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Megaspore competition in F_1 and F_2 hybrids between *Oenothera hookeri* and *Oe. suaveolens*

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Abstract

Megasporogenesis and development of the embryo sac were investigated in F_1 and F_2 hybrids from crosses of *Oe. hookeri* and *Oe. suaveolens*. All hybrids form heteropolar and homopolar magaspore tetrads; the embryo sac, however, usually develops from the micropylar megaspore. Its development may occur immediately after degeneration of three other megaspores or after a period of competition between both apical megaspores. They develop simultaneously for a relatively short time, after which the growth of the chalazal megaspore is inhibited, although the latter does not degenerate. The micropylar megaspore as a rule develops without disturbances into the embryo sac, but in some ovules it is formed from the chalazal megaspore or double ones arise from both apical megaspores of the tetrad. The frequency of the micropylar embryo sac formation seems to be dependent above all on the hybrid plant genome and not on the haploid genome of the megaspore.

Key words: megaspore competition, hybrid Oenothera megasporogenesis

INTRODUCTION

In the ovules of most *Onagraceae* species a heteropolar megaspore tetrad forms a functional megaspore on the micropylar pole (Rodkiewicz and Śnieżko 1978). In such tetrad the presence of callose in the wall surrounding the meiotic cells is considered to be important (Kuran 1972). Callose completely surrounds the young meiocyte. At the beginning of prophase I it disappears from the micropylar apex of the cell, and after meiosis it is at this apex of the tetrad where a functioning megaspore is formed and develops into the embryo sac (Rodkiewicz 1970). Callose is believed to make difficult penetration of large molecules through the

cell wall, therefore, it may affect the development of megaspores on the chalazal side of the tetrad (Rodkiewicz 1973).

The presence of callose in the wall surrounding the meiocyte and later the tetrad is not indispensable polarising factor since in some species of the genus *Oenothera*, in spite of the absence of callose, polarization of the tetrad takes place and the embryo sac always originates from the micropylar megaspore as in *Oe. hookeri* (Noher de Halac and Harte 1975) and *Oe. silesiaca* (Rodkiewicz and Śnieżko 1978). In some ovules of *Oe. hookeri* the delay of the meiotic division II in one cell of a diad indicates the heteropolarity but in many ovules the tetrads are homopolar, very similar to those of *Oe. biennis* and *Oe. muricata* (Renner 1921, Rodkiewicz et al. 1971) where 50% of embryo sacs develop from the micropylar megaspore, and the remaining 50% from the chalazal one.

Oe. suaveolens is on intermediate species between *Oe. hookeri* (100% micropylar embryo sacs) and *Oe. biennis* (50%). In the ovules of *Oe. suaveolens* heteropolar and homopolar tetrads are formed but 70% of the embryo sacs develop on the micropylar side.

In the present study the development of embryo sac from megaspores in homo- and heteropolar tetrads was followed in generations I and II of *Oe. hookeri* × *Oe. suaveolens* hybrids.

MATERIALS and METHODS

Oenothera hookeri, genotype ^{h}*hookeri* × ^{h}*hookeri*, were crossed reciprocally with *Oe. suaveolens*, genotype *flavens* × *albicans*. Three F_1 hybrids were obtained: *Oe. hookeri* × *Oe. suaveolens*, genotype ^{h} *hookeri* × *flavens*, *Oe. suaveolens* × *Oe. hookeri*, genotype *flavens* × ^{h} *hookeri*, and albicans × × ^{h} *hookeri*. The latter were not analysed.

Table 1

Phenotype of F_2 hybrids of *Oenothera hookeri* x *suaveolens*

Hybrids No	Bud	Flower	Stem	Leaves
I	long strips with red anthocyanin	small, dark yellow	reddish in upper part	nervation white, weakly pilose
II	completely green	small, light yellow	green	broader than in No I, strongly pilose
III	broad stripes with red anthocyanin	large, dark yellow	deep red at top and base	narrow, weakly pilose

After selfpollination of the hybrid *Oe. hookeri* × *Oe. suaveolens* plants of diverse appearance occurred. For further investigations three hybrid plants differing distinitly in phenotype were chosen (Table 1). The ovaries from the fifth to the seventh bud over the first opened flower contained ovules in the megasporogenesis stage. These ovaries were fixed in an alcohol acetic acid mixture (3:1). The ovules were hydrolysed in 1N HCl and, after washing, stained with aniline blue for callose fluorescence or subjected to PAS reaction for polysaccharides. Squash preparations were examined in a fluorescence microscope and light microscope. Ovaries in various stages of development were embedded in paraffin, 5-7-micrometre sections were stained with PAS.

RESULTS

In the examined hybrids megasporogenesis had a similar course. A meiocyte in the ovule elongated, its plastids grouped at micropylar and chalazal poles and the nucleus lay in the middle (Fig. 2). After staining with aniline blue there was no meiocyte wall fluorescence, except some which occurred very rarely in fragments of the wall (Fig. 6).

After the first meiotic division a dyad of two similar cells (Fig. 3) separated by a callose-containing wall was formed (Fig. 4). In the young tetrads the walls formed after division II were of unequal thickness, and the wall formed later showed weaker fluorescence (Fig. 8). Most of the mature tetrads had transverse walls of similar thickness (Figs. 5 and 9).

Most tetrads found on the sections of older ovules were considered homopolar, because their transverse walls were of equal thickness, somewhat bent towards the tetrad centre and both aplical megaspores contained plastids with starch. Heteropolar tetrads were less numerous, the wall separating the chalazal megaspore was thinner than two other transversal walls of the tetrad (Fig. 10) and it was often bent towards the chalaza instead of the centre like in the homopolar tetrad.

In heteropolar tetrads degeneration started most frequntly on the chalazal pole (Fig. 11); sometimes two megaspores on the chalazal side degenerated simultaneously. The central micropylar megaspore also degenerated relatively early (Fig. 12). At the same time the apical micropylar megaspore became vacuolised, the plastids previously aggregated at its apex dispersed throughout the cytoplasm, and the nucleus shifted towards the micropyle showing a distinct nucleolus. This megaspore grew towards the micropyle of the ovule. A trace of the degenerated megaspores could be seen in the ovules with embryo sac more advanced in development.

In homopolar tetrads one of the middle megaspores (Fig. 13) was the first to degenerate (Fig. 14). At this stage both apical megaspores seemed

equally viable. They had a distinct nucleus with a large nucleolus, and the plastids containing strach in a greater quantity than during megasporogenesis. A vacuole was formed on the chalazal side of each apical megaspore and the nucleus shifted in a micropylar direction. The growing chalazal megaspore filled the place left by degenerating central megaspores. Their trace soon became obliterated so that both developing megaspores were separated by the relatively thin walls (Fig. 1).

In numerous ovules growth inhibition of the chalazal megaspore occurred relatively early in development. The megaspore preserved the ability of starch accumulation, but it did not grow or grew very slowly in comparison with the micropylar megaspore (Fig. 15). The micropylar embryo sac reached its fulll development with the egg apparatus differentiated, whereas the chalazal embryo sac was sometimes still binucleate.

In several ovules the embryo sac developed from a chalazal megaspore and then three megaspores on the micropylar side degenerated. In six ovules of F_2 hybrids both apical megaspores developed into embryo sacs. Other than micropylar sac was formed rather rarely (Table 2).

DISCUSSION

In ovules of the F_1 hybrids two types of tetrads are formed: homopolar and less frequently, heteropolar ones (Noher de Halac and Harte 1975, Śnieżko and Harte 1984). Similar tetrads were found in ovules of F_2 hybrids. It is possible that some young heteropolar tetrads with visible delay in the second meiotic division on the chalazal side became homopolar

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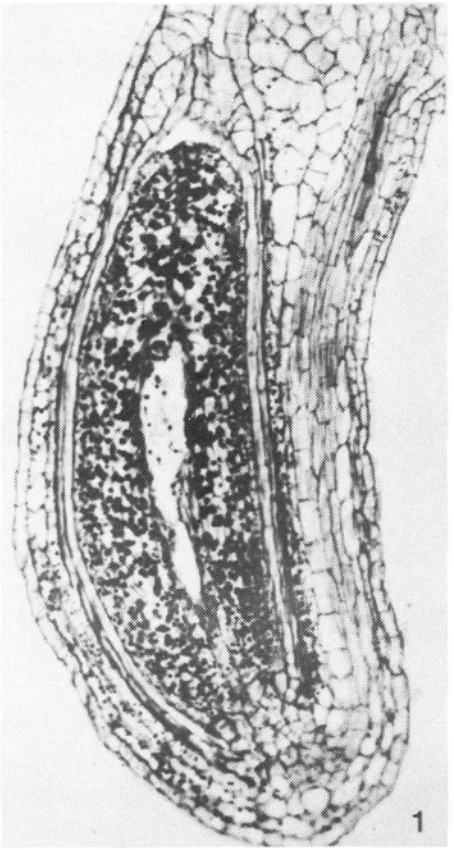
The photographs show meiotic cells of F_1 hybrids from crosses between *Oe. hookeri* and *Oe. suaveolens*. The same stages were seen in all ovules of the inspected hybrids

Fig. 1. Ovule with micropylar embryo sac more advanced in development than the chalazal one, x 350. Fig. 2. Meiocyte: at poles darkly stained starch grains. x 800. Fig. 3. Dyad. x 800. Fig. 4. Tetrad with thinner wall between chalazal megaspores. x 800. Fig. 5. Homopolar tetrad: transverse wall bent towards centre. x 800. Fig. 6. Callose fluorescence in meiocyte wall. x 800. Fig. 7. Callose in transverse wall of dyad. x 800. Fig. 8. Heteropolar tetrad, callose fluorescence weaker on chalazal side. x 800. Fig. 9. Homopolar tetrad: uniform fluorescence of walls. x 800. Fig. 10. Heteropolar tetrad in nucellus tissue, starch visible as black grains. x 500. Fig. 11. Degeneration of chalazal megaspore. x 500. Fig. 12. Developing micropylar megaspore and three degenerated chalazal ones. x 500. Fig. 13. Tetrad with degenerated central megaspore. x 500. Fig. 14. Tetrad after degeneration of both central megaspores. x 500. Fig. 15. Micropylar megaspore grows towards micropyle, chalazal megaspore contains strach but does not grow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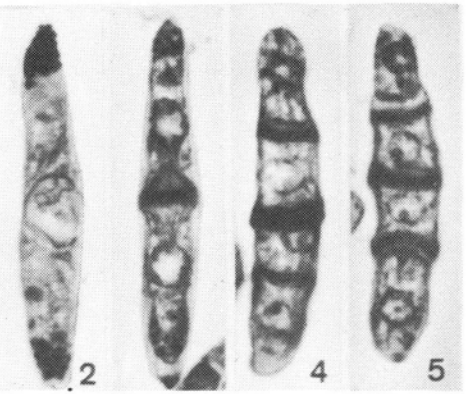
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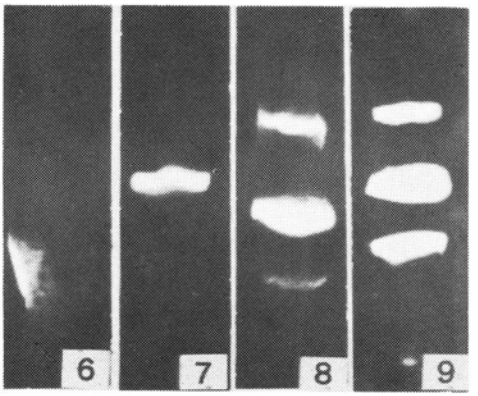
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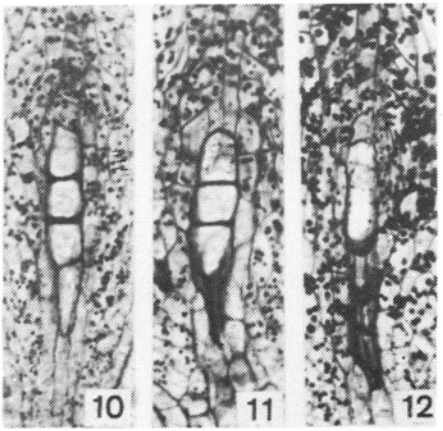
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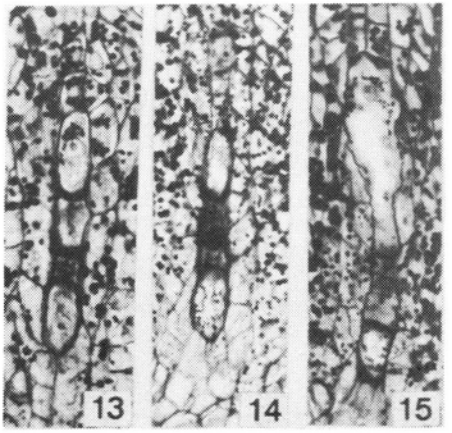
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as they mature. Although the hybrid plants mostly formed homopolar megaspore tetrads, the embryo sac usually developed on the micropylar side. Polarization of the tetrads occurred during degeneration of central megaspores and was manifested by inhibition of chalazal megaspore growth.

Two pathways of development of the finally heteropolar tetrad with functional megaspore at the micropylar apex can be indicated (Fig. 16).

Development of the tetrad according to pattern A is the same as in *Onagraceae* forming heteropolar tetrads with a micropylar functional megaspore

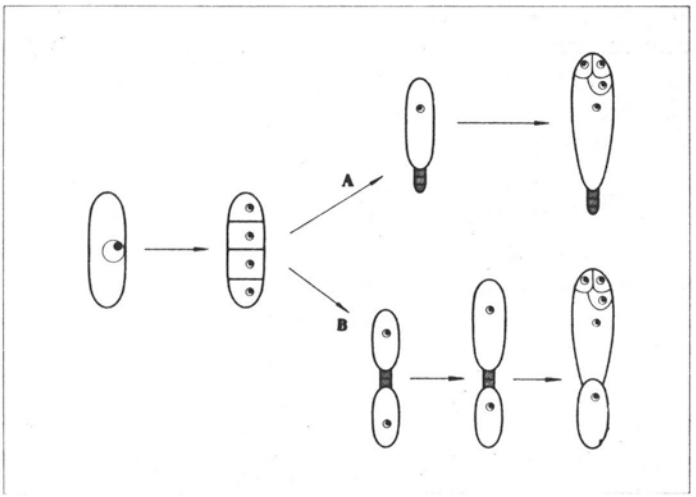

Fig. 16. Development of embryo sac in F_1 and F_2 hybrids of *Oenothera hookeri* and *Oe. suaveolens*

and callose wall surrounding the chalazal megaspores (Rodkiewcz and Śnieżko 1978). In this pattern only the micropylar megaspore gives rise to the embryo sac, whereas the remaining three degenerate rather quickly. In a certain number of hybrid ovules, in spite of the lack of callose in the lateral walls polarization of the cells takes place already during megasporogenesis.

Pattern B shows that tetrads become polarized in a later stage, after degeneration of the central megaspores and a short period of simultaneous growth of both apical megaspores. The micropylar megaspore prevails over the other one, but this is not associated with degeneration of the chalazal megaspore. It can develop very slowly even when there is already a mature embryo sac which originated from the micropylar megaspore.

The development of the embryo sac in most ovules of two F_2 plants (No I and II) took place according to pattern B.

Renner (1917, 1921) believes that the viability and development of the

megaspore in the tetrad is determined by its haploid chromosome complex which plays a decisive role in the megaspore competition.

The hybrid plant No III by its appearance and the constant micropylar position of the embryo sac resembles *Oe. hookeri*, one of the parental species (Noher de Halac and Harte 1975, 1977). It supposely has the genotype ^{h}*hookeri* × ^{h}*hookeri*, therefore both aplical megaspores have a ^{h}*hookeri* complex. Polarization occuring in tetrads of F_2 hybrids cannot therefore be explained by the prevalence of one of the genetic complexes in the micropylar megaspore.

Hybrids No I and No II are not similar to either of parental species. The embryological processes are here also somewhat different. Probably in both hybrids chromosome complexes *flavens* and ^{h}*hookeri* are present. According to the theory of Renner (1921), such complexes are randomly transmitted to the poles during meiotic division I, thus, in hybrids the complex *flavens* may be found with equal frequency in the micropylar and chalazal megaspores. In many tetrads both the aplical megaspores begin to develop simultaneously. The chalazal megaspore persistD much langer than the central ones; in spite of growth inhibition, sometimes even both aplical megaspores develop into embryo sacs. It seems, therefore,

Table 2

Types of tetrads and embryo sacs of F_1 and F_2 hydrids from crosses between *Oenothera hookeri* and *Oe. suaveolens*

Hybrids	Tetrads		Embryo sacs	
	hetero-polar	homo-polar	micro-pylar	others
F_1				
Oe. suaveolens x *Oe. hookeri*	69	72	18	–
Oe. hookeri x *Oe. suaveolens*	47	71	26	4
F_2				
Oe. hookeri x *Oe. suaveolens*				
Plant No I	6	112	34	8
Plant No II	36	102	15	9
Plant No III	20	51	53	–

that the complexes *flavens* and ^{h}*hookeri* are equally viable. Notwithstanding whether the *flavens* or ^{h}*hookeri* complex is present in the micropylar megaspore, the latter develops without obstacle, whereas the chalazal megaspore, although potentially viable is inhibited in growth independently of its chromosome complex. It cannot be decided which complex is more favourable to development, and which accelerates degeneration. Investigations of a large number of F_2 hybrids may supply more information.

On the basis of these observations the difference in tetrad polarisation between F_1 and F_2 hybrids and between the particular F_2 hybrids may be indicated (Table 2).

There is a strong tendency in the F_2 to homopolar tetrad formation. In the plant No I only six tetrads were heteropolar of the inspected 118, and in the plant No II 36 of 138. Hybrid plants No I and No II differ in the frequency of micropylar embryo sac formation: plant No I forms about 80% such embryo sacs, plant No II about 70%, Hybrid plant No III develops only micropylar embryo sacs.

It seems that in the ovules of F_1 and F_2 *Oenothera* hybrids the conditions on the micropylar side are more favourable for the embryo sac development. These conditions may be complex; probably the tetrad is polarized under the influence not of a single factor, but several interacting ones, dependent on both the genetic complexes and cytoplasmic properties of the ovular tissues and meiocyte. Similar conclusions can be drawn from analysis of tetrad polarization in the F_1 *Oenothera* hybrids (Śnieżko and Harte 1984).

The suggestion that tetrad polarization depends on the diploid plant is confirmed by descriptions of embryo sac development in homozygotic *Oenothera* species. In the *Oe. hookeri* tetrad the micropylar megaspore develops faster than the chalazal one, although in both the same viable complex ^{h}*hookeri* is present (Noher de Halac and Harte 1975). In *Oe. purpurata* the *rubens* complex is present in both apical megaspores of the tetrad. The apical megaspores of this species compete for a long time; embryo sacs are formed either on the chalazal apex or on both apices of the tetrad (Rudloff 1929).

In *Oenothera hookeri* the "favourable micropylar position" factor seems to act stronger than in *Oe. purpurata*. The *Oenothera* F_2 hybrids differ from one another in the action of this factor. Therefore, polarization of the tetrad cannot be explained solely by the difference in the action of the haploid genetic complexes in the megaspores.

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Degeneracja megaspor w zalążkach I i II pokolenia mieszańców między Oenothera hookeri i Oe. suaveolens

Streszczenie

Prześledzono megasporogenezę i rozwój woreczka zalążkowego u mieszaniców F_1 i F_2 z krzyżowań *Oe. hookeri* i *Oe. suaveolens*. Wszystkie mieszańce tworzą heteropolarne i homopolarne tetrady megaspor, jednak woreczek zalążkowy najczęściej rozwija się z mikropylarnej megaspory. Rozwój może następować bezpośrednio po degeneracji trzech chalazalnych megaspor lub po okresie współzawodnictwa obu wierzchołkowych megaspor. Megaspory wierzchołkowe stosunkowo krótko rozwijają się równocześnie, po czym następuje zahamowanie wzrostu chalazalnej megaspory, która mimo to nie degeneruje. Magaspora mikropylarna rozwija się bez zakłóceń w woreczek zalążkowy. W niektórych zalążkach tworzą się woreczki zalążkowe z chalazalnej megaspory lub podwójne pochodzące z biegunowych megaspor tej samej tetrady. Częstotliwość tworzenia mikropylarnego woreczka zalążkowego wydaje się zależeć przede wszystkim od diploidalnej rośliny mieszańcowej a nie od haploidalnego genomu megaspory.