



Early stages of anther development in flowering plants

Ivan I. Shamrov^{1,2}, Galina M. Anisimova² & Anastasija A. Babro^{2*}

Ivan I. Shamrov^{1,2}
e-mail: ivan.shamrov@gmail.com

Galina M. Anisimova²
e-mail: galina0353@mail.ru

Anastasija A. Babro^{2*}
e-mail: ABabro@binran.ru

¹ Herzen State Pedagogical University,
St. Petersburg, Russia

² Komarov Botanical Institute RAS,
St. Petersburg, Russia

* corresponding author

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ABSTRACT

The analysis showed that when studying the anther of flowering plants there is a complex of unsolved embryological problems. First of all, they relate to the first stages of microsporangium initiation and time of archesporium differentiation. Many of classifications of microsporangium wall formation were worked out on the base of parietal tissue formation features. Of the 4 types of microsporangium wall formation proposed by Davis (1966), 2 types (basic and reduced) are controversial. In the classification by Teryokhin et al. (1993, 2002), the basic type was included as a complicated variation in the centripetal type, and the reduced type as a reduced variation in the centrifugal type. In our opinion, in angiosperms only two types of formation of microsporangium wall layers can be distinguished: centrifugal and centripetal. As our study has shown, the reduced type, as well as, the basic one, are variations of the centripetal type, while the reduced type is a modification of the typical variation. Various points of view on the formation of microsporangium layers are discussed not only from the outside of the parietal cells, but also from the lateral and internal sides.

Key words: anther, microsporangium wall formation, development, flowering plants

РЕЗЮМЕ

Шамров И.И., Анисимова Г.М., Бабро А.А. Ранние стадии развития пыльника у цветковых растений. Проведенный анализ показал, что при изучении строения и развития пыльника цветковых растений существует комплекс нерешенных эмбриологических проблем. Они касаются, прежде всего, начальных стадий заложения микроспорангия и времени дифференциации археспория. Большинство классификаций по формированию стенки микроспорангия разработаны на основе признаков парietальной ткани. Из 4 типов образования стенки микроспорангия, выделенных Дэйвис (1966), 2 типа (основной и редуцированный) являются спорными. В классификации, предложенной Терехиным и др. (1993, 2002), основной тип включен как компликатная вариация в центростремительный тип, а редуцированный тип рассматривается в качестве редуцированной вариации центробежного типа. По нашему мнению, у цветковых растений можно выделить только 2 типа: центробежный и центростремительный. Как показало наше исследование, редуцированный тип, как и основной, являются вариациями центростремительного типа, при этом редуцированный тип представляет собой модификацию типовой вариации. Обсуждаются различные точки зрения о формировании слоев микроспорангия не только с наружной стороны из клеток парietального слоя, но и с латеральных и внутренней сторон.

Ключевые слова: пыльник, формирование стенки микроспорангия, развитие, цветковые растения

Angiosperm anther is rather well studied. Some of its features discovered during comparative embryological investigations can be used to resolve controversial questions in systematics and phylogeny. First of all, types of microsporangium wall formation, types of microsporogenesis and microspore tetrad formation, structure of microsporangium wall, especially of endothecium and tapetum are among these features. A number of embryological problems making difficulties to appreciate the importance of many characteristics of male reproductive sphere have accumulated nowadays. The published data is subject to be systemized and re-thought. That is why the analysis of debatable problems in anther study was carried out.

Formation of microsporangium wall layers

Microsporangium wall is in a great importance in formation of haploid microspores in tetrads and male gametophytes (pollen grains). The developmental changes in its structure are coordinated with microsporogenesis and microgametogenesis. The 3 stages of its development are found out (Shamrov 2015). The first stage is initiation and differentiation of microsporangium wall layers (from archesporium up to sporogenous tissue). The second stage is the definitive formation of microsporangium wall (microsporocytes are before or in the beginning of meiosis). The wall (formed microsporangium wall) consists of epidermis, endothecium, middle layers, tapetum. The third stage in-

cludes transformation processes of microsporangium wall layers (from tetrad formation up to maturation of pollen grains). The layers are: epidermis, endothecium with fibrous thickenings or without them (mature microsporangium wall). The stages of microsporangium wall formation are congruent with premeiotic, meiotic and postmeiotic periods of anther development (Budell 1964, Kamelina 1981).

There is a discussion in literature about the way of development of layers in microsporangium wall. It is supposed usually that these layers on the distal side (it is situated opposite the connective) are being initiated by the activity of archesporial cells, differentiating in sub-epidermal layer in every microsporangium. In the inner and lateral sides the layers arise from meristem cells of connective (Davis 1966). That is why inner and outer segments of tapetum were singled out (Periasamy & Swamy 1966). According to Bhandari (1968), in *Anemone rivularis* Buch.-Ham. ex DC. (Ranunculaceae) tapetum formation from the connective tissue in the inner part of microsporangium (inner tapetum) is proved as it arises earlier than tapetum in the outer part of microsporangium (outer tapetum) does and it has a different structure. In *Coleus scutellarioides* (L.) Benth. and *Berberis vulgaris* L. outer tapetum originates from parietal cells (the cells, arising by the means of archesporial cell divisions), and inner one – from cells being situated near the sporogenous cells (Budell 1964).

The first classification of types of microsporangium wall formation was presented by Batygina et al. (1963). Using the ideas by Warming (1873) about two ways of layer formation in microsporangium wall two types were distinguished: Solanaceae (centrifugal way) and Umbelliferae (centripetal way). Later on, Carniel (1961) proved correct the centripetal way of formation of microsporangium wall layers in *Zea mays* L. Other types (Poaceae and Ericaceae) from this classification (Batygina et al. 1963) were selected because of tapetum arising from the derivatives of secondary archesporial cells (these types were not confirmed in literature further).

The classification by Davis (1966) is being used extensively in the literature. Depending on the direction of cell divisions in two initial parietal layers and the time of tapetum differentiation in microsporangium wall 4 types are distinguished. Parietal layers may be called differently: initial layer originated on the base of archesporium is the primary parietal layer, and its derivatives are secondary parietal layers (outer and inner parietal layers). There are following types of formation of microsporangium wall layers recognized by Davis: basic type (cells of the both of parietal layers divide almost synchronously and produce all the layers of the wall except epidermis), dicotyledonous type (cells of the inner parietal layer differentiate into tapetum, while cells of the outer one produce endothecium and middle layers through multiple divisions), monocotyledonous type (cells of the outer parietal layer differentiate into endothecium and the middle layers, when tapetum is formed as a result of cell divisions of inner parietal layers) and reduced type (inner parietal layer forms tapetum, and outer one – endothecium).

The basic type, according to Davis (1966), is described in 9 families, dicotyledonous type in 43 families, monocotyledonous type in 26 families, reduced type in 2 families.

Kamelina (1991, 2009) provides updated data at the time of their analysis. In her opinion, among 165 families of angiosperms the next types of microsporangium wall formation were found: dicotyledonous type (83), basic (36), monocotyledonous (44), reduced (2). But these data are not definitive. Many families of flowering plants remain embryologically unexplored. On the other hand, the study of the anther, as well as of many other embryonal structures, has turned only into a compilation of characteristics for the purposes of taxonomy, solving problems of developmental biology. Detailed studies on the genesis of structures have become rare and not always accurate. So, to explain the directions of evolution of dioecy in 3 species of *Echinocereus* (Cactaceae), two illustrations are given, which, according to the authors (Hernández-Cruz et al. 2018), indicate a monocotyledonous type of microsporangium wall formation. However, in the provided photographs it is clear that the tapetum differentiates first followed by the middle layer and endothecium, i.e. the microsporangium wall develops according to the dicotyledonous type.

The basic and the reduced types proposed by Davis (1966) are very controversial in this classification. When substantiating a basic type, it is very important to find out synchronous cell divisions in the both parietal layers but there are extremely few documented examples of this pattern. Reduced type is characteristic for some species of Araceae and Hydrocharitaceae families (according to APG 1998). Analysis of the literature data shows that in *Wolffia microscopica* (Griff.) Kurz (Maheshwari 1954) and *Lemna paucicostata* Hegelm. (Maheshwari & Kapil 1963) cells of the parietal layer divide only one time. Two layers arise – endothecium and tapetum. But in *L. gibba* L. and *L. trisulca* L. (Lodkina 1971) and *Najas lacerata* Rendle (Swamy & Lakshmanan 1962) the middle layer forms in microsporangium wall as a result of cell divisions of inner parietal layer as well. In other words, these species have not reduced type of microsporangium wall formation, but the monocotyledonous one.

By the other classification (Teryokhin et al. 1993, 2002) all the diversity of formation types of microsporangium wall may be reduced to two: centripetal and centrifugal ones. There are 3 variations distinguished in the centripetal type: typical (monocotyledonous type, according to Davis 1966), complicated (basic type, by Davis 1966), and prolonged (multiple cell divisions of inner parietal layer and the later differentiation of tapetum). In the centrifugal type there are also 3 variations: typical (dicotyledonous type, by Davis 1966), complicated (multiple cell divisions of the outer parietal layer) and reduced (reduced type by Davis 1966). The naturality of inclusion of basic type within the complicated variation into the centripetal type especially can be seen by examination of microsporangium wall formation in *Begonia manicata* Brongn. (Begoniaceae) (Anisimova 1983). In this case, the cells of the outer parietal layer have already undergone divisions and two sub-epidermal layers have formed, at the time when the cells of the inner parietal layer still divide and differentiation of tapetum cells have not happen yet. In our opinion, the reduced type is closer to

centripetal, too. For example, it is demonstrated by the data on microsporangium wall formation in *Zannichellia pedunculata* Rchb. (Zannichelliaceae) (Kamelina & Teryokhin 1990). The cells of inner parietal layer divide periclinally, producing middle layer and tapetum as in the case of typical variation of centripetal type. The middle layer disintegrates during early stages of microsporogenesis and often it is not taken into account in the formed microsporangium wall characteristics. As a matter of fact, the type of microsporangium wall formation in this species is centripetal, but not centrifugal (Teryokhin et al. 1993, 2002). Thus, the basic and reduced types (according to Davis 1966) are the modified variants of the centripetal type. Inclusion of the basic type into the centripetal type as a complicated variation is quite obvious by reference to the genesis peculiarities of layers in microsporangium wall. As for the reduced type, the absence of the middle layer in microsporangium wall in some species, its presence in others and instability of this feature within the related species and families Lemnaceae, Najadaceae, Zannichelliaceae and other families of Alismatales order characterized by ephemeral middle layer (Aponogetonaceae, Potamogetonaceae, Ruppiaceae, Scheuchzeriaceae), indicate the tendency to reduction of middle layers number in these plants, that probably came into existence during adaptation to life in water.

It should be mentioned that Kordyum (1978) also names the dicotyledonous type as centrifugal, and monocotyledonous – as centripetal one. This author does not distinguish any variations in the types. Like Davis (1966), she considers that all the types are the derivatives of the basic type. It is difficult to agree with this opinion. We have already mentioned that synchronous divisions in the both of parietal layers are extremely few and the monocotyledonous type may be easily mistaken for the basic one without finding out the consequence of derivative layers formation. In both types tapetum is the last to differentiate.

Only one type of microsporangium wall formation is considered to exist within each angiosperm family (Davis 1966). Nevertheless, the study of a large number of representatives of Solanaceae has shown that this process comprises a combination of different cell division consequences in parietal layers and their derivatives. Possible combinations of types were revealed on the base of this fact (Garcia 2003).

Based on the presented facts, in angiosperms only two types of formation of microsporangium wall layers can be distinguished: centrifugal (typical variation – dicotyledonous type, according to Davis, 1966; complicated variation) and centripetal (typical variation – monocotyledonous type, according to Davis 1966; complicated – basic type, according

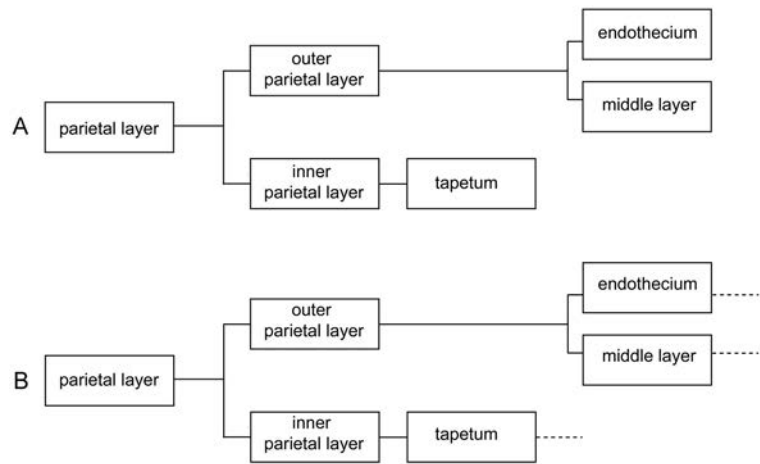


Figure 1 Centrifugal type of microsporangium wall development. Note: Triple dots indicate the possibility of divisions and formation of new layers inside of each tissue type. A – typical variation; B – complicated variation

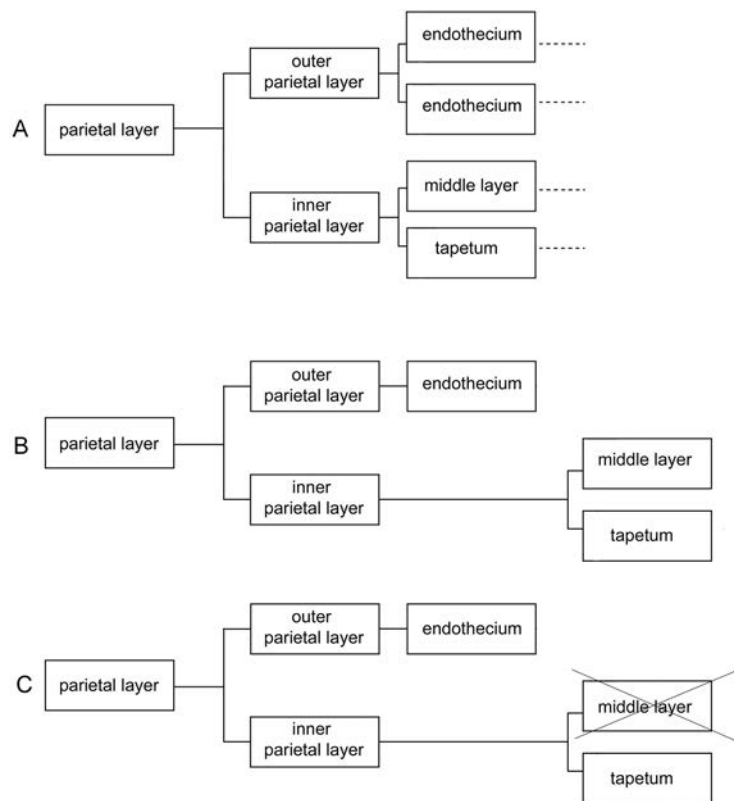


Figure 2 Centripetal type of microsporangium wall development. Note: Triple dots indicate the possibility of divisions and formation of new layers inside of each tissue type. Cross indicates the ephemeral state of middle layer, or its reduction. A – complicated variation; B – typical variation; C – reduced variation

to Davis 1966; reduced variation – reduced type, according to Davis 1966) (Fig. 1A–B, 2A–C). It should be noted that our understanding of complicated variation differs from the understanding adopted in the classification by Teryokhin et al. (1993, 2002). These authors considered that the main sign of complicated variation is the ability to form multi-layered tissues. They noted a similar thing for typical variation. We believe that only cases of microsporangium wall formation should be attributed to typical variation, when it consists

of only 4 layers (epidermis, endothecium, middle layer, and tapetum). The main argument for highlighting complicated variation is the phenomenon of the formation of multi-layered endothecium. An analysis of the available data indicates that in plants with the basic and dicotyledonous types of wall formation of microsporangium (according to Davis 1966), fibrous thickenings are formed not only in the subepidermal, but also in the remaining underlying layers. Consequently, the formation of multi-layered endothecium is characteristic of both centrifugal and centripetal types.

The problem of layer formation in microsporangium wall is preconditioned by the use of the term “archesporium” to a great extent. Many of the researchers often do not take into account the stage of sporangium development when archesporium differentiation occurs so the terms “sporangium initial” and “archesporium” became equated. The cells differentiating first in the corners of forming microsporangia within the anther or in the ovule primordium are usually called archesporium (Maheshwari 1950, Singh 1978, Bouman 1984, Kamelina 2002).

Peculiarities of archesporium genesis in other higher plants may help to solve this problem. We can see numerous cell divisions and the series of processes from sporangium initial up of archesporium differentiation when analyzing the development of sporangium according to leptosporangiate type. An archesporium in sub-epidermal layer of sporangium emerges only after the end of layer setting off in microsporangium wall on its inner and lateral sides. Only after that the archesporial cell divides forming sporogenous cell (inwards) and tapetal, or parietal ones (outwards). While sporangium forms according to eusporangiate type (from a number of epidermal cells) the complex of initial cells of sporangium arises in epidermis. Lateral and inner cells of this complex organize lateral and inner parts of the sporangium, respectively (Bower 1935, Smith 1938, Camefort et al. 1997). The archesporium produce sporogenous cells (centripetally) and epidermal cells, that compose the outer part of sporangium wall further (centrifugally) as a result of its divisions. Ultimately, all these cells of developing sporangium form the complex of cells of epidermal origin while the influence of sub-epidermal and underlying cells on its genesis usually is not taken into account (Shamrov 2008b).

Just because of the difficulties arisen in the rendering of the term “archesporium” some researchers (Sladkov & Grevtsova 1988) proposed to specify the sub-epidermal cells in distal part of locule wall (and also in the apical part of nucellus) as “sporangium and archesporium initial cells”, and to use the term “archesporium” for the cells producing spores only, i.e. instead of the words “sporogenous cell” or “micro- and megasporocyte”. But the question of the origin of microsporangium wall layers on its lateral and inner sides stays unresolved with such a rendering of archesporium.

Micro- and megasporangium formation in higher plants, including flowering plants, have common features. In the case of epidermal or sub-epidermal origin in gymnosperms (Brunkner 1973, Singh 1978) and sub-epidermal origin in angiosperms (Shamrov 2008a, b) the initials of the entire sporangium arise first of all. In megasporangium (nucellus) of flowering plants, the derivatives of inner area (which

transforms into the basal zone of nucellus) detach themselves firstly by means of differentiating divisions of central initials; the archesporium cells arise centrifugally. The lateral zone of nucellus forms as a result of division of lateral initials (Shamrov 2008a, b). Archesporial cells (usually in crassinucellate ovules) also undergo divisions and produce parietal cells outside (centrifugally) and sporogenous ones inside (centripetally). In developing tenuinucellate ovules with nucellus represented by epidermal layer only, the initials transform into sporogenous cells and in megasporocytes further without any divisions.

We have already mentioned, that the majority of authors describe the layer formation on a distal side of a microsporangium by means of activity of archesporial cells. There are other points of view regarding the formation of microsporangium wall layers. In *Helianthus ciliaris* DC. and *H. tuberosus* L. the wall layers of the inner part of microsporangium are to form from the parietal layer which is situated under the sporogenous cells (Babro & Voronova 2018). Other researchers describe initially cell division in sub-epidermal layer of microsporangium that results in formation of the archesporial cell and lying under it parietal cell. Division of archesporial cells leads to formation of the parietal cell over it likewise, and further – to formation of the parietal layer, that is from what microsporangium wall on the outer side develops (Torshilova & Batygina 2005). In opinion of some authors (Carniel 1961, Batygina et al. 1963, Bhandari & Khosla 1982), the whole microsporangium wall including tapetum has parietal origin.

Researchers came to this conclusion by analyzing data on the development of anther, mainly *Arabidopsis thaliana* (L.) Heynh (Scott et al. 2004). In each microsporangium archesporial cells arise, during the division of which primary parietal cells (outward) and primary sporogenous cells (inward) are formed. Primary sporogenous cells divide and are transformed into meiocytes, while primary parietal cells divide periclinally, separating outward endothecium, and inward – secondary parietal cells. The latter are divided again, forming the middle layer and tapetum. Based on the genes involved in the first stages of development, the authors proposed a model for the development of microsporangium, in which sporogenous cells play the main organizing role. Primary sporogenous cells and their derivatives (secondary sporogenous cells) create radial fields around themselves and cause surrounding cells to divide periclinally, regardless of their origin, i.e., both primary and secondary parietal cells, as well as any adjacent cells. Thus, even with the involvement of molecular-genetic data, the question of the origin of the layers of the lateral and internal parts of the wall of microsporangia remains unresolved.

We have already noted that in the megasporangium (nucellus) of flowering plants of sub-epidermal origin, the initials of the entire sporangium arise first of all. In megasporangium, the derivatives of inner area detach themselves firstly by means of differentiating divisions of central initials. The archesporial cells arise centrifugally. The lateral zone of nucellus forms as a result of division of lateral initials.

During microsporangium formation in some angiosperms (*Allium caspium* (Pall.) M. Bieb., Alliaceae – Shamrov

2006, *Rhododendron schlippenbachii* Maxim., *R. luteum* Sweet, Ericaceae – Shamrov & Babro 2008, *Kalanchoe nyikae* Engl., Crassulaceae – Anisimova 2016, *Euphorbia seguieriana* Neck., *E. stepposa* Zoz ex Prokh., Euphorbiaceae – Anisimova 2019) the group of initial cells (central and lateral) differentiates in sub-epidermal layer which produce both microsporangium wall layers and archesporium. As a result of periclinal division of the central sub-epidermal cell, the archesporial cell arises centrifugally, and the cell forming proximal (situated closer to the connective) part of microsporangium wall - centripetally. Lateral sub-epidermal initial cells form lateral parts of microsporangium wall by the means of divisions. The outer layer (parietal cells) and the inner one (sporogenous cells) arise as a result of periclinal divisions of archesporial cells. The cells of parietal layer divide periclinally and anticlinally producing layers of the distal part of microsporangium wall (Fig. 3A–H, 4A–B, 5A–D). Sporogenous cells undergo a number of divisions and then transform into microsporocytes, or microspore mother cells.

Characteristics of microsporangium wall layers

The layers of microsporangium wall differ not only by the features of their genesis but by the structural characteristics, too. Every layer differs by the structure of its cell and carries out the specific function (Dafni 2000, Quilichini et al. 2014). Epidermis cells vary by size and perform various functions: protects, ensures gas exchange and transpiration, and also takes part in attraction of pollinators (Goldberg et

al. 1993, Rezanejad 2008). The cells often stretch, small vacuoles combines in a single one, driving the nucleus away to the periphery. Outer tangential cell wall thickens and covers itself by cuticle (Fig. 4E, 5E).

The layer located just under the epidermis i.e. the outermost of the layers originated by parietal cell divisions forms endothecium achieving the higher grade of development at the time of pollen distribution (Fig. 4E). Many fibrous thickenings depart from the inner walls of endothecium cells. The cells of this layer lose their content early; they provide anther dehiscence in the area of stomium, usually by longitudinal slits in every theca. Dehiscence of thecae occur ordinary after disintegration of their septa.

Endothecium with fibrous thickenings can be found only in the outer side of anther in some plants: in *Drymis winteri* (Winteraceae) (Bhandari & Venkataraman 1968), *Bauera capitata* (Cunoniaceae) (Prakash & McAlister 1977). In addition, in some plants with basic and dicotyledonous types of microsporangium wall formation, fibrous thickenings appear not only in sub-epidermal layer, but in other remaining subjacent layers. Such layers are often named as endothecium and middle layers with fibrous thickenings. However, some authors (Teryokhin et al. 1993) consider that only the cells with fibrous thickenings may be named endothecium (when the thickenings form in sub-epidermal and subjacent layers endothecium is multilayered). It is suggested to specify all the cell layers between tapetum and epidermis as “the tissue of middle layers” on the early

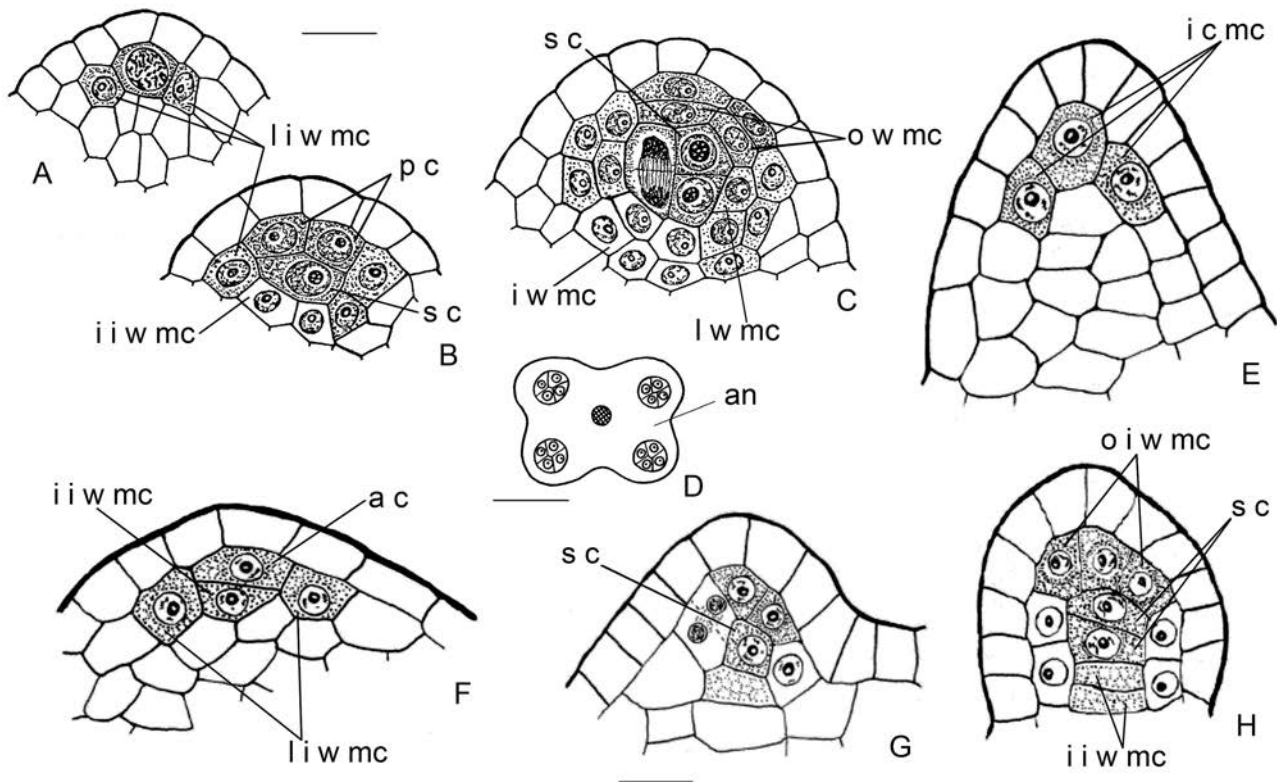


Figure 3 Early stages of microsporangium formation. A–D – *Allium caspium* (Pall.) M. Bieb. (Alliaceae), E–H – *Rhododendron schlippenbachii* Maxim. (Ericaceae). an – anther, ac – archesporial cell, icmc – initial cells of microsporangium, iiwmc – inner initials of microsporangium wall, iwmc – inner part of microsporangium wall, liwmc – lateral initials of microsporangium wall, lwmc – lateral part of microsporangium wall, oiwmc – outer initials of microsporangium wall, owmc – outer part of microsporangium wall, pc – parietal cell, sc – sporogenous cell. Scale: A–C, E–H – 10 µm, D – 50 µm

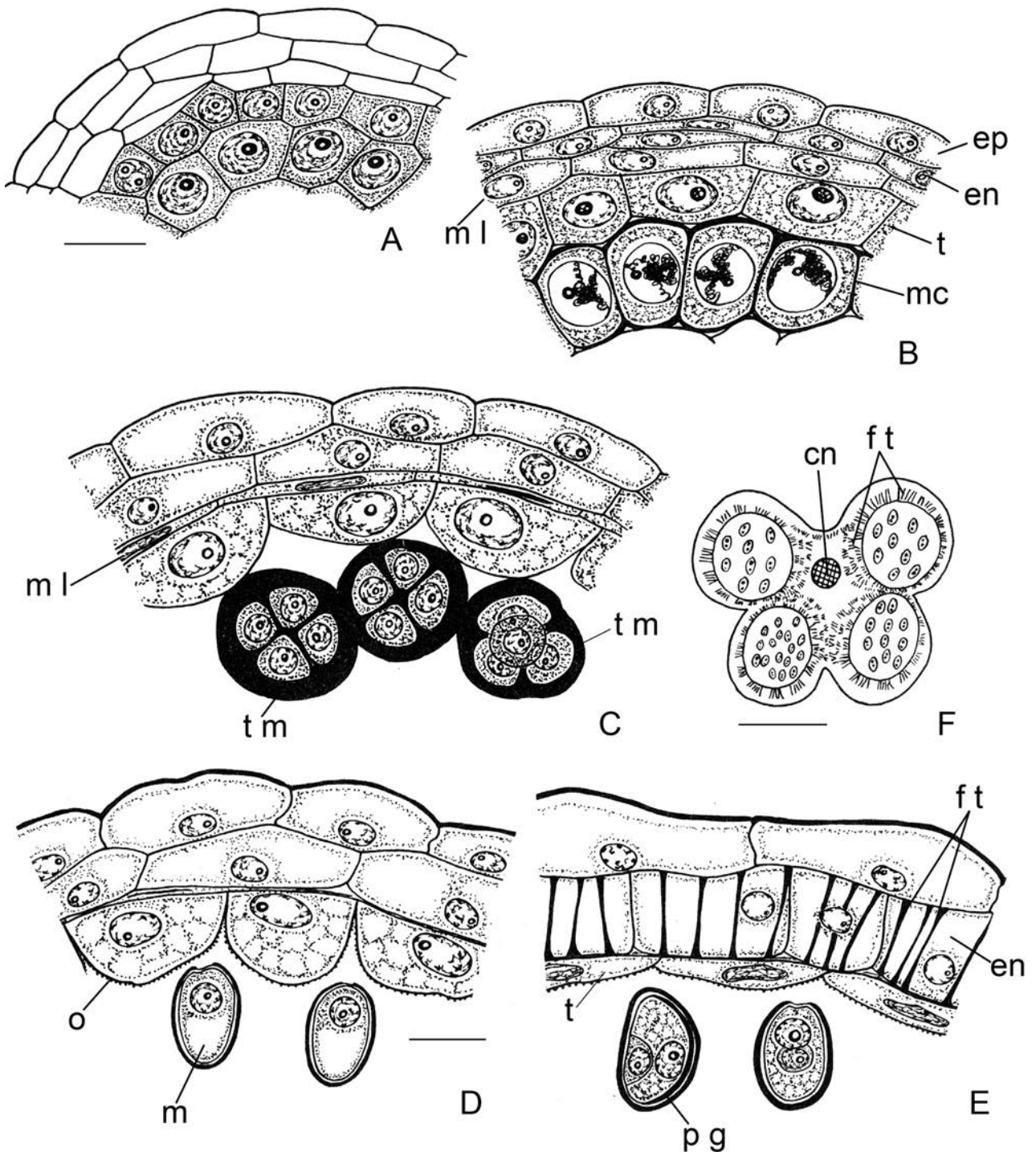


Figure 4 Cell differentiation and specialization of wall microsporangium layers in *Allium caspium* (Pall.) M. Bieb. (Alliaceae). A – sporogenous tissue stage, B – microsporocytes at meiosis beginning, C – microspore tetrads, D – vacuolized microspores, E – mature pollen grains, F – anther scheme before dehiscence. cn – connective, en – endothecium, ep – epidermis, f t – fibrous thickenings, m – microspore, mc – microsporocyte, m l – middle layer, o – orbicules on tapetal membrane, p g – pollen grain, t – tapetum, t m – microspore tetrad. Scale: A–E – 10 μ m, F – 50 μ m

stages of their development and to estimate presence and characteristics of endothecium (is it present, consists of one or more layers, or absent) only after formation of the fibrous thickenings. It should be noted that fibrous thickenings arise also in the most of connective cells surrounding vascular bundle in a number of plants (Fig. 4F).

Neither the plants with flowers developing in water in the case of hyphydrogamy (Ceratophyllaceae, Cymodoceaceae, Najadaceae, Hydrocharitaceae) nor the species with

cleistogamous flowers have fibrous thickenings in microsporangium wall. The plants with anthers opening by apical pores (Asclepiadaceae, Ericaceae, Solanaceae) have no fibrous thickenings either. The walls of epidermal cells produce the thickenings (therefore such a layer is called exothecium by analogy with endothecium) and also the walls of cells underlying pores like a roll (Shamrov & Babro 2008, Shamrov 2015). The special case is described in *Chrysanthemum morifolium* Ramat. (Asteraceae) (Fei et al. 2016). Its cul-

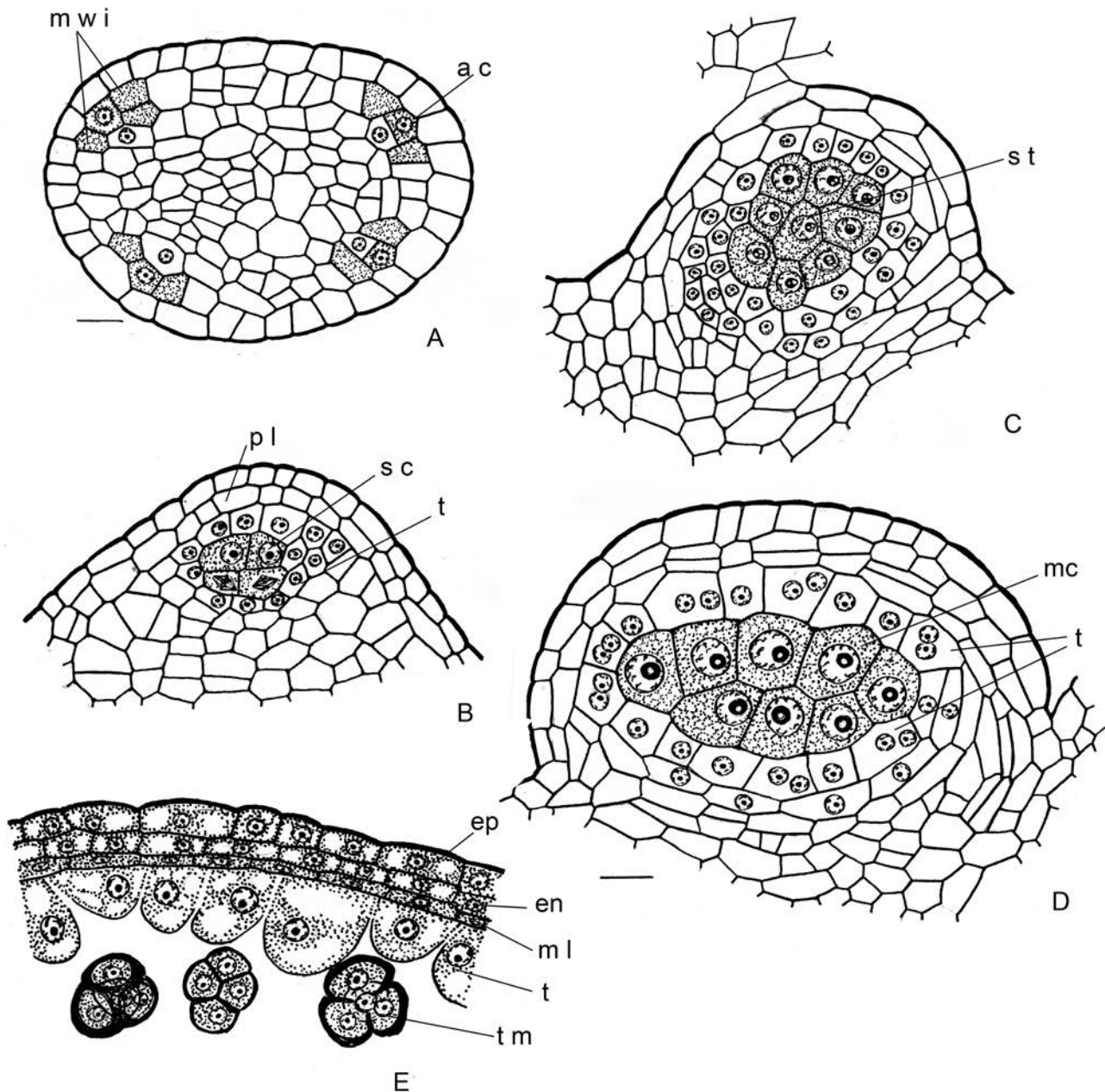


Figure 5 Structure and development of the anther in *Euphorbia seguieriana* Neck. A – differentiation of microsporangium wall initials and archesporium appearance; B–D – microsporangium wall formation and sporogenous tissue, cells of which are before microspogenesis, E – first signs of cellular secretory tapetum reorganization into ameboid tapetum at microspore tetrads, cell walls are destroyed. a c – archesporium cell, en – endothecium, e p – epidermis, mc – microsporocyte, m l – middle layer, m w i – microsporangium wall initials, p l – parietal layer, s c – sporogenous cells, s t – sporogenous tissue, t – tapetum, t m – tetrad of microspores. Scale: A–E – 10 μ m

tivar with dehiscent anthers (Qx-097) has endothecium with fibrous thickenings. In another cultivar (Qx-007) anthers do not dehisce as a result of fibrous thickenings missing in endothecium. In addition the latter have septa in theca and stomium completely remained, enlarged cells of the anther, high hydration and high quantity of K^+ and Ca_2^+ ions.

There are one or more middle layers under endothecium; they consist of not very large cells and as a rule disintegrate after completion of meiosis in microspores mother cells. In the course of development the transitory starch may accumulate in cells of middle layers (Clément & Pacini 2001).

The innermost layer of microsporangium wall is tapetum. Its cells often have tabular shape, are filled with dense

cytoplasm and contain one or sometimes several nuclei. Tapetum may be homomorphous (its cells have similar structure) or heteromorphous (cells placed near connective are often larger, papilliform, and the number of layers may be more) (Kamelina 1981, 2009). The main function of tapetal tissue is to provide microsporocytes, developing microspores and pollen grains with nutrients, the most important of which are polysaccharides, enzymes, hormones, accumulating within microsporangium locule (Pacini 2010).

Tapetal cells secrete callase – the enzyme for dissolution of callose walls of microspore tetrads; they produce sporopollenine for exine and orbicules (Ubish bodies) that are usually located on the inner membrane and contact the

developing pollen grains; they take part in composition of pollenkit (consists of lipids and carotenoids) and trifina (consists of a mixture of hydrophilic and hydrophobic substances) encouraging pollination by insects (El-Ghazaly 2002). In addition, the substances inhibiting processes of endothecium cell specialization during all the period of microsporogenesis are being synthesized in tapetum. After the completion of sporopollenin production the inhibiting action terminates and tapetum begins to disintegrate; usually it takes place at the stage of vacuolated microspores. The formation of thickenings in endothecium cells occurs only after that (Chauhan 1977). When tapetum persists for a long time, especially in plants with cytoplasmic male sterility the abortion of pollen can be seen, endothecium cells do not pass to fibrous thickening formation, anthers do not dehisce (Chauhan 1979, Chauhan & Gupta 2006). In some plants such processes are normal. In *Escallonia rubra* (Escalloniaceae) (Kamelina 1985) the start of fibrous thickenings formation in the cells of 2–3-layered endothecium takes place only on the stage of 2-celled pollen grain with the generative cell being situated near the sporoderm. Mature pollen in this species is 3-celled. Tapetum persists for a long time and disintegrates just before anther dehiscence. The longevity of tapetum, which ceases in the period of pollen grains formation, results in more late start of fibrous thickenings genesis in endothecium in some Euphorbiaceae (*Euphorbia iberica*, *E. petrophila*, *E. seguieriana*, *E. stepposa* – Anisimova 2019). As a rule, tapetum cells do not persist in mature anther.

CONCLUSION

At present, there is a complex of unresolved problems in anther study of flowering plants. They concern the initial stages of microsporangium development, the ways of layer formation in microsporangium wall and characteristics of layers in course of development. It is difficult to agree with the opinion on parietal origin of the layers on all of the parts of microsporangium wall. Parietal layer can not arise on the lateral parts or near the connective. The cells, layers, or tissues, originating by the means of archesporial cell divisions are called parietal. It concerns both male and female reproductive spheres. Parietal tissue can be easily detected visually in the nucellus of crassinucellate ovule by the character of cells (they have more dense cytoplasm). It has a shape of a segment and spreads from epidermis to megasporocyte (or megasporocytes, if there are more than one) and, further, to embryo sac. The number of layers in this tissue depends on nucellus massiveness; its width is an average of 3–5 cells in *Paeonia lactiflora* Pall. (Paeoniaceae), 3–7 cells in *Ceratophyllum demersum* L. (Ceratophyllaceae) (Shamrov 2008a). The parietal tissue composing the distal part of microsporangium wall in *Datisca cannabina* L. (Datiscaceae) (Kamelina 1983), *Acer rufinerve* Siebold & Zucc. (Aceraceae) (Alimova 1985) can be identified in a similar way. Many of classifications of microsporangium wall formation were worked out on the base of parietal tissue formation features. Of the 4 types of microsporangium wall formation proposed by Davis (1966), 2 types (basic and reduced) are controversial. As we have already

noted, in the classification by Teryokhin et al. (1993, 2002), the basic type was included as a complicated variation in the centripetal type, and the reduced type as a reduced variation in the centrifugal type. As our study has shown, the reduced type, as well as, the basic one, are variations of the centripetal type, while the reduced type is a modification of the typical variation.

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