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Distribution of Polisaccharides during Megasporogenesis in *Onagraceae*

Rozmieszczenie polisacharydów w megasporogenezie u *Onagraceae*

Размещение полисахаридов в мегаспорогенезе *Onagraceae*

INTRODUCTION

Linear megaspore tetrad with micropylar megaspore functioning as the mother cell of a four-nucleate embryo sac is characteristic of the majority of *Onagraceae* (4, 5). Occurrence of callose in megasporocyte and megaspore wall was described in several species of this family (7, 11). The starch was noticed in the apices of megasporocyte and in polar megaspores of two species: *Oenothera lamarckiana* (6) and *Epilobium palustre*. (13).

MATERIAL AND METHODS

The following species of *Onagraceae* were investigated: *Epilobium palustre* L., *Oenothera fruticosa* L., *Oe. grandiflora* L. *Oe. rosea* Art., *Oe. silesiaca* Renner, *Godetia grandiflora* Lindl., *Clarkia elegans* Dougl., *Fuchsia hybrida* hort.

Ovules were fixed in ethyl alcohol and acetic acid (3:1), hydrolyzed for 10—15 min in 1N HCl and rinsed in water; afterwards the PAS reaction (periodic acid, Schiff) was carried out in toto. Callose was detected in a fluorescent microscope after aniline blue treatment (1). The squash preparation were made after both procedures.

RESULTS

Epilobium palustre. Callose occurs during the first meiotic prophase in the entire wall of a meiocyte (Pl. I. 1), in the somewhat later first

meiotic prophase the fluorescence typical for callose disappears from the micropylar pole of the cell, whereas the chalazal part fluoresces very strongly (Pl. I, 2). At the end of the Ist prophase there is futher change in callose distribution, then a strongly fluorescing ring occurs in the submicropylar region. This ring is also visible in dyads (Pl. I, 3). The cross walls, set up between cells of dyads and tetrads, show very strong fluorescence (Pl. I, 5); however, there is no obvious fluorescence on the micropylar pole during the whole megasporogenesis. The second meiotic division is usually asynchronous, delayed in the chalazal cell. As a result of this delay a transitory triad stage is formed (Pl. I, 4). The tetrad fluorescence gradually disappears, at first from the lateral walls of the micropylar megaspore, later from lateral walls of inactive megaspores and finally from the cross walls.

The walls of meiocyte, dyads and tetrads give a positive PAS reaction during the entire megasporogenesis (Pl. I, 6—10). An especially strong reaction occurs in the cross walls of dyads. The reaction is shown by the middle lamella already in the early cytokinesis (Pl. I, 7). Callose fluorescence can also be seen in a forming middle lamella as in e.g. the chalazal cell of the tetrad (Pl. I, 5).

PAS reaction reveals two groups of grains situated at two meiocyte poles (Pl. I, 6). The grains are localized in plastids (13) and may be considered as starch grains. In heavily flattened preparations the polar group of starch grains is spread to one layer, then it is possible to evaluate their number as about 300 in *Epilobium*. It seems that at the micropylar apex the grains are somewhat more numerous than at the chalazal pole. These grains are rather small, their diameter being up to 0,3 nm; they are much smaller than starch grains of 2,0 nm diameter in somatic cells of the ovule. The groups of grains remain at the poles of a dyad and tetrad without any noticeable changes until the conclusion of megasporogenesis. In tetrads the starch grains are conspicuously lacking in two middle megaspores. Only in one tetrad for more than 100 seen, several starch grains were present (Pl. I, 15). At the beginning in young megaspores the starch grains are concentrated in a region between the cell nucleus and the apex (Pl. I, 9). Later the number and size of starch grains decrease gradually in the chalazal megaspore, whereas in the micropylar megaspore there appears a reverse process — the starch grains become larger, reaching 1,0 nm in diameter, and more numerous. Finally, starch grains disappear completely from the degenerating chalazal megaspore and fill up the micropylar megaspore, which functions as an embryo sac mother cell. Picture (Pl. I, 10) shows a tetrad with chalazal cell undivided by a cell wall. The small starch grains are scattered at the chalazal region and larger grains fill up the micropylar megaspore.

Oenothera. In three investigated species: *Oenothera fruticosa* (Pl. I, 13—15), *Oe. rosea* (Pl. II, 10), *Oe. grandiflora*, callose occurs and disappears in similar fashion as in *Epilobium*. This process is in some respect different in *Oenothera silesiaca*, where fluorescence typical of callose was not revealed in the megasporocyte, and in the lateral walls of the tetrad (Pl. II, 4); small, weakly fluorescing grains were sometimes seen at the apices of tetrads and in the lateral walls of middle megaspores. The walls in all investigated species of *Oenothera* were PAS-positive. A particularly strong reaction occurs in the cross walls of the tetrad (Pl. II, 3). The starch grains also occurred at the poles in megasporocytes in all species (Pl. I, 11, Pl. II, 1, 9). In *Oe. silesiaca* more or less similarly large groups of starch grains remain at the poles of dyads (Pl. II, 2) and tetrads (Pl. II, 3), whereas in other species the number of starch grains in dyads and tetrads seems to decrease. Later, during the growth of the micropylar megaspore, starch entirely disappears in the chalazal megaspore.

Clarkia elegans. Localization of callose in cell walls during megasporogenesis is like that in *Epilobium* (Pl. II, 5). Among 88 meiotic figures there were 36 meiocytes, 6 dyads, 18 triads and 28 tetrads. In most triads chalazal cells were degenerating before conclusion of the second division. In half of the seen tetrads the wall between chalazal megaspores was much thinner than the remaining two cross walls, which were 2,0 nm thick. All cross walls were strongly PAS-positive. A weaker reaction was shown by the lateral walls of inactive megaspores. The cell wall of a functioning megaspore is much thinner than that of others (Pl. II, 12). Starch grains appear, as in other genera, at the megasporocyte poles (Pl. II, 11) but already at first meiotic metaphase the number of grains obviously decreases and is not visible in some dyads and tetrads; however, in other tetrads starch grains occur again but only in functioning megaspores (Pl. II, 12).

Godetia grandiflora. Localization of callose during megasporogenesis remains like that in Epilobium. One more prominent difference is the occurrence of a stronger fluorescing segment in the submicropylar part in the meiocyte at the late first prophase, the other is the absence of strong fluorescence at the chalazal apex in the course of megasporoginesis (Pl. II, 6—8). Cell walls are PAS-positive; particularly thick and heavily stained are the cross walls in tetrads. Starch grains appear at the poles of meiocytes, their number seems to decrease so that in some dyads starch grains were not detected. Only micropylar megaspores in tetrads contained a small number of starch grains.

Fuchsia hybrida. Callose in megasporogenesis occurs according to the *Epilobium* pattern. Older meiocytes in *Fuchsia* differ from those of the other species by a thickening in the submicropylar and chalazal part of

the wall (Fig. 1). It is possible to distinguish two layers in cell wall. The outer layer gives a stronger PAS reaction than the inner one which is almost PAS-negative. Similarly, the middle part of the cross walls gives a stronger PAS reaction than the inner layer (Pl. II, 15).

Distribution of starch grains is not uniform in all meiocytes. There are some with compact groups of starch grains at the poles. In the megasporocyte (Pl. II, 13), there are two groups of starch grains at the micropylar pole, one group closely pressed to the nuclear envelope at the place where the chromosomes are gathered. This starch grain group is in fact much larger because part of it lies out of focus. In many megasporocytes the starch grains are rather loosely dispersed over a large area (Pl. II, 14), nevertheless in tetrads starch appears in both polar megaspores or only in the micropylar one (Pl. II, 15).

Only in *Fuchsia* were abnormal meiocytes and tetrads noticed. Some meiocytes are 2—3 times longer and narrower than typical ones, some others were almost of izodiametrical shape (Fig. 1b), several postmeiotic cell arrangements in the shape of a tetrad were composed from 5 cells, and some of them were binucleate (Fig. 1c).

DISCUSSION

The pattern of callose distribution in seven out of eight species of investigated *Onagraceae* is very closely related. During the first meiotic prophase callose occurs in the PAS-positive wall of meiocyte and afterwards it disappears from the micropylar apex of the cell. The micropylar wall does not give a positive callose reaction during the following megasporogenesis. The cross walls in dyads and tetrads show strong callose fluorescence and PAS reaction. In most species the wall between the two chalazal megaspores is thin, not completed or lacking.

Starch grains in all the investigated species are gathered at the meiocyte poles and remain there still in the tetrad at the micropylar apex of the functional megaspore and chalazal apex of the chalazal megaspore. There are no visible starch grains in the two middle cells of a tetrad. Starch grains gradually disappear in the chalazal megaspore and grow in the micropylar one. In some part of *Fuchsia* meiocytes a number of starch grains are loosely scattered. There are also some abnormal meiocytes and tetrad-like arrangements. Dispersion of starch grains in many meiocytes may be considered a result of disturbed meiocyte development. In less than 1% of *Epilobium* tetrads few starch grains were placed in one of the middle megaspores; however, the megasporogenesis was normal.

Starch grains were observed in few species: *Oenothera lamarckiana*

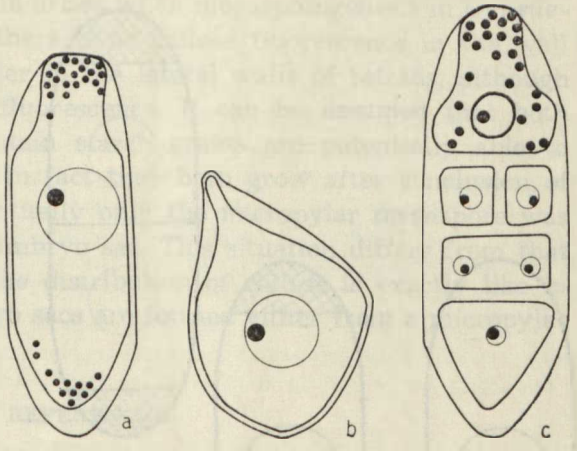

Fig. 1. *Fuchsia hybrida:* a — typical megasporocyte, b — atypical megasporocyte, c — atypical arrangement of postmeiotic cells

(6), *Cypripedium insigne* (2), *Paphiopedilium spicerianum* (3), and as referred to by Maheshwari (8), in *Loranthus pentandrus* in megasporocyte mother cell (T r e u b 1883), in dyads of *Psychotria* (F a g e r l i n d 1937), in functioning megaspore of *Castalia odorata* (C o o k 1902), *Acacia* (G u i g n a r d 1881), *Sedum* (D'H u b e r t 1896), *Pentas, Richardsonia, Cephalanthus* (F a g e r l i n g 1937).

During megasporogenesis in *Onagraceae* starch grains appear at the poles of the meiocyte in the Ist meiotic prophase. In two species: *Epilobium palustre* and *Oenothera silesiaca,* the groups of starch grains remain at the same place without any distinct changes until the early tetrad stage (Fig. 2a). In *Oe. fruticosa, Oe. rosea, Oe. grandiflora, Clarkia elegans, Godetia grandiflora* and *Fuchsia hybrida* the groups of starch grains set in meiocytes decrease significantly and even disappear before formation of dyads, but later are visible again, either in both polar megaspores or only in the micropylar one (Fig. 2b). Starch grains become more numerous and larger in micropylar functioning megaspores of all species.

The developing meiocyte grows along the micropylar-chalazal axis of the ovule. In this polarized surrounding the meiocyte shows callose and starch distribution which may be considered as a visible feature of cell polarization. Callose originally present in the whole meiocyte wall disappears from the part at the micropylar apex which later becomes a part of the functioning megaspore wall. Distribution of starch grains corresponds to that of the meiocyte plastids (13).

In *Onagraceae* dyads and tetrads are respectively composed of cells of equal size, and therefore the Ist and IInd meiotic divisions may be considered as symmetrical. The IInd division, however, is obviously asymmetrical when the starch and organelles distribution are taken into account. Both middle megaspores contain no starch grains and plastids (as was

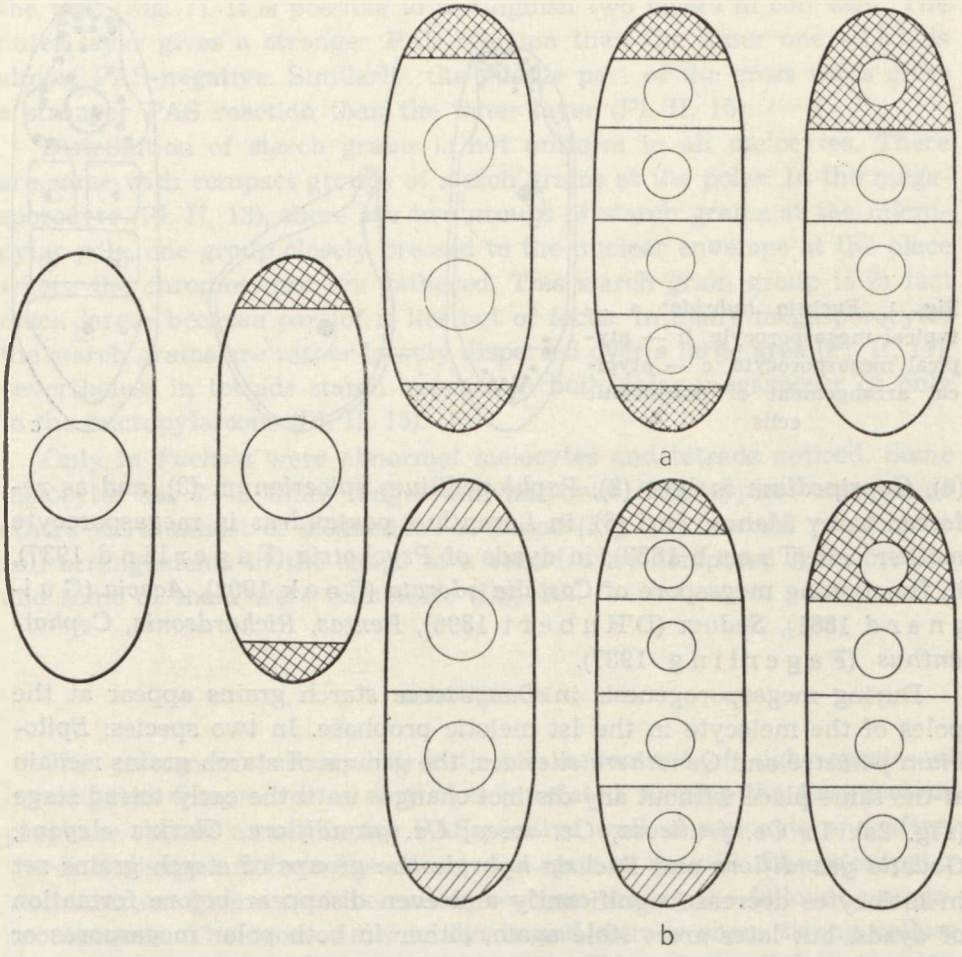

Fig. 2. Diagrammatic presentation of distribution of starch grains in megasporogenesis; a — in *Epilobium palustre* and *Oenothera silesiaca*, b — in *Oe. fruticosa*, *Oe. rosea*, *Oe, grandiflora*, *Clarkia elegans*, *Godetia grandiflora* and *Fuchsia hybrida*. Localization of starch grains shown by hatching. Density of hatching shows the changes in amount of starch grains

observed in an electron microscope) and therefore it may be assumed that their development into an embryo sac is excluded. Two apical megaspores with starch grains and plastids are potentially able to further development as cells fully equipped in organelles. They differ, however, in their position in the ovule and chemical constitution of their walls: the micropylar megaspore having the wall without callose, and the chalazal megaspore enveloped by a thick callose layer. There is an assumption that callose decreases the permeability of the cell wall, and consequently the chalazal megaspore was less accessible to the nutrients coming from the surround-

ing tissues. A separate problem arises when megasporogenesis in *Oe. silesiaca* is considered, because there is no callose fluorescence in the wall of the megasporocyte and later in the lateral walls of tetrads, although the cross walls give strong fluorescence. It can be assumed that both apical megaspores which contain starch grains are potentially able to develop into an embryo sac. In fact they both grow after conclusion of the meiotic division, but eventually only the micropylar megaspore was observed to develop into an embryo sac. This situation differs from that in *Oe. muricata* (12) where the distribution of callose is exactly like in *Oe. silesiaca* but where embryo sacs are formed either from a micropylar or chalazal megaspore (10).

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STRESZCZENIE

W ścianach młodych mejocytów gatunków z rodziny *Onagraceae* pojawiała się kaloza, która następnie znikała z bieguna mikropylarnego komórki. Obecność kalozy stwierdzono w ścianach bocznych i poprzecznych podczas całej megasporogenezy.

Wyjątkiem była *Oenothera silesiaca*, gdzie kaloza występowała tylko w poprzecznych ścianach diad i tetrad. Ściany mejocytów diad i tetrad u wszystkich gatunków dawały reakcję PAS, szczególnie silną w ścianach poprzecznych.

Na biegunach mejocytów wszystkich gatunków gromadziły się plastydy ze skrobią. W dalszych stadiach megasporogenezy wystąpiły różnice w ilości skrobi. U *Epilobium palustre* i *Oe. silesiaca* w czasie całej mejozy grupy ziaren skrobi utrzymywały się w tych samych miejscach bez wyraźnych zmian. U pozostałych gatunków podczas I i II podziału mejotycznego ilość skrobi malała. W tetradzie zawsze obserwowano ziarna skrobi w megasporze mikropylarnej i chalazalnej. Z czasem ziarna skrobi w megasporze mikropylarnej powiększały się, a w chalazalnej zanikały.

U *Fuchsia hybrida* obserwowano zaburzenia w megasporogenezie, przejawiające się w nietypowych kształtach megasporocytów, rozproszeniu w nich ziaren skrobi i tworzeniu 5-komórkowych układów w formie tetrad.

РЕЗЮМЕ

В оболочках молодых мейоцитов видов из семейства *Onagraceae* появлялась калоза, которая потом исчезала с микропильного полюса клетки. Установлено присутствие калозы в боковых и поперечных оболочках во время всего мегаспорогенеза. Исключением была *Oenothera silesiaca*, где калоза выступала только в поперечных оболочках диад и тетрад. У всех видов оболочки мейоцитов диад и тетрад давали реакцию PAS; особенно сильная реакция наблюдалась в поперечных оболочках.

На полюсах мейоцитов всех видов накапливались пластиды с крахмалом. В последующих стадиях мегаспорогенеза наблюдались разницы в количестве крахмала. Во время всего мейоза группы зерен крахмала в *Epilobium palustre* и *Oe. silesiaca* сохранялись на тех же самых местах без отчетливых изменений. Во время I и II мейотического деления количество крахмала у других видов уменьшалось. В тетраде зерна крахмала всегда наблюдались в микропильной и халазальной мегаспоре. Зерна крахмала в микропильной мегаспоре увеличивались, а в халазальной исчезали.

Кроме того, в *Fuchsia hybrida* наблюдались нарушения в мегаспорогенезе, что проявлялось в нетипичных формах мегаспороцитов, рассеянии в них зерен крахмала и образовании 5-клеточных систем в форме тетрад.

LEGENDS

Plate I:

1—5. *Epilobium palustre*. Callose fluorescence in cell wall of: 1, 2 — megasporocyte, 3 — dyad, 4 — triad, 5 — tetrad (×1100).

6—10, 15. *Epilobium palustre*. Starch grains and cell walls after PAS reaction: 6 — meiocyte, 7 — young dyad, 8 — dyad, 9 — triad, 10 — tetrad, 15 — tetrad (× 1100).

11. *Oenothera fruticosa*. Meiocyte after PAS reaction (×1100).

12—14. *Oenothera fruticosa*. Callose fluorescence in cell wall of: 12 — meiocyte, 13 — dyad, 14 — tetrad (×1100).

Plate II:

1—3. *Oenothera silesiaca*. Cells after PAS reaction: 1 — meiocyte, 2 dyad, 3 — tetrad (× 1100).

4. *Oenothera silesiaca*. Callose fluorescence in cell walls of tetrad (× 1100).

5. *Clarkia elegans*. Callose fluorescence in cell walls of triad (×1300).

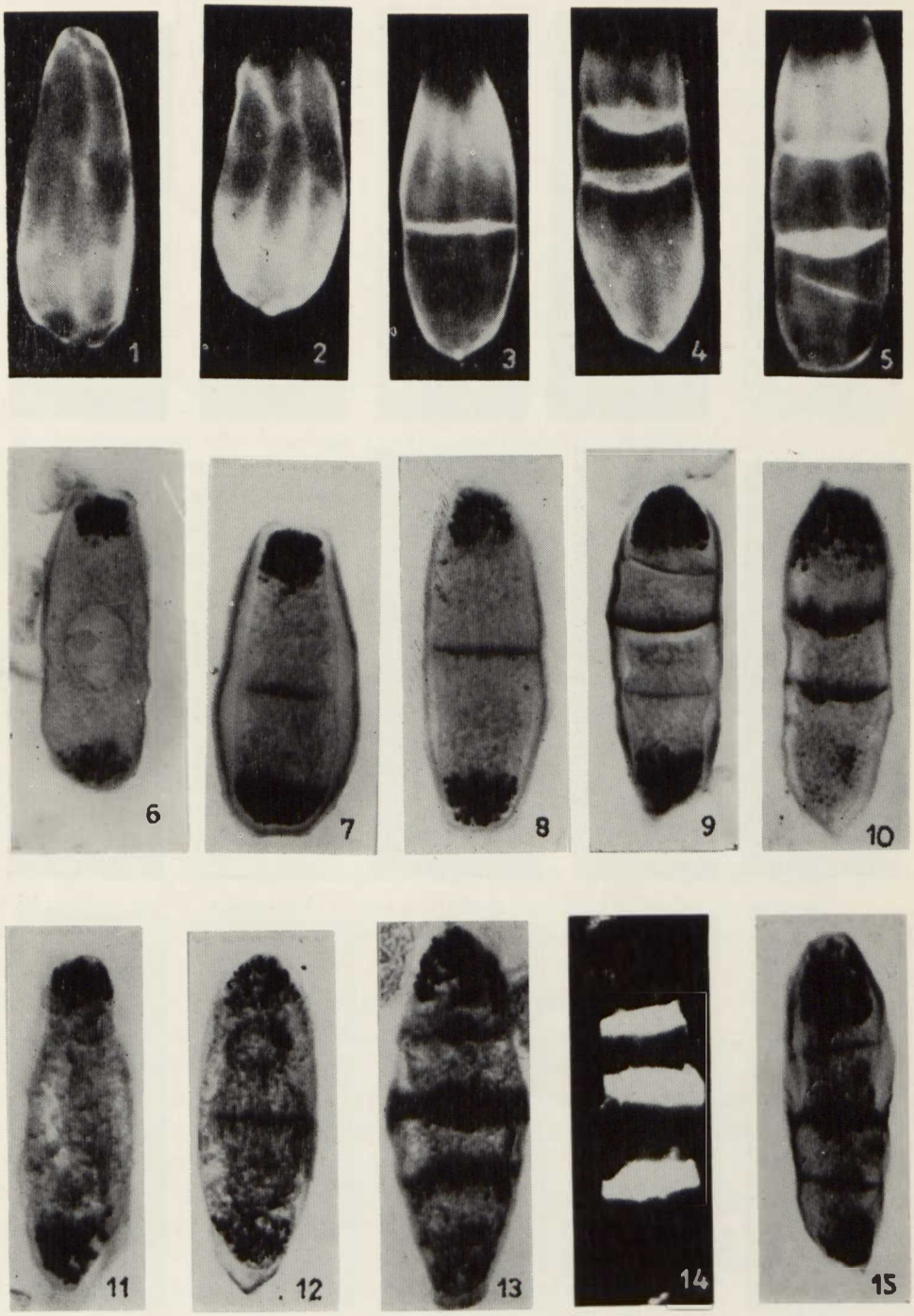
6—8. *Godetia grandiflora*. Callose fluorescence in cell walls of: 6, 7 — meiocytes, 8 — triad (× 1100).

9. *Oenothera rosea*. Meiocyte after PAS reaction (× 1100).

10. *Oenothera rosea*. Callose fluorescence in cell wall of meiocyte (× 1100).

11, 12. *Clarkia elegans*. After PAS reaction: 11 — meiocyte, 12 — triad (× 1300).

13—15. *Fuchsia hybrida*. After PAS reaction: 13, 14 — meiocytes, 15 — triad (× 700).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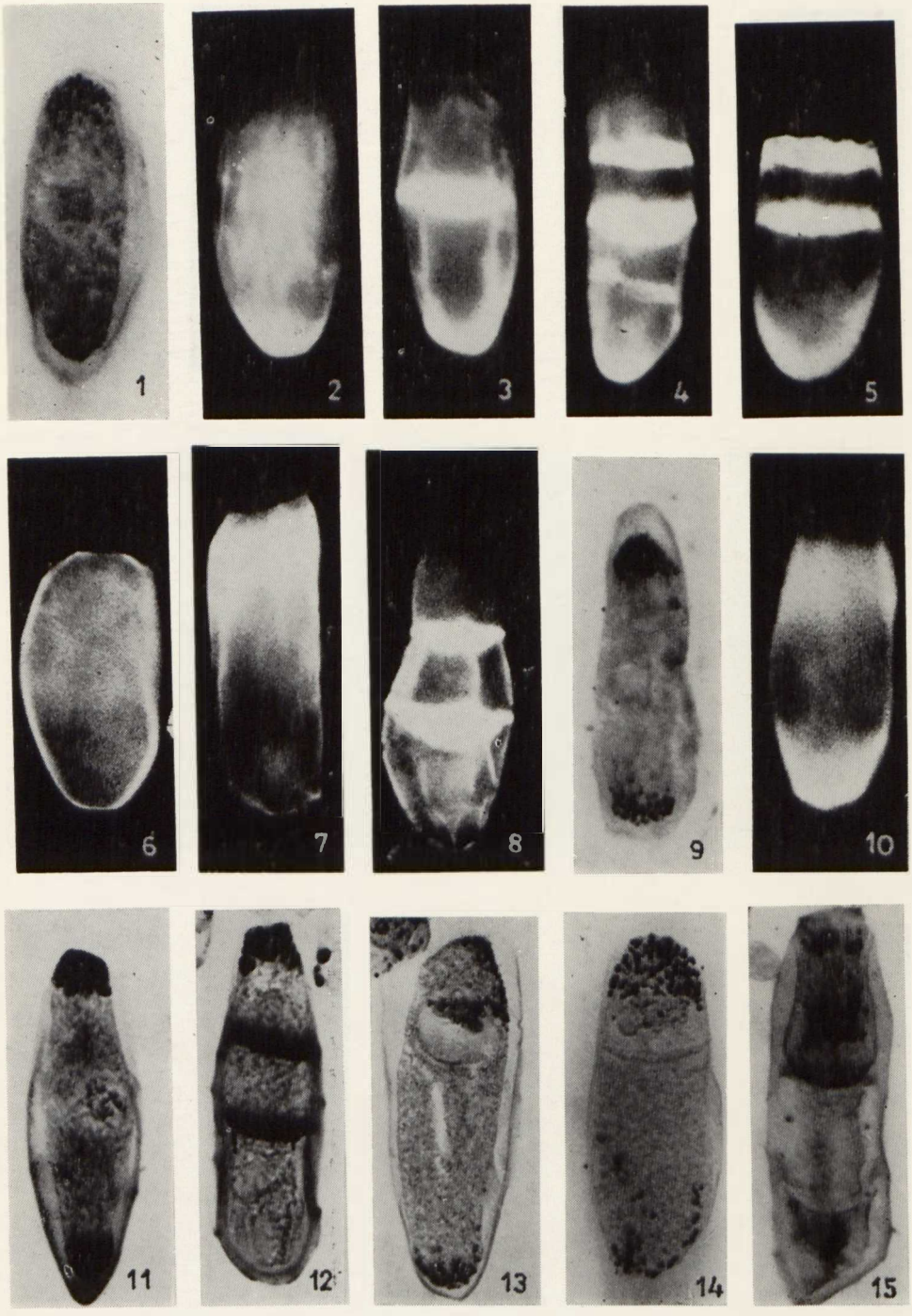
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