^A homozygotes are identified by their distinctive morphology as described in the text as well as cytologically by the 7 pairs of chromosomes at meiosis.
⊙ = circle of.

In general, no differences in germination behavior can be detected between the homozygotes and heterozygotes, nor do they show differences in the seedling stage. However, the races Castleberry A-1 and A-4 are exceptions. The heterozygotes of Castleberry A-4 segregate homozygotes which have pale green leaves and grow less vigorously than the heterozygotes; it is advantageous to separate the two types early and provide special care for the less vigorous type. Later, plants of the latter turn a deeper green and recover their vigor so that the rosettes can be planted in the field at the same time as the heterozygotes. Two different lines have been isolated from Castleberry A-1, one of which behaves as described above; the other corresponds with those of the remaining races in which the seedlings do not deviate phenotypically from the heterozygotes.

Like the homozygous grandifloras, the B^AB^A homozygotes break rosette early; they produce strong basal branches. They tend to be distinctly shorter than the heterozygotes; only in the case of Brewton and Chastang 1 and 7 are the homozygotes as tall as the heterozygotes. The leaves of the B^AB^A homozygotes are narrower than those of the heterozygotes. The gray green color of the homozygotes results from the strigose pubescence of the leaves which is also characteristic of the buds and fruits. As a result, the B^AB^A homozygotes differ distinctly from the relatively glabrous homozygous grandifloras.

The B^AB^A homozygotes derived from Brewton, Castleberry A-9, and Chastang 1 and 7 begin blooming in Düsseldorf about the middle of August, while the remainder only flower from the middle of September (Castleberry A-4) to the end of October (A-1). Time of flowering can be induced earlier through short day treatments. The late blooming races of the homozygous *O. grandiflora* are characteristically well-branched at the flowering tips. Bud and flower size show considerable variation; in Castleberry A-4 the petals are 0.8 cm across, in the other races 2.7, 3.5, and 4.0 cm, respectively.

Cytological Investigations

In order to determine the segmental arrangements of the chromosomes of the races listed in Table 1, crosses were carried out with a series of races with known segmental arrangements of their complexes (see Appendix I, Cleland, 1972). The Brewton strain was also utilized, its B^A complex having the following segmental arrangement (Steiner und Stubbe, 1986):

1·6 3·2 5·11 7·10 9·4 12·8 13·14

The results of our analysis show that the above arrangement is also found in the B^A complexes of Castleberry A-1/1, A-1/2, A-4, A-9 and Chastang 7; on the other hand, the B^A complex of Castleberry A-7 is one translocation removed, i.e. it has the 7·14 and the 13·10. The

arrangement for the B^A complex of Chastang 1 is probably the same as the B^A of Brewton, but still requires confirmation.

The arrangements of the B complexes that produce the typical *grandiflora* phenotype are identical or close to the arrangement of the Johansen race of *O. elata* ssp. *hookeri*, considered by Cleland to be primitive for the Euoenotheras. Some of these were published earlier (Steiner and Stubbe, 1984). Arrangements of the B complexes of newly determined races are shown below:

Castleberry A-1
1·4 3·2 5·6 7·10 9·8 11·13 12·14
Castleberry A-4
1·2 3·4 5·6 7·10 9·8 11·13 12·14
Castleberry A-9
1·2 3·4 5·6 7·10 9·8 11·12 13·14
Chastang 7
1·4 3·2 5·6 7·10 9·8 11·12 13·14

Observations upon the interaction between genome and plastome

As already established by Steiner and Stubbe (1986), when these complex-heterozygotes are used as pollen parent and combined with an egg complex, e.g. *albicans* Grado, twin hybrids are produced, one of which corresponds to a typical $^{G}albicans \cdot {}^{h}grandiflora$ (AB) type while the other is modified in the direction of an AA phenotype. Accordingly, the newly analyzed large-circled forms can be designated as BB^A, since they consist of a typical and a modified B complex. In all combinations so far tested the B complex behaves as in the combination square of Stubbe (1959) which is reproduced here with the modifications of 1966 and 1989 (Fig. 1).

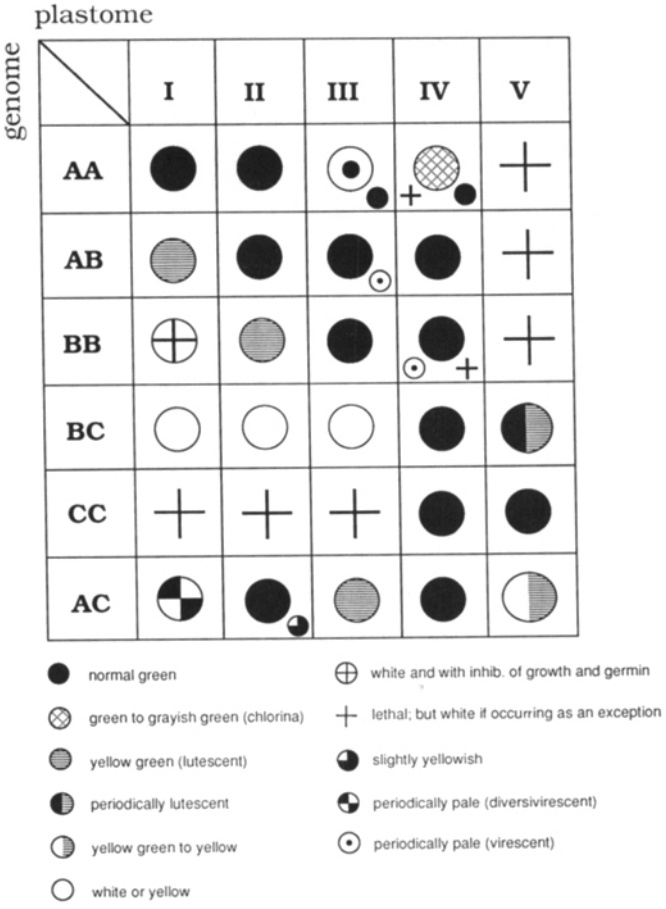


Fig. 1 Compatibility relations between different diploid genotypes and plastid types (after Stubbe, 1959). More than one symbol in some squares indicates certain differences among the A and B complexes.

How the B^A complexes fit into the pattern of genome-plastome interaction now remains to be determined. As already pointed out, with two exceptions the B^AB^A homozygotes are a normal green. Since they naturally occur with plastome III, it is logical to compare them with the combinations BB-III, AB-III, and AA-III. While BB-III and AB-III develop into a normal green, the combination AA-III exhibits the *virescens* character, namely, a bleaching of the cotyledon bases and the primary leaves (Figs. 2 and 3); as successive leaves develop they become progressively greener. The mature plants are often somewhat weaker than the AA-I and AA-II types which are green from the beginning of development. However, they flower and fruit abundantly. In rare cases the AB-III combinations may also show a light virescence.

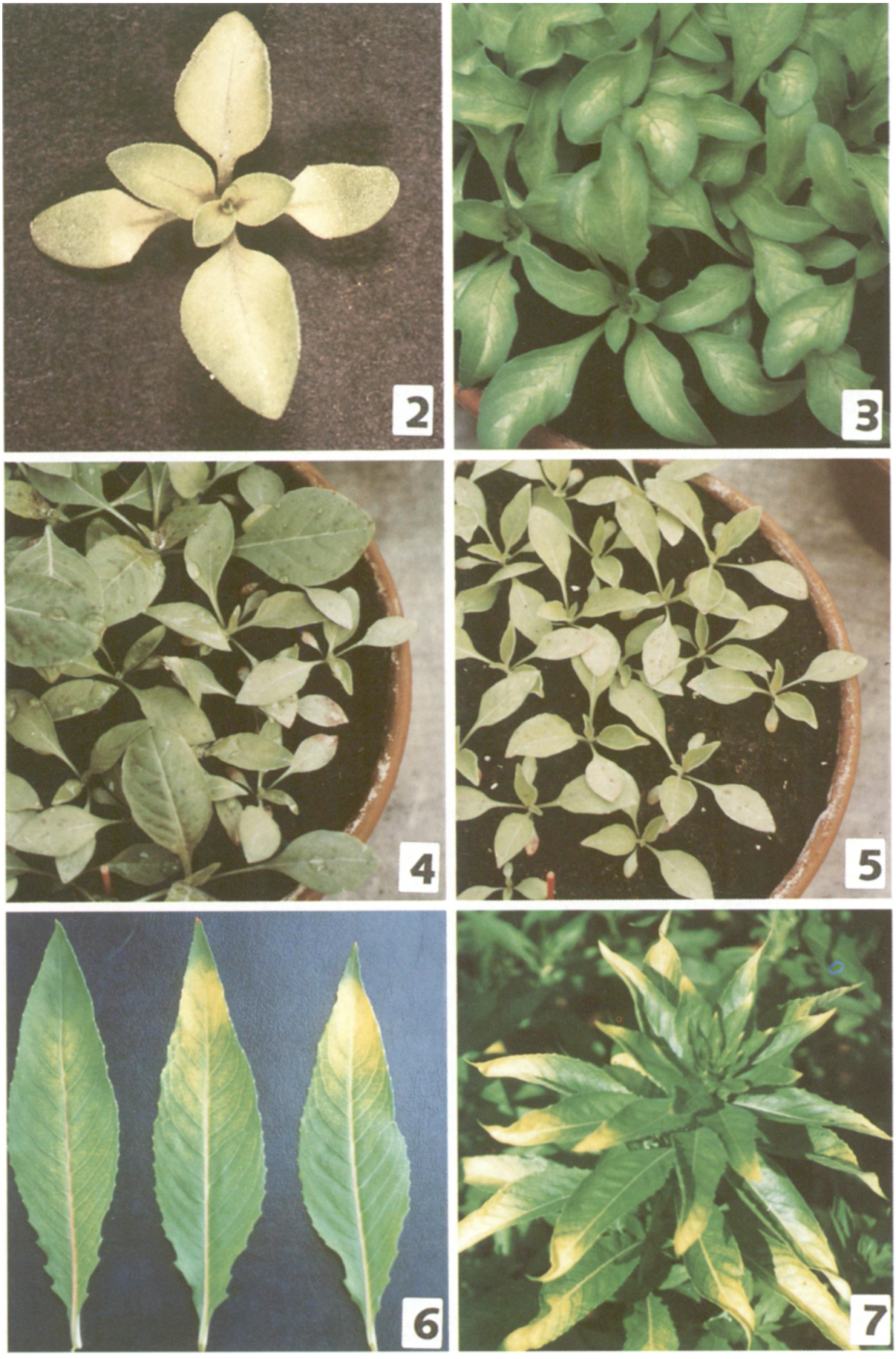
The B^AB^A homozygotes Castleberry A-1/1 and A-4 (Figs. 4 and 5), which are pale green in early development, do not show the typical virescent phenotype, although they display a certain tendency toward it. Hybrids produced between different B^A genotypes when combined with plastome III developed normally green in most cases; the hybrid B^AB^A-III from the cross Castleberry A-1/1 × A-4 (89/754) showed no bleaching.[a]

[a] However, the hybrid B^ACastleberry A-4 × B^A Brewton (^{88}/289) shows virescent bleaching.

The evaluation of the B^A complexes as B genotypes modified by A elements raises the question whether the assumed A genome elements also influence the greening capacity of B^A hybrids with pure A and B genotypes and different plastomes.

The first combination to consider is the hybrid AB^A-III which is easily constructed by crossing the B^AB^A homozygotes with AA homozygotes such as races of *O. elata* ssp. *hookeri*. Others are available from earlier experiments with plastomes I, II, III, and IV as well as with mutated plastids and can be utilized for crosses and their reciprocals. Finally, the *albicans* complex of the Grado race of *O. suaveolens* ($^{G}albicans$), which is transmitted exclusively through the egg, has been combined with various plastomes in heterogamous complex-heterozygous hybrids (e.g. $^{G}albicans \cdot undans$) and was used in appropriate crosses. These and other combinations and their phenotypic appearence are summarized in Table 2.

Of the tested AB^A-III idiotypes those whose A complex was derived from the races *hookeri* Standard and Johansen were clearly virescent in their early development, i.e. the cotyledons are bleached at the base and the primary leaves are pale. The subsequent series of leaves are decreasingly pale. The strength of the virescence is not significantly different from strain to strain, but is not



Figs. 2–7

Table 2 Overview of the greening capacity of plants which combine the B^Acomplexes of the newly investigated races with the A complexes of *hookeri* Standard, Johansen and ^{G}*albicans* and different plastomes of the subsection *Oenothera*.[a]

	Plastome III			Plastome I				Plastome IV	
B^A complexes of:	*hookeri* St.	Johansen	^{G}*albicans*	*elata*	*hookeri* St.	Johansen	^{G}*albicans*	Johansen	^{G}*albicans*
Brewton	vir	vir	green		green	green	green		green
Castleberry A-1/1	vir	vir	green	green → lut	green → lut	green → lut	green → lut	green	green
Castleberry A-1/2	vir	vir	green		green	green	green → lut		green
Castleberry A-4	vir		green				green → lut	green	green
Castleberry A-7	vir	vir	green	green	green	green	green → lut	green	green
Castleberry A-9	vir	vir	green			green	green		green
Chastang 1	vir					green	green		green
Chastang 7	vir		green			green → lut	green		green
Route 4-1	vir	vir	green			green	green → lut		green

[a] In cells of table where arrows are shown, the plants are green when young, but display a light *lutescens* as they become older. Abbreviations: vir = *virescens*; lut = *lutescens*.

quite as great as in the homozygous *hookeri* race with plastome III. The *virescens* character is absent in the combination ^{G}*albicans* · B^A-III. This matches the reaction of ^{G}*albicans* in other AA-III combinations, e.g. ^{G}*albicans* · *undans*-III. Here the greening is nearly normal and the plants show only a slight chlorophyll deficiency at low temperatures.

We can thus conclude that the B^A complexes differ from the pure B genomes in that in combination with *hookeri* Standard and Johansen and plastome III, they cannot neutralize the virescent action of the latter complexes.

The few AB^A-II combinations so far tested show normal greening, as known for the AB-II types (not presented in Table 2). More numerous are the previously tested AB^A combinations with plastome I. These are interesting because the typical AB-I combinations show the *lutescens* type of bleaching (Figs. 6 and 7), although the strength of expression varies from genotype to genotype. Ordinarily the distal regions of the cotyledons are yellowish, while the primary leaves exhibit a greenish yellow. The older rosette leaves frequently show a yellowish stripe on the otherwise green leaf surface. Such a typical *lutescens* bleaching was not observed in the AB^A combinations with plastome I, although a certain tendency toward it was frequently seen. This was particularly true for those AB^A-I combinations in which the A complex was represented by ^{G}*albicans*. They are normally green when young. Older rosettes with the B^A complexes from the races Castleberry A-1/1, A-1/2, A-4 and A-7, as well as Chastang 7, showed a moderate *lutescens* bleaching. However, those with the B^A complexes of the races Castleberry A-9, Brewton, and Chastang 1 were a normal green. When the A complex in the combination AB^A came from *hookeri* Standard or Johansen, the hybrids were generally variegated. In cases in which the seed parent carried plastome III and plastome I was contributed through the pollen, the normal greening expected with the AB genotype and plastome III along with *lutescens* spotting caused by plastome I, did not occur. Instead a virescent type of bleaching was produced by plastome III and a normal green spotting by plastome I.

If Johansen or a ^{G}*albicans* complex together with plastome I in the seed parent was contributed to the AB^A hybrid, no variegation occurred and the plants were a normal green. The plastome III of the pollen parent was not expressed in the progeny due to its weaker position in the hierarchy of plastid competition (Schötz, 1954). Unexpected in this combination was a strong autumnal *lutescens* bleaching of the cauline leaves which was observed in the hybrid Johansen · Castleberry A-1/1 (AB^A) with plastome I (from Johansen as the seed parent) (90/517).

The *chlorina* type bleaching characteristic of many AA-IV combinations could not be established with certainty for combinations in which the A complex was introduced from the Johansen strain as seed parent or from ^{G}*albicans*. The AB^A-IV plants were healthy and green.

More interesting results are expected when B^AB^A homozygotes with the plastomes I, II and IV are grown; a portion of these became available in the 1991 season. The B^AB^A-I of Brewton, Castleberry A-1, A-4, A-9, and Cha-

Figs. 2, 3 Young rosettes of the *virescens* phenotype, genome-plastome combination AA-III.

Fig. 4 Progeny from a selfed plant, strain Castleberry A-4. The smaller pale green plants are homozygous B^AB^A, the taller, normal green plants are complex-heterozygous BB^A.

Fig. 5 Progeny from a selfed homozygous B^AB^A, strain Castleberry A-1/1.

Figs. 6, 7 Older leaves and shoot of the *lutescens* phenotype, genome-plastome combination AB-I.

stang 7, derived from selfed AB^A-I, most likely are lethal, as indicated by the high proportion of empty seeds.

Discussion

Cytogenetic analysis has shown that the newly discovered large-circled forms involve a complex-heterozygote with two clearly distinguishable genomes; one of the complexes is a typical *grandiflora* complex while the other is a hybrid complex in which B and A elements are combined. The B elements appear to be dominant when the overall phenotype is considered. The early breaking of the rosette, the late blooming, and the pronounced branching in the flowering region are all expressions of the B genome. Nevertheless, not all B^A genotypes are alike in this respect. Some B^AB^A homozygotes resemble *O. grandiflora* more strongly, while others are more similar to *O. villosa*, particularly those with narrow leaves and a strigose pubescence. The latter character gives the plants a gray-green color. That differences are observed among the B^A genotypes suggests that they did not result from a single evolutionary event. On the other hand, the fact that the B^A complexes, with one exception, all possess the same chromosome formula is evidence for their common origin.

The extent of the differences between the B and the B^A complexes can hardly be explained through an accumulation of mutations, but must have occurred through hybridization. Thus, it is useful to investigate the greening capacity in hybrids and in B^AB^A homozygotes with different plastome types for obtaining evidence on the origin of the foreign elements in the B^A genome.

The B^AB^A homozygotes are relatively compatible with plastome III; however, in the combinations of the A complexes of the *hookeri* Standard and Johansen races the B^A complexes cannot compensate for the bleaching effect exerted by the former on plastome III plastids. Further, the B^A complexes differ from the B genomes in that in the combinations of B^AA with plastome I the usual *lutescens* bleaching of the AB-I combinations is strongly diminished or absent completely. Both results indicate that the B^A genomes lack a certain number of factors characteristic of the B genome. Which elements have been substituted can only be determined after the B^AB^A homozygotes in combination with plastomes I and II are evaluated. The same is true for the combination B^AB^A-IV which also exhibits an incompatibility in the pollen. The pollen inactivation known to be associated with certain B-IV combinations has been observed in 1990 for most of the pollen types of the B^A-IV combinations (Stubbe, unpublished). In this respect the B^A complexes are similar to the true B complexes. Further, the lethality of B^AB^A-I conforms well to the character of typical B complexes (as shown in Fig. 1).

The results so far obtained support the hypothesis that in the B^A complex the original B complex has been modified through hybridization by a significant number of genes from an A complex. Crossing-over is rare for the genes responsible for the phenotypic characters that distinguish the various complexes in races of *Oenothera*, since these genes lie in the central heterochromatic segments of the chromosomes, where they are protected from crossovers. Therefore, the most likely explanation lies with either an exchange of entire chromosomes or of chromosomal segments through translocation. Such an exchange could readily occur in a hybrid between *O. grandiflora* and the sympatric *O. biennis* in which the B genome of *O. grandiflora* has been combined with the beta complex of *O. biennis* (A genome). As established earlier (Steiner and Stubbe, 1986), the newly discovered complex-heterozygotes cannot be simple hybrids between the two species, because of the phenotype of the B^AB^A homozygotes as well as their viability.

To determine from which beta *biennis* complexes the integrated chromosomes or chromosome segments of the B complex have been derived must remain the subject of a separate study. If our interpretation of the B^A complexes is correct, the occurrence of a mixed complex between two basic genome types in nature is established, as is theoretically predictable from the compatibility relationships between genome and plastome shown in the combination square (Fig. 1). There are no plastome-limited barriers to crosses between *O. grandiflora* and *O. biennis*.

The new forms are relatively vigorous as a result of their complex-heterozygosity. Their phenotypic similarity to *O. grandiflora* suggests good survival potential in the species range nor should they be serious competitors of *O. biennis*. The segregation of homozygotes is an indication of the relatively recent origin of the large-circled forms, as is also the large flowered character which favors outcrossing in contrast to the more anciently established small flowered, self pollinating complex-heterozygotes. How the systematist will classify these new forms will depend upon the extent to which morphological or other characters receive the greatest emphasis.

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W. Stubbe

Botanisches Institut der
Heinrich-Heine-Universität Düsseldorf
Universitätsstr. 1
D(W)-4000 Düsseldorf 1
Federal Republic of Germany

E. Steiner

Department of Biology
The University of Michigan
Ann Arbor, MI 48109-1048
USA