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Gene-dependent male sterility and plastomes in *Oenothera*

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Abstract The plastids in the cells of the tapetum in anther of *Oenothera* are involved in the development of male sterility (mst). We combined nuclear homozygosity for each of the two mst genes with the four different plastomes of *Oenothera* and demonstrated that in both cases the sterile anther phenotype is independent of the plastome. The experiments provide additional information on competition between megaspores and embryo sacs in the ovule.

Key words *Oenothera* · Male sterility · Plastome · Gametophyte competition

Introduction

In *Oenothera* two nuclear genes for male sterility mst, *fr* and *ster*, have been identified (Oehlkers 1926; Harte 1942, 1948). Both genes act on the sporophyte. The pollen of heterozygotes, *fr*/+*fr* and *ster*/+*ster*, is fertile, irrespective of the genotype of the individual pollen grain. The phenotypes of the mutant homozygotes, *fr*/*fr* and *ster*/*ster*, are characterized by the degeneration of the contents of the anther after meiosis. The degeneration process has been described by several authors (Oehlkers 1927; Harte and Bissinger 1952; Noher de Halac 1985, Noher de Halac et al. 1990). One of the most characteristic deviations in the development of the male-sterile anthers of the type *sterilis* (*ster*/*ster*) has been identified as a malfunction of the plastids in the tapetal cells (Noher de Halac et al. 1992) that influences lipid metabolism in the anthers.

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In *Oenothera* it is a well-known fact that genetic differences occur in the plastome, the DNA located in the plastids (Stubbe 1989). The question that therefore arises is whether the influence of the nuclear genes on plastid metabolism, which is involved in the development of male sterility, depends on the plastome with which they are combined. To solve this problem, nuclear homozygosity for each of the male-sterility alleles, *fr* and *ster*, must be combined with different plastomes. This is made possible by the availability of test stocks that combine an identical nuclear genetic constitution with each of the different plastomes I, II, III, IV. Such stocks have been constructed by W. Stubbe, (University of Düsseldorf, FRG), to whom we are indebted for providing plants and seeds of his stocks for our experiments.

Our study consists of two parts. The first was concerned with the genetics of the experiment and the second part with histological, histochemical and developmental investigations of the different genotypes that are characterized by the alleles of the male-sterility genes and the plastomes. The first part on the combination of the mst genes with four different plastomes is presented here.

Materials and methods

The following genotypes were used in the experiments. The nomenclature of the species and Renner complexes is according to Harte (1993) who also describes the pedigree of the stocks. The species consisted of *Oe. hookeri* de Vries (*^{h}hookeri*·*^{h}hookeri*), *Oe. strigosa* de Vries (*deprimens*·*stringens*). *Oe. suaveolens* Desf. (*albicans*·*flavens*) and *Oe. blandina* (*^{h}blandina*·*^{h}blandina*). The plastones, all from the cultures of Prof. W. Stubbe, were *Oe. albicans*·*undans* with plastome I, *Oe. albicans*·*undans* with plastome II, *Oe. albicans*·*undans* with plastome III and *Oe. albicans*·*undans* with plastome IV. The male-sterility alleles were *Oe. ^{h}hookeri*·*velans ster*/*ster* × *ster*/+ as source of *ster*, *Oe. strigosa* de Vries and hybrids as source of *fr*, *Oe. hookeri* de Vries × *strigosa* de Vries (*^{h}hookeri*·*stringens*)+/*fr*, *Oe. suaveolens* Desf. × *strigosa* de Vries (*flavens*·*stringens*)+/*fr* and *Oe. blandina* × *strigosa* de Vries (*^{h}blandina*·*stringens*)+/*fr*.

Oe. blandina is a homozygous mutant of *Oe. Lamarckiana* de Vries. The Renner complex *^{b}blandina* is nearly identical to *velans*, but without the sporophytic lethal. The mutant *sterilis* was found in the

F_2 progeny of *Oe. Hookeri* × *Lamarkkiana brevistylis* (Harte 1948) and has been maintained for nearly 40 generations by crossing of sister plants *sterilis* × *brevistylis*, *ster* $+^{br}$/*ster* $+^{br}$♀/*ster br* × *ster br*/$+^{ster}$*br*♂. The Renner complexes *hookeri* and *velans* are very similar to one another, differing only by one reciprocal translocation of chromosome arms, some alleles for quantitative characters and the sporophytic lethal in *velans*. As a consequence of the long period of inbreeding the stock contains a mixture of chromosomes and alleles from both parental complexes. It has lost the lethal allele from *velans* and is essentially homozygous for all loci with the exception of *ster* and *br*. The genetic stock *Oe.* ^{h}*blandina·stringens* with *fr* has been created by backcrossing of the F_1 *Oe. blandina* × *strigosa*, ^{h}*blandina·stringens*, with pollen of *Oe. strigosa* and subsequent crossing of sister plants *fr*/*fr* × $+^{fr}$/*fr* for many generations. The stocks of *Oe.* ^{h}*hookeri·stringens* carrying *fr* were obtained either from the F_2 generation of the hybrid *Oe. Hookeri* × *strigosa* or by backcrossing of the hybrid with pollen of *Oe. strigosa* and subsequent crossing of sister plants *fr*/*fr* × *fr*/$+^{fr}$, which gives segregating families. The same method has been followed in constructing the stocks *Oe. flavens·stringens fr*/$+^{fr}$.

The gametes of these heterozygous stocks contain not the pure original Renner complexes but a haploid mixture of the components of each. These gene complexes are designated, according to the *Oenothera* nomenclature, as haplo- (^{h}) with the added names of the two Renner complexes that are involved, e.g. ^{h}(*hookeri·stringens*).

The genetic basis of the experiment

The genetic Renner complexes used in the experiment are characterized not only by their chromosome formulae and their allelic constitution, but also by their compatibility with the various plastomes of *Oenothera* and the influence of their alleles on the development of the haploid generation and on the competition between meiospores and gametophytes in both sexes (Harte 1969a, b; Sniezko and Harte 1984). These latter factors determine which genotypes will be represented in the active gametes.

The Renner complexes *albicans* of the test stocks, derived form *Oe. biennis* L., and *deprimens* of *Oe. strigosa* de Vries are active only in the egg cells. The Renner complexes *undans* in the test stocks and *stringens* of *Oe. strigosa* are only active in the pollen. The Renner complexes ^{h}*hookeri*, ^{h}*blandina*, *velans* and *flavens* are active in both sexes. In the hybrids, competition between the megaspores in the ovule and between pollen tubes is influenced by the genetic constitution of the gametes. These selection processes determine which genotypes will be represented in the population of fertilizing gametes.

In order to combine homozygosity for each of the male-sterility alleles with the various plastomes, test stocks with a homogeneous egg cell population, containing *albicans* and the plastids with the particular plastome, must be used as the female parent. The mst alleles are introduced by the male parent. This can be either a constant hybrid with the mst allele in its pollen complex (*Oe. strigosa* with *stringens fr*) or a segregating hybrid, e.g. *Oe.* ^{h}*hookeri·velans ster*/$+$ *ster*, or a hybrid with *stringens* from *Oe. strigosa*.

Hybrids of the genetic constitution *Oe. albicans·*^{h}(*hookeri·velans*), which have as chromosome configuration a ring of 14 chromosomes, are obtained by crossing a test stock with a particular plastome as female parent with plants containing the *ster* allele. In these hybrids only pollen grains containing ^{h}(*hookeri·velans*) are active. Nearly all egg cells contain ^{h}(*hookeri·velans*); the frequency of egg cells with *albicans* is very low. Self-pollination of such a hybrid results in the formation of an F_2 family of *Oe.* (^{h}*hookeri·velans*). The plants are homozygous for all of the alleles contributed by the pollen grain that gave rise to the F_1 plant that was the parent of this particular F_2 family. In addition, only a few plants of a constitution similar to that of the F_1, *Oe. albicans·*^{h}(*hookeri·velans*), are observed. In these, the genetic segregation of single gene loci only occurs by crossingover.

The hybrids *Oe.* (*albicans·undans* × *strigosa*) *albicans·stringens* (chromosome configuration: a ring of 12 chromosomes plus a bivalent) have only *albicans* egg cells and *stringens* pollen, irrespective of the plastome constitution, and are constant hybrids. In the F_2 families obtained by self pollination all of the plants are identical with the F_1, with the exception of a few genes segregating as a result of crossingover, especially *fr* and *s* (sulfurea flower colour).

To increase the probability of obtaining homozygous *fr*/*fr* plants in the F_2 families, the allele *fr* was also introduced by pollen from hybrids with *Oe. strigosa*. Due to the small chromosome rings formed in the hybrids *Oe. flavens·stringens* (4 2 2 2 2 2), ^{h}*hookeri·stringens* (4 4 2 2 2) and ^{h}*blandina·stringens*, the Renner complexes in these hybrids break down during meiosis. The gametes contain a mixture of chromosomes and genes from both complexes. This makes it impossible to predict the genetic contitution of a particular gamete and thus of an individual F_1 plant arising from pollination with such mixed Renner complexes. However, the mixed gene complexes will have a 0.5 probability of containing the *fr* allele from *stringens* together with combinations of alleles at other loci derived from the two partners that will differ in each gamete.

Because *stringens*, in contrast to the other Renner complexes with which it is combined in the hybrids used for transfer of *fr*, is not active in the embryo sacs, it can be expected that the mixed gene complexes will also contain a mixture of the alleles that influence embryo sac competition. One can expect to find gene complexes which will transmit the *fr* allele through the egg cells in hybrids with *albicans*, but neither the frequency of this event nor the behaviour in a particular case is predictable. The same holds for the genes that are responsible for the incompatibility of some genomes with particular plastomes.

The phenotype "male sterility", which is determined by homozygosity for recessive alleles of both loci, is obviously expressed in combination with the plastome of the species in which they were first observed. This is the plastome I of *Oe. Hookeri* de Vries for *ster* and of *Oe. Hookeri* de Vries and *Oe. suaveolens* Desf. for *fr*. Our study is concerned with the question of cooperation with the other plastomes.

Results

Male-sterile type *sterilis*, *ster*

The test stocks with the different plastomes were crossed with pollen of the heterozygote *Oe.* ^{h}*hookeri·velans ster*/$+^{ster}$. The F_1 plants and the F_2 progeny have the plastome of the female parent of the F_1 hybrid. The male parent is heterozygous, so only one-half of the F_1 plants will have the required constitution *ster*/$+^{ster}$. Self pollination of the F_1 hybrids gives rise to F_2 families, each derived from a single F_1 plant, of *Oe.* (^{h}*hookeri·velans*). All of the plants of any one family will have either normal fertile anthers or male-sterile anthers of the *sterilis* type. Both kinds of F_2 families are expected to occur with nearly equal frequencies. The phenotypes of male-sterile plants with different plastomes in a common genetic background can be compared directly. The genetic formula is presented in Table 1.

The design of the experiment to obtain homozygotes *ster*/*ster* in different plastomes is simple. The aim is to achieve a high probability of observing the required genotypes. The size of the F_1 generation must be large enough to provide a sufficient number of plants that are heterozygous *Oe. albicans* $+^{ster}$ ^{h}(*hookeri·velans*) *ster*. The F_2 families obtained by individual self pollinations can be rather small because the sister plants are genetically identical. Approximately 50 families of 20 plants each were raised for every plastome test. With this number one can expect to find enough families of the genotype *sterilis*, if this allele is expressed with the

Table 1 Scheme to obtain homozygous *ster/ster* in a given plastome

Parents	♀ *Oe. albicans·undans* with required plastome × ♂ *Oe.* ^{h}*hookeri·velans ster/*$+^{ster}$
Gametes	♀ *albicans* $+^{ster}$ × ♂ ^{h}*(hookeri·velans)* $+^{ster}$ or *ster*
F_1	*albicans·(*^{h} *hookeri·velans)* $+^{ster}/+^{ster}$ or $+^{ster}$*/ster*
F_2 from F_1	$+^{ster}/+^{ster}$:
	^{h}*hookeri·velans* $+^{ster}/+^{ster}$
and	*albicans·*^{h}*(*^{h}*hookeri·velans)* $+^{ster}/+^{ster}$
Phenotype	all plants with fertile pollen
or F_2 from F_1	$+^{ster}$*/ster:*
	^{h}*hookeri·velans ster/ster*
and	*albicans·*^{h}*(*^{h} *hookeri·velans) ster/ster* due to crossingover
Phenotype	male-sterile, type *sterilis*
and	*albicans·*^{h}*(*^{h} *hookeri·velans)* $+^{ster}$*/ster*
Phenotype	fertile pollen

plastome, and also to obtain some *Oe. albicans·*^{h}*(*^{h}*hookeri·velans)* individuals in families with the allele *ster*, which can be used to obtain homozygous *ster/ster* plants in the next generation.

For the plastomes I, II and IV this approach succeeded. Families of male-sterile plants were observed in sufficient frequency.

The Renner complex ^{h}*(hookeri·velans)* is, like its ancestors ^{h}*hookeri* and *velans*, homozygous incompatible with plastome III. The seedlings develop a few small yellow leaves and then die. Homozygosity for *ster/ster* in plastome III can only be realized when the allele *ster* is transferred by crossingover from ^{h} *hookeri* to *albicans*. The exact frequency of crossingover for this locus is unknown, but in any case this event is rare. Egg cells with *albicans* appear at a low frequency of less than 5%. Furtermore, only one-half of the F_1 plants are expected to contain the *ster* allele. As a consequence of these uncertainties, it is impossible to predict the number of progeny (number of families and plants per family) that it will be necessary to score to detect *Oe. albicans·*^{h}*(hookeri·velans) ster/ster* homozygotes in combination with plastome III. The decision was made to take the chance to find such a plant from among the same number of progeny as used for the other tests. One plant of the desired genotype was observed, which demonstrates that the phenotype *sterilis* can be expressed with plastome III.

In conclusion, the observation of mst plants of the genotype *ster/ster* in combination with all plastomes, all of which have been tested in our experiments, proves that the expression of *sterilis* is independent of cooperation with a particular plastome.

Male sterile *fr*

The phenotype of the homozygous *fr/fr* has been described from segregating progenies of the hybrids *Oe. flavens·stringens* and *Oe.* ^{h}*hookeri·stringens*. This demonstrates that its expression is possible with the plastomes I from *Oe. Hookeri* de Vries and II from *Oe. suaveolens* Desf.

In the design of experiments to combine homozygosity for *fr/fr* with different plastomes, difficulties which arise due to genetic peculiarities of *Oenothera* have to be considered. In the F_1 hybrids *Oe. albicans·undans* × *Oe. strigosa, albicans·stringens*, the chromosome configuration is a ring of 12 chromosomes plus a bivalent, irrespective of the plastome. The Renner complex *stringens*, the source of the *fr* allele, is strictly a pollen complex. This makes the hybrid *Oe. albicans·stringens* a constant hybrid, in whose progeny homozygous *fr/fr* plants can only appear following the crossingover of *fr* from *stringens* to *albicans* in a megaspore mother cell. This occurs only with a very low frequency (Renner 1942). To assure a high probability of finding such plants, extremely large F_2 families of several hundred plants each would be required. Male-sterile plants were observed in the offspring of the hybrids with plastome I and II, but were not found in combination with plastomes III and IV. This result, however, does not allow any conclusions to be drawn about the cooperation of *fr* with these plastomes, because the numbers of the progenies could have been too small in relation to crossingover frequency.

In order to avoid this difficulty, *fr* was introduced into hybrids with different plastomes from segregating hybrids with *Oe. strigosa*. The Renner complexes ^{h}*hookeri*, ^{h}*blandina* and *flavens*, which are favoured over *albicans* in competition between megaspores in the ovule (Sniezko and Harte 1986), were chosen for this purpose. All form small chromosome rings with *stringens* (Harte 1948). As in the experiments with *ster*, the probability of the pollen transferring the *fr* allele is only $P = 0.5$.

It can be expected that the mixtures of chromosomes from two Renner complexes that are generated during meiosis in hybrids with low chromosome catenation will also have a mixture of the alleles of the two Renner complexes that influence megaspore competition. Segregation in the F_2 families will give rise to two types of plants. One type is formed from *albicans* egg cells and resembles the F_1; the other type will be different in each F_2 family, but the plants are homozygotes and correspond to the mixture of *stringens* and the Renner com-

plex with which it was combined in the pollen parent of the F_1. The ratio of these two types will differ in every F_2 family. The mixing of the alleles from the two Renner complexes will also involve the gene loci that influence competition in pollen as well as in the ovules. The behaviour of a mixed gene complex carrying *fr* in the plastome hybrids can be expected to lie between that of the two Renner complexes from which it has arisen. Consequently, the segregation pattern in the F_2 families of the individual F_1 plants is unpredictable.

In some cases the plants in the F_2 can resemble a type from the offspring of *Oe. flavens·stringens*, but only when the mixed gene complex ^{h}(*flavens·stringens*) does not carry the lethal of *flavens*. These families can be either *fr/fr* or $+fr/+fr$.

The design of the experiment for investigating the expression of male sterility of the type *fr/fr* has to differ from that for investigating *sterilis*. This design is presented in Table 2. The factors to be considered are: (1) the expected variability in the frequency of the heterozygous F_1 plants, which results from pollen-tube competition in the male parent; (2) the variabililty in the results of competition between megaspores in the ovules; and (3) the low frequency of crossingover of the *fr* locus. For these reasons, the number of F_2 families and the number of plants in each family has to be larger in the investigations with *fr* than in those with *ster*. However, the required population size cannot be calculated in advance. The only option is to start with several hybrids of the constitution $fr/+^{fr}$ in which the female parents differ in their competitive ability in the megaspore tetrad relative to *albicans* and to consider the linkage relations of the *fr* locus. The available hybrids, which have already been mentioned above, have different advantages as pollen parents. The approach chosen was to use three hybrids as pollen parents for the crosses with the test stocks, then raise a reasonable number of F_1 plants and generate an F_2 family from each one by self pollination, growing the maximum number of plants that could be handled in the available area. We expected this experimental design would give us a reasonable chance to find the desired *fr/fr* genotypes.

The competition between the pollen tubes from the hybrid *Oe.* ^{h}*hookeri*·(chromosome configuration 44222) favours the *hookeri* chromosomes of the ring 1·2·3·4 that carry the allele $+^{fr}$. Consequently only a small fraction of the F_1 plants obtained from crosses involving the test stocks *Oe. albicans·undâns* as female and *Oe.* ^{h}*hookeri·stringens* as pollen parent will carry the *stringens* chromosome arm with the *fr* allele. Therefore, the number of F_1 plants that must be self pollinated in order to find at least one family with *fr/fr* homozygotes will be very large. Furthermore, the chance of observing the male-sterile plants depends on the particular combination of alleles from both Renner complexes that influence the competition behaviour in the ovules; those from ^{h}*hookeri* will enhance competitive ability over *albicans*, those from *stringens* will inhibit it.

The results of our observations on the progenies of the hybrids *Oe. albicans·*^{h}(*hookeri·stringens*) in combination with the different plastomes are in agreement with these considerations. In some families, all plants were similar to *Oe. Hookeri*, with fertile pollen. In other families, the plants resembled the F_1, or a mixture of both types was observed, which indicates recombination from both Renner complexes of alleles involved in megaspore competition in the ovules. In the progeny of the hybrid with plastome III, the incompatibility of ^{h}*hookeri* with this plastome led to families of either lethal yellow seedlings, when the gene complex introduced by the pollen contained the incompatibility factors from ^{h}*hookeri*, or *albata* plants similar to the F_1. In one family

Table 2 Scheme to obtain *fr/fr* homozygotes in combination with different plastomes

Parents	♀ *Oe. albicans·undans* with different plastomes	♂ *Oe. strigosa*
Gametes	♀ *albicans* $+^{fr}$	♂ *stringens fr*
F_1	*albicans* $+^{fr}$*·stringens fr*	
F_2	*albicans* $+^{fr}$*·stringens fr* and *albicans fr·stringens fr*	
	fertile pollen	male sterile
		only by crossingover

Scheme for hybrids of *Oe. strigosa*:

Parents	♀ *Oe. albicans·undans* with different plastomes	♂ *Oe.* ^{h}*hookeri* $+^{fr}$*·stringens fr*
Gametes	♀ *albicans* $+^{fr}$	♂ ^{h}(*hookeri·stringens*) $+^{fr}$ or *fr*
F_1	*albicans·*^{h}(*hookeri·stringens* $+^{fr}/+^{fr}$ or $+^{fr}/fr$	
F_2	from *albicans·*^{h}(*hookeri·stringens*) $+^{fr}/+^{fr}$ only plants with fertile pollen	
	from *albicans·*^{h}(*hookeri·stringens*) $+^{fr}/fr$ albata plants with fertile pollen and homozygotic mail-sterile plants in variable proportions	

The same scheme describes the experiments with pollen of *Oe.* (^{h}*blandina·stringens*) $+^{fr}/fr$.

Parents	♀ *Oe. albicans·undans* with different plastomes	♂ *Oe. flavens·stringens* $+^{fr}/fr$
Gametes	♀ *albicans* $+^{fr}$	♂ ^{h}(*flavens·stringens*) $+^{fr}$ or *fr*
F_1	*albicans·*^{h}(*flavens·stringens*) either $+^{fr}/+^{fr}$ or $+^{fr}/fr$.	
F_2	similar to *albicans·flavens* or *albicans·stringens*	
	from $+^{fr}/+^{fr}$ all plants with fertile pollen,	
	from $+^{fr}/fr$ nearly all plants with fertile pollen, rare male-sterile *fr/fr* due to crossing over of *fr* to *albicans*	

with plastome II, a homozygote *albata fr/fr* appeared, the result of a crossingover of *fr* from the arm of *stringens* chromosome no. 3 to *albicans*.

The experiments with the hybrids *Oe. albicans·undans* × *Oe.*^{h}*blandina·stringens* were more successful. In the mutation process of *velans* to ^{h}*blandina* the sporophytic lethal of *velans* has been lost. Many F_2 families consisted of plants similar to *Oe. blandina* or a mixture of these types and *albata* in variable proportions. Families of homozygous male-sterile plants have been observed in combination with plastome I. Male-sterile plants of the genotype *Oe. albicans·*^{h}*(*^{h}*blandina·stringens)fr/fr* have been observed in the experiments with the plastomes I and II. From the hybrid *Oe. albicans·*^{h}*(*^{h}*blandina·stringens*) with plastome III, one F_2 family was found with homozygous male-sterile *fr/fr* plants, which resembled *Oe. blandina* and some sister plants with fertile pollen similar to those of the F_1. With plastome IV an F_2 family arose in which the majority of plants was homozygous for *fr/fr* and also for another gene ("Knirps", *kr/kr*, very small plants). Both of these recessive alleles were contributed by the Renner complex *stringens*.

These observations demonstrate that in *Oe.* ^{h}*blandina·stringens* competition between pollen tubes with *fr* or $+^{fr}$ is less severe than in pollen derived from hybrids with the Renner complex ^{h}*hookeri*. The frequency of heterozygous *fr*/$+^{fr}$ F_1 plants was sufficient to give rise to several F_2 families with male-sterile offspring. The viability of these plants with plastome III indicates the loss of the incompatibility factors from *velans*.

In *Oe. suaveolens* × *strigosa, flavens* $+^{fr}$*·stringens fr*, the chromosome configuration is 4 2 2 2 2 2 and *fr* is located in a bivalent that enables free segregation. As the bivalent carrying the *fr* locus is not involved in competition between spores or gametophytes, the *fr* allele will be transferred by 50% of the pollen grains into the hybrids with the different plastome stocks. The hybrids *Oe. albicans·*^{h}*(flavens·stringens)* have a large chromosome ring (chromosome configuration: 12 2), irrespective of the combination of *flavens* and *stringens* chromosomes in the individual F_1 plant, and they are similar to *Oe. suaveolens* (*albicans·flavens*) or to the F_1 *Oe. albicans·stringens*. Both types are constant hybrids due to the sporophytic lethal of *flavens* and the genes that restrict *stringens* to be active only in the pollen. The complications arise from the sporophytic lethal in the ring chromosomes of *flavens*, which will elimilate the homozygous embryos, and the incompatibility with plastome III, which eliminates the remaining homozygotes as yellow seedlings. Male-sterile F_2 families with *fr/fr* homozygotes should be observable. However, mixed gene complexes which combine the *fr* allele with the competion behaviour of *flavens* but without the lethal and the incompatibility factors, were not observed. Homozygous male-sterile plants can only arise in the F_2 as result of crossingover in the arm of chromosome no. 3 thereby transferring *fr* to *albicans*.

In summary, homozygotes from all of the hybrids with *fr* from *stringens fr/fr* can be expected only with such a low frequency that they are unlikely to be found in progenies of limited numbers. However, the procedure followed in the experiments was successful: male-sterile phenotypes were in fact observed in combination with all four plastomes. The results demonstrate that the male-sterile phenotype associated with homozygosity at the *fr* locus can be expressed in combination with all of the plastomes tested in this experiment.

Discussion

The main purpose of these experiments was to test whether nuclear gene-dependent male sterility in *Oenothera* is influenced by the plastome. We concluded that both of the mst genes are expressed independently of the plastome with which they are combined, in spite of the fact that disturbances in plastid metabolism are involved in the process of pollen degeneration, at least in *sterilis*. The question that remains is whether only the result of the degeneration of the microspores and the contents of the anthers is similar with the different plastomes or whether further investigations will reveal differences in the details of the morphological or physiological processes in the anthers (Noher de Halac et al., in preparation).

In addition, some of the observations made during the experiments provide information on competition between megaspores or embryo sacs in the ovules. Earlier investigations on the genetics of competition in the ovule led to the identification of several genes influencing this phenomenon (Harte 1953, 1969). Observations on the development of polarity in the ovule, which makes the competition between megaspores and young embryo sacs possible (Noher de Halac and Harte 1975), revealed the presence of all least two loci that influence this process (Sniezko and Harte 1984, 1985, 1986). The result of genetic segregation in the progeny of a hybrid with *stringens* (not active in egg cells) and ^{h}*hookeri* or any other Renner complex which is genetically favoured over *albicans* (only active in the egg cells) cannot be predicted for an individual gamete. It should be possible to find a recombination of alleles from *stringens* and the other Renner complexes like ^{h}*hookeri*, that would influence competition in the ovule. The segreation patterns observed in the F_2 families varied from complete preference for *albicans* to preferences for the mixed complex, similar to the behaviour of ^{h}*hookeri*; there were also families with approximately 50% of both gene complexes in the female gametes. In all families, however, the homozygous plants were similar to *Oe. hookeri* or *Oe. blandina*, and not to the hybrids of these species with *stringens*. This demonstrates that several gene loci in different chromosomes are involved in determining the outcome of competition in the ovule. These can be recombined in the segregating hybrids with low chromosome catenation. The result is that a new competi-

tion behaviour can arise that enables several parts of the original Renner complex *stringens* to pass through the egg cells.

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