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Final stage of progamic phase in different plant species: *Rosa* cv. 'Sonia', 6 taxons of *Oenothera* and 4 of *Brassicaceae*

Końcowe stadium fazy progamicznej u różnych gatunków roślin: *Rosa* cv. 'Sonia', 6 taksonów *Oenothera,* 4 gatunki z rodziny *Brassicaceae*

SUMMARY

The progamic phase begins after pollination; it takes place during the pollen tube growth through the style and ovary to the ovule and its final event is fertilization. In every stage of the progamic phase the pollen tube growth is affected by different factors. The last part of the pollen tube growth inside the ovary is considered to be chemotropically directed by the exudates originating from the micropyle of a fertile ovule. At that stage, the patterns of the pollen tube growth strongly suggest an active role of the ovules in attracting the pollen tube to the micropyle. The pattern of pollen tube growth in the pistil, especially in the ovary varies and depends on the plant taxonomic position.

Studies of the progamic phase were carried out in 3-year cycles on hundreds of pistils taken from buds and flowers of: *Rosa* cv. 'Sonia', *Oenothera brevistylis* (female sterile and fertile form), *Oe. hookeri, Oe. biennis, Oe. suaveolens. Oe. lamarckiana, Oe. franciscana, Brassica oleracea, Sinapis alba, Sisymbrium loeselii, Capsella bursa-pastoris.* The model plants represent examples of a different anatomy of ovaries and ovules, different biology of flowering and some specific characteristics determined by genetic factors (female sterility in *Oenothera brevistylis*, destruction of nucellar tissue in *Brassicaceae* ovules).

In the ovary, a pollen tube has to find a fertile ovule and penetrate into it. This process depends on the interaction between the ovule and the pollen tube. In different plants this interaction is modified. Detailed observations show that the attracting factors are emitted only from fertile ovules (containing a mature embryo sac). In all the investigated species the pollen tubes ignored the presence of infertile (too young or sterile) ovules and then grew inside the ovary without any defined direction. Such growth leads to a formation of knots, swellings or rolling the end part of the pollen tube into balls.

In the upper part of the style of *Rosa* a great number of pollen tubes are stopped. Only one pollen tube has access to the ovary, where only one ovule is fertile. This kind of selection of the pollen tubes before fertilization diminishes the competition and variability of gametophytic interactions. Such a system is defined as evolutionary older than that in *Oenotheraceae* and *Brassicaceae*. In *Rosa* the pollen tube can grow in the ovary without a fertile ovule but its growth is unusual and not directed.

Ovules of *Brassicaceae* are campylotropous, the way to their micropyle leads on a relatively long funiculus. The ovules get their receptivity step by step. Pollen tubes growing on the placenta are not efficiently attracted. They grow at the base of the funiculus towards and away from the micropyle. An effective penetration takes place after a period of winding, which can be explained as adaptation of pollen tubes to the long period of maturation of the ovules.

In the ovary of each *Oenothera* species there are hundreds (400–800) of anatropic ovules containing embryo sacs of a different genotype. Among the fertile ones some unfertile are scattered. Exudates from many ovules are dispersed on the placenta and the signal of attraction is not clearly directed. The pollen tube has to find the fertile ovule with an embryo sac of a preferable genotype. Pollen tubes of *Oenothera* produce many branches, which can help to explore the placenta in searching for a proper ovule. In the vicinity of its micropyle the end part of the pollen tube develops many short branches. The main branch penetrates into micropyle and takes part in fertilization. Short branches grow to the top part of the integument. They could serve as haustoria or strengthening structures, supporting the main branch.

The interaction between pollen tubes and ovules is performed in a different way: 1. In *Rosa* male partners are selected before entering the ovary. Established system of early selection creates a matching number of fertile ovules and pollen tubes, which is favourable for an individual seed to develop without competition with its brother ovules. 2. Competition between numerous pollen tubes during the progamic phase like in *Oenothera* and *Brassicaceae* is more beneficial for the next generation. Pollen tubes in the final stage of the progamic phase show a different strategy: they can wait till the ovule reaches its maturity (*Brassicaceae*) or actively penetrate the placenta in search for a preferable ovule (*Oenotheraceae*). Both patterns of pollen tube growth ensure the effective fertilization for a great majority of ovules.

STRESZCZENIE

Interakcja między płodnym zalążkiem a łagiewką pyłkową jest ważnym czynnikiem decydującym o zapłodnieniu, a w konsekwencji o płodności rośliny. Do takich interakcji dochodzi w zalążniach roślin kwiatowych pod koniec fazy progamicznej. Końcowy odcinek wzrostu łagiewek pyłkowych jest ukierunkowany sygnałami płynącymi z dojrzałych do zapłodnienia zalążków i uznawany za chemotropizm. Jeśli ten okres wzrostu łagiewki przebiega efektywnie, trafia ona do mikropyle zalążka, penetruje go i zapładnia znajdujący się w nim woreczek zalążkowy.

Proces ten przebiega nieco inaczej u różnych roślin. Modyfikują go takie czynniki, jak: różnice w biologii kwitnienia, zapylenie, a także różna budowa anatomiczna zalążni i zalążków oraz czynniki genetyczne.

Do badań wybrano takie rośliny, których cechy pozwalają uwzględnić wymienione powyżej czynniki modyfikujące przebieg fazy progamicznej: *Rosa* cv. 'Sonia', *Oenothera hookeri, Oe. lamarckiana Oe. franciscana, Oe. biennis*, *Oe. suaveolens, Oe. brevistylis* (forma żeńska sterylna i płodna), *Brassica oleracea, Sinapis alba, Sisymbrium loeselii, Capsella bursa-pastoris.* Badania prowadzono w cyklach trzyletnich. Słupki z pąków i otwartych kwiatów były macerowane i barwione błękitem anilinowym w celu ujawnienia obecności łagiewek pyłkowych w tkankach (7,

28). Obserwacje i zdjęcia wykonano w mikroskopie fluorescencyjnym Nikon Labophot 2 A. Wyniki obserwacji były jednoznaczne i nie wymagały oceny metodami statystycznymi.

W zalążniach łagiewki pyłkowe rosną po powierzchni łożyska w sposób, który wskazuje na tropizm tylko ku płodnym zalążkom. W żadnej z badanych zalążni nie stwierdzono reagowania łagiewek na obecność sterylnych zalążków. Nigdy nie penetrują one mikropyle sterylnych, ani zbyt młodych zalążków. Brak płodnych zalążków nie zahamowuje wzrostu łagiewek w zalążni. Rosną one nadal, tworząc przy zakończeniu kłębki lub pętle, a łagiewki *Oenothera* pozostają zakończone drzewkowatym rozgałęzieniem.

U *Rosa* niezależnie od obfitości zapylenia do zalążni wrasta tylko jedna łagiewka pyłkowa. Wzrost pozostałych zostaje zahamowany pod znamieniem lub w szyjce słupka. W zalążni tylko jeden zalążek osiąga płodność. Jest to ewolucyjnie stary system wczesnej selekcji gametofitów, który ogranicza ich interakcje i współzawodnictwo łagiewek. System ten sprzyja rozwojowi pojedynczego nasienia, które nie jest zmuszone do konkurencji o miejsce i pokarm w zalążni.

U gatunków z rodziny *Brassicaceae* i *Oenotheraceae* liczba łagiewek wrastających do zalążni zawsze przewyższa liczbę oczekujących na zapłodnienie zalążków. Stwarza to konieczność konkurowania łagiewek i możliwość doboru genetycznego między nimi a woreczkami zalążkowymi. Szczególnie wyraźnie zaznacza się to zjawisko w systemie reprezentowanym przez taksony *Oenothera.* Setki anatropowych zalążków emituje sygnały swej dojrzałości w kierunku powierzchni łożyska, gdzie sygnały te ulegają rozproszeniu w występującej tam wydzielinie. Łagiewki rozgałęziają się, co ułatwia im penetrację łożyska w różnych kierunkach i dokładną lokalizację płodnego zalążka o właściwym genotypie. W pobliżu mikropyle takiego zalążka łagiewka wytwarza krótkie odgałęzienia wtórne, które wrastają do szczytowej części osłonki wokół mikropyle. Główne odgałęzienie przerasta zwartą tkankę ośrodka i zapładnia woreczek zalążkowy. Rola krótkich odgałęzień może polegać na wspomaganiu głównej części w sposób mechaniczny — umacniają one jej zakotwiczenie w mikropyle, kiedy natrafia ona na opór tkanki ośrodka, lub też stanowią haustoria pobierające substancje odżywcze z osłonki. Taką rolę przypisano podobnym odgałęzieniom łagiewek u *Fuchsia.* Zdolność rozgałeziania się łagiewek *Oenothera* jest niewątpliwie pomocna w interakcjach koniecznych do efektywnego zapłodnienia.

U *Brassicaceae* łagiewki napotykają sygnał z zalążków u podstawy sznureczka, ponieważ kampylotropowe zalążki mają inaczej skierowane mikropyle. Wzrost łagiewek przez pewien okres nie zostaje skutecznie ukierunkowany, lecz rosną one wokół sznureczka, w górę i w dół, tworząc pętle i pierścienie zanim stosunkowo bezpośrednio przerosną ku mikropyle. Ten okres zmiennego kierunku wzrostu można tłumaczyć pulsacyjnym wydzielaniem sygnałów wabiących przez zalążki lub stopniowym dojrzewaniem zalążków do stanu pełnej receptywności. Przez okres zmiennego wzrostu łagiewki zachowują gotowość do zapłodnienia. Podczas tego okresu większa liczba zalążków może osiągnąć receptywność, co sprzyja zwiększeniu płodności rośliny.

K e y w o r d s: pollen tube, progamic phase, fertilization, ovule, micropyle, tropism, *Rosa, Oenothera, Brassica, Sinapis, Sisymbrium, Capsella bursa-pastoris*.

INTRODUCTION

Plant fertility is, to a large extent, dependent on fertilization — the final event of progamic phase. Sperm cells have to be provided by a pollen tube to the embryo sac, which is hidden inside a special organ — the ovule. A very important role of the pollen tube is to find the ovule with a mature, fertile embryo sac and to penetrate into it in an efficient way, as it was described by many authors (14, 16, 27, 36, 39). This phenomenon is modified in different plant species by the

anatomy of ovules and their distribution inside the ovary. Fertilization is a result of the final part of the pollen tube growth, which is determined by the signals from a fertile ovule (2, 4, 5, 8, 12, 17, 24, 32, 27, 38, 42). In present paper different strategies of pollen tubes growth aiming at penetration of ovules are discussed on the example of *Rosa* cv. 'Sonia', several taxons of *Oenothera* and four species from *Brassicaceae.*

MATERIAL AND METHODS

Model plants were chosen with the aim of comparing pollen tubes growth inside the differently built ovaries containing different types of ovules. Besides anatomy, a genetic factor and pollination preference were also taken into consideration. The model plants represent 3 types:

1. *Rosa* cv. 'Sonia' — ovary of one carpel, parietal placenta, 2–3 crassiinucellar ovules, only one of which or none being fertile. The difference in the fertility of ovules allows us to compare the pollen tube growth in the presence and absence of a fertile ovule.

2. 6 taxons of *Oenothera* represented an ovary with central placenta, in 4 chambers containing hundreds of anatropic, crassinucellar ovules. *Oenothera hookeri, Oe. brevistylis, Oe. lamarckiana* and *Oe. franciscana* have to be cross pollinated, *Oe. biennis* and *Oe. suaveolens* are selfpolinated. *Oe. brevistylis* occurs in two forms — female fertile or female sterile one, which is genetically determined. The chosen taxons differ in pollination preferences and ovules fertility, which allows us to observe an effect of the genetic factor and attraction by fertile ovules during the final stage of the progamic phase.

3. *Brassicaceae* species were represented by: *Sisymbrium loeselii* L., *Capsella bursa pastoris* L., *Sinapis alba* L., and *Brassica oleracea* L. The species differ in the number of ovules in the ovary, rate of ovules maturation and their fertility.

In earlier studies the anatomy of pistils was investigated by histological methods in light, fluorescence and electron microscope (1, 9, 31, 3, 4, 5, 29). A long period of investigations on plants grown in experimental garden at UMCS Institute of Biology in Lublin gave the opportunity to learn about the flowering biology of the studied plants. Investigations on the pollen tube growth in the pistil of plants were conducted in 3-year cycles depending on plant biology in the period between 1985–2001. Additionally some experiments on *Oenothera* pollen tubes grown *in vitro* were carried out in 1999–2001.

Some flowers were left for natural pollination, others were manually pollinated with pollen grains freshly taken from opening flowers of the same plant and from other plants to compare self- and cross-pollination effect. All the samples were not less than 30 pistils. The results concerning final events of the progamic phase were obtained from hundreds of ovules and appeared so consistent that statistics was not necessary.

The pistils were collected in the middle of flowering season. They were taken from buds, just opening flowers and at a different time after anthesis till the corolla withered. The time intervals were from 3 hours to 6 days depending on the biology of flowering species.

Pistils were macerated in 1N NaOH, rinsed a few times in water and stained with aniline blue (28). After staining they were gently squashed on the slide and observed in the fluorescence microscope NIKON LABOPHOT 2 A, in UV with BG filter (420 nm). The method allowed us to find fluorescence of callose in the walls and plugs of the pollen tube. The pollen tubes were photographed on ASA 200 KODAK film.

In the *in vitro* experiment *Oe. hookeri* pollen tubes were grown on semi-solid medium particularly described in a separate paper (26).

OBSERVATIONS

The flowers of *Rosa* cv. 'Sonia' contain 70–120 pistils growing inside the receptaculum. There is a slight difference in the developmental stage between pistils growing on the side-wall of the receptaculum (more advanced in development) and pistils growing on the bottom (delayed); where among well developed pistils some underdeveloped ones are scattered. The pistils in the same receptaculum have styles of different length, which allows all of them to expose their stigmas over the receptaculum. Nearly every pistil contains two ovules inside the ovary. Both grow from the wall (parietal placenta). One ovule is bigger and it contains a well-developed embryo sac (*Polygonum* type), the other ovule remains smaller, and shows an inhibition of megaspores or embryo sac development. In some ovaries 3 ovules are present, but one is dominating, two others are underdeveloped. Sometimes all three ovules are of the same size (smaller than the fertile ovules in other pistils).

The ovules of *Rosa* represent the crassinucellar type — many layers of nucellar cells cover the embryo sac. The inner integument forms a micropylar canal. The top parts of inner and outer integuments develop abundant secretional papillae. Similar papillae are also present on the ovary wall forming the placenta. Papillae are covered by a thick layer of exudate forming a droplet on the micropyle of a fertile ovule. Papillae on the placenta and around the micropyle form a final part of the transmitting tract and support the pollen tube growth inside the ovary.

Immediately after anthesis the stigma of the pistil is dry, but sticky. In nature, in a rose flower hundreds of pollen grains are deposited on each stigma by bees. After pollination the stigma becomes stimulated to secretion and on the second day after anthesis it covers with a fluid. The process of pollen grains germination takes place after 2–3 days. During this time the stigma produces a thick layer of the fluid, the pollen grains get swollen and the pollen tube starts to protrude. The pollen tube grows for several hours than turns under the surface of the stigma. On 3–4th day after pollination most of the pollen tubes reach the place beneath the stigma. At the top part of the style their growth is stopped. The tips of many pollen tubes become swollen and covered by a thick layer of callose. On 5th day only few pollen tubes (2–7) are growing down the style. Some callose plugs are formed in the tubes. They grow at a different rate and only one of them — the fastest one enters the ovary. After this event other pollen tubes are inhibited in their development. More than 2000 pistils were observed but only twice 2 pollen tubes were found inside the same ovary. In the ovary the pollen tube grows more or less directly to the micropyle of the bigger, fertile ovule. After its penetration the pollen tube grows along the micropylar canal and further on it enters the

nucellus. Here, it turns few times while growing between the cells, and finally it touches the embryo sac. The contact between male and female gametophyte is at the level of the egg apparatus but not always from the top, sometimes it occurs from the side. The pollen tube gets the contact with the embryo sac on the 7th or 8th day after pollination.

If 2 (sometimes 3) ovules are of a similar size the pollen tube does not direct to any of them. It grows on the placenta making loops or knots (Fig. 1). A similar pattern of growth was found in an ovary with underdeveloped ovules. The pollen tube grows even on the secretional papillae surrounding the micropyle of such an infertile ovule, but it never penetrates into it. When fertilization does not happen, the pollen tube continues its growth for 2 days looping and turning in different directions on the placenta. Some pollen tubes, when they are not aiming at the fertile ovule, grow slowly and many side bubbles are formed on them (Fig. 2).

Pollen tube growth in *Oenothera* is very similar in every taxon. All the plants pollinated in a natural way by moths, or manually, appeared very fertile and set seeds. Pollen grains are deposited on dry stigmas in great amount, because they are joint in big clusters by viscine threads. This phenomenon, characteristic of *Oenothera*, ensures abundant pollination. Anthesis and pollination by moths occurs in the evening and, in consequence the progamic phase occurs at night — time. The temperature has an influence on the pollen tube growth rate, and at about 12–15°C the whole progamic phase takes no longer than 12 hours. On the stigma hundreds of pollen tubes germinate in 2 hours after pollination and quickly grow down the style. After 6 hours they cover a 3–5 cm distance and enter the ovary. During the next 2 hours most ovules can be fertilized.

Pollen grains of all taxons of *Oenothera* have 3, sometimes 4 colpi with poruses. The pollen tube can germinate from one or more poruses, which is a very characteristic phenomenon. Each pollen tube growing from the same pollen grain is able to develop for a long time. Another specific feature of *Oenothera* pollen tubes is the ability to split into branches. It can occur immediately after germination or later at different distance of growth (Figs 3, 4). Very characteristic branches are produced when the pollen tube is already growing in the transmitting tissue beneath the placenta. At this stage the last callose plug is formed, separating the end part of the pollen tube (Fig. 5). After turning to the surface of the placenta, the pollen tube produces many branches. Sometimes it looks like a brush. Each branch grows independently on the placenta winding among the ovules. The placenta is covered with an exudate, which contains pectines and free calcium ions. Similar substances are present in the micropyle of fertile ovules. A pollen tube near such a micropyle divides into two kinds of branches. One branch penetrates the micropylar canal, then the nucellus of the ovule. This branch can be

regarded as the main one, aiming at fertilization (Figs 6, 9). It grows through the nucellar tissue to the embryo sac. The last part of this pollen tube branch becomes swollen, and shows bright callose deposit at the tip (Figs 5, 6, 8). It penetrates into the embryo sac through the filiform apparatus in one of the synergids. The other branches, formed near the micropyle of the ovule, direct to the top part of the integument and grow in its tissue (Figs 6–9).

In the presence of sterile ovules a pollen tube also divide into many branches in its end part. The branches grow in different directions on the placenta, but they ignore the micropyle of the ovules. Sometimes, the pollen tube can grow around the ovule making loops or knots, and then the short branches form tree-like endings (Fig. 10).

The flowers of *Brassicacae* have to be cross-pollinated to set seeds. The ovary is built of 2 carpels. The placenta is placed in the middle on the septum formed during the pistil development. In the ovaries there can be 6 ovules in *Sinapis alba*, 8–10 in *Brassica oleracea*, and more than 15–18 in *Sisymbium loeselii* and *Capsella bursa-pastoris*. Marginal ovules very often stay underdeveloped; in some cases also ovules in other places of the placenta can be sterile. Especially in *Sisymbrium* sterile ovules are scattered among fertile ones. Ovules start to develop as crassinucellar type, its tissue originating from a parietal cell is formed before megasporogenesis and survives till the beginning of the embryo sac development. This tissue degenerates during gamethophyte maturation. This process differs in some details in each of the investigated species, but it is not important for the present studies. A fertile ovule of each species has only some remnants of the nucellar cells in the form of a jelly substance at the apex of the embryo sac. The ovules are campylotropous. The micropyle is turned to the side from the placenta and the pollen tube has to grow up the funiculus to reach it.

A transmitting tract located inside the septum leads from the stigma to the ovules. The pollen tubes grow along it for several hours and then emerge on the placenta surface. Here they grow turning and winding among the funiculi of ovules (Figs 11, 12). Some pollen tubes are stopped at the base of the funiculus, some others grow up directly to the micropyle. Very often the pollen tube grows to the funiculus and away from it a few times, thus making loops. At other ovules the pollen tube grows up the funiculus but does not reach the micropyle. It turns around many times making a thick ring (Figs 13 and 14). After a period of not strictly directed growth, the pollen tube finds its way to the micropyle and penetrates it. In every observed ovule, the pollen tube interacts with the embryo sac through the filiform apparatus. If any ovule is not receptive (smaller, too young or without a mature embryo sac) the pollen tubes neither stop at it, nor grow up their funiculus. Such ovules are never penetrated by any pollen tube.

DISCUSSION

The penetration of the micropyle by the pollen tube can be regarded as a fertilization process. There is a very strong correlation between the number of penetrated ovules and the number of seeds set in the ovary, as it was estimated in different plant species (27, 3, 34). In the present studies, the pattern of the pollen tube growth in its end part before fertilization was the main object of observations. Differences of final stages of the progamic phase in various model plants could be explained by adaptation to a different anatomy of ovules and an unequal rate of their maturation. In every species sterile ovules are ignored by the pollen tubes. There is a strong argument that a fertile ovule must play a very active role in the interaction with a male gametophyte. It becomes a source of signals attracting the pollen tube (8, 12, 21, 24).

In *Rosa* only one pollen tube enters the ovary, the others are stopped in the style. The fastest pollen tube evokes in the style a physiological barrier for the other pollen tubes. This barrier, localized under the stigma, is effective not only on the second day after pollination, but for 7–8 days, till the end of the progamic phase. This mechanism of closing the entrance to the ovary is active in spite of the presence of more than one ovule. Judging by the size and shape of ovules, it is sure that only one ovule dominates in the pistil and that it is destined for fertilization. In *Rosa,* a well established biological system provides an opportunity to develop an achene from one ovule, without disturbing the competition for place and nutrients from brother ovules which they have started to develop, but inhibited before megasporogenesis, they stay sterile and not receptive. Later, in the following stages of the relatively long progamic phase (4–10 days' span between pollination and fertilization) they look similarly small. It means that the pollen tube growth does not stimulate an embryo sac development in such ovules. The phenomenon of only one ovule dominance was also found in other *Rosaceae* species (11, 36).

The progamic phase in *Rosa* reveals specific features different than in other angiosperm plants, which represent the evolutionary old system of sexual reproduction. The female organ developed a series of barriers limiting male partners to the necessary minimum. The progamic phase is relatively long, but the interaction between the pollen tube and the ovule is limited. During several days some unfortunate events can happen disturbing or even killing a single pollen tube before it becomes able to fertilize the ovule. It is a factor decreasing plant fertility. Developing progeny is preserved from the stress of competition, which is positive for the individual seed, but not very beneficial for the species (22, 20).

In most angiosperm plants the progamic phase opens up more possibility of fertilization than it is observed in *Rosa*. The progamic phase is shorter —

takes one day or a few hours, which allows sustaining more stabile conditions. A most obvious benefit of an excess of pollen tubes in the ovary is ensuring fertilization to many ovules. The negative side of this reproductive system is that developing seeds have less favourable conditions than in *Rosa*. They have to compete for the nutrients and place in the same ovary. The other difference in the biology of reproduction between *Rosa* and evolutionary more advanced angiosperms lies in a more bilateral interaction between pollen tubes and ovules. Pollination and pollen tube growth in the pistil often stimulates ovule maturation and has a positive effect on their receptivity (39, 40, 37).

In the ovaries of *Oenothera* and *Brassicacae* pollen tubes competition is not limited by strict barriers in the style, but takes place in the ovary, where many more pollen tubes enter than there are receptive ovules. This competition has a positive effect on the vigour of progeny, as it was described in many species (9, 20, 22). In spite of a strong incompatibility reaction on the stigma and in the style (a characteristic feature of plants belonging to *Brassicaceae* (6, 18, 19, 33)), a great number of pollen tubes search for the fertile ovules which send them chemotropic signals. The reaction is mutual: these signals stimulate and direct the pollen tube growth (16) and the pollen tubes presence stimulates the maturation of ovules. In *Brassicaceea* the stimuli from fertile ovules are emitted by the micropyle and passed (proceed) down the funiculus where they are caught by the pollen tubes. The pattern of the pollen tube growth with stopping, growing up and down the funiculus can be explained by changes in the emission of chemotropic substances from ovules. It looks as if the attractant were secreted in portions from time to time. The ovules may get their receptivity step by step or emit the exudates in pulses. The final part of a pollen tube is more or less directed to the embryo sac, which can be explained by a strong and permanent signal coming from the micropyle, when the embryo sac reaches the full maturity.

However, the interaction between fertile ovules and pollen tubes is clear in all model plants, specific features of the pollen tube growth in *Oenothera* give an example of very effective system of sexual plant reproduction. A great number of pollen tubes enter the ovary, which is necessary for fertilization of hundreds of ovules. Pollen tubes originate from grains with different genotypes (most taxons of *Oenothera* are heterozygotic and haploid genomes are segregated during macro- and microsporogenesis) (9, 25). In the ovary a big population of genetically different pollen tubes compete for these ovules in the embryo sac which contain a more or less preferable genotype. This competition leads to a selective fertilization — it was statistically proved that in *Oenothera* the genetic constitution of the progeny is not created at random (10, 15, 23). Selective fertilization was also found in other plants (13, 20).

A very specific feature in all *Oenothera* taxons is the pollen tube branching observed in pistils and *in vitro*. Branching of pollen tubes during their growth in the nucellus was described in spinach, but the pattern was different (41). It was most obvious in *Oe. hookeri*, but it is a common character for some other genera of *Oenotheraceae* (*Onagraceae*) family (26, 35). All *Oe. hookeri* pollen grains contain the same genotype and the competition of pollen tubes cannot be determined by a genetic factor. The pattern of pollen tube growth inside the ovary is the same after cross- and self-pollination. Pollen tubes can branch at every stage of the progamic phase, but the pattern of growth of the final part is especially interesting. After the formation of the last callose plug, the pollen tube divides into many branches. They grow independently on the placenta surface. This stage of the progamic phase is connected with searching for the fertile ovule. In other plants, where a smaller number of ovules are formed, the pollen tube can grow more or less directly to them. After getting the signal of receptivity the pollen tube penetrates the micropyle, as it was observed in many plants (14, 39, 41, 34). In *Oenothera* the situation is different. In the ovary of every *Oenothera* taxon hundreds of anatropic ovules send their signals towards the surface of the placenta, where the chemotropic signal can be accumulated and become strong. It is not limited to a few points, but spread on the placenta surface. The pollen tube reacts to such a signal by dividing into many branches. It was proved in an *in vitro* experiment — the pollen tubes produce more branches when a squashed placenta tissue is added to the medium (27, 30). In the ovary, among fertile ovules, there are some sterile ones and the pollen tube has to distinguish and find the fertile one, which becomes more ensured when the receptivity signal is caught by branches growing in different directions. After localization of the fertile ovule the nearest branch is directed and stimulated to penetrate the micropyle. At that stage, additional short branches are formed. They grow into the top part of the inner integument. The role of the main branch is clear — it takes part in fertilization, because it carries sperm cells and a great part of the cytoplasm. Additional short branches have to be observed in the electron microscope. In the light microscope the tube structure looks completely empty. The branching of the pollen tube near the ovule micropyle was also described in *Fuchsia boliviana* (35). The short branches growing into the integument were considered to be haustoria feeding at first the pollen tube and the embryo sac growing quickly after fertilization. In *Oenothera* it is very probable that the short branches could be haustoria feeding the pollen tube and giving an active strengthening to the main branch. They look like claws catching a stabile support. The main branch has to grow through the compact nucellus and has to overcome this obstacle, so short branches can help in a mechanical way. When fixed around the micropyle, they can help to keep the main branch in a proper position. Considering all

possible roles of the specific branching of the pollen tube in *Oenothera* ovary, we can conclude that the effective interaction with ovules during final events of progamic phase ensures high fertility of these plants.

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PODPISY FOTOGRAFII

Fig. 1. Loops formed at the ending of *Rosa* pollen tube in absence of fertile ovule. ×120.

Fig. 2. *Rosa* cv. 'Sonia' pollen tube growing along ovary wall shows bubbles on a sides and swollen tip covered by thick callose layer. ×120.

Fig. 3. Pollen grains of *Oenothera hookeri* germinated *in vitro* form more than one pollen tube. ×90.

Fig. 4. Pollen tubes of *Oe. hookeri* branching *in vitro* after 2 h of incubation. ×250.

Fig. 5. Pollen tube of *Oe. hookeri* branching during its growth in ovary (part from last callose plug to the tip touching the embryo sac). ×120.

Fig. 6. Fertilized ovule of *Oe. hookeri*. Main branch of pollen tube in nucellus is swollen. Short branches grow into top part of inner integument. ×120.

Fig. 7. Micropylar part of fertilized ovule of *Oe. biennis.* Short branches seen as bright aggregate at the micropyle, main branch of pollen tube seen in nucellus. ×120.

Fig. 8. Similar like in Fig. 7 ovule of *Oe. franciscana*, well visible short branch of pollen tube in the integument. Arrow shows place of contact between pollen tube tip and embryo sac. ×120.

Fig. 9. Similar like Fig. 7 — *Oe. brevistylis* (fertile form), well visible main branch in the nucellus. ×120.

Fig. 10. Tree-like ending of pollen tube growing in ovary of female sterile *Oe. brevistylis*, no branch penetrates any ovule. ×250.

Fig 11. Pollen tubes and ovules in ovary of *Sisymbrium loeselii*. ×200.

Fig. 12. Pollen tube growing on funuculus of ovule *Brassica oleracea* makes few loops before fertilization. ×200.

Fig. 13. Fertilized ovule of *Sinapis alba* with pollen tube forming a ring on funiculus. ×200.

Fig. 14. Similar stage like in Fig. 13 in *Capsella bursa-pastoris* — pollen tube made a knot on funiculus before penetration to the embryo sac (place of gametophytes contact shown by arrow). ×200.