



Suggested mechanisms underlying pollen wall development in *Ambrosia trifida* (Asteraceae: Heliantheae)

Nina Gabarayeva¹ · Svetlana Polevova² · Valentina Grigorjeva¹ · Elena Severova² · Olga Volkova² · Stephen Blackmore³

Received: 6 May 2018 / Accepted: 11 October 2018
© Springer-Verlag GmbH Austria, part of Springer Nature 2018

Abstract

By a detailed ontogenetic study of *Ambrosia trifida* pollen, tracing each stage of development with TEM, we aim to understand the establishment of the pollen wall and to unravel the mechanisms underlying sporoderm development. The main steps of exine ontogeny in *Ambrosia trifida*, observed in the microspore periplasmic space, are as follows: spherical units, gradually transforming into columns, then to rod-like units; the appearance of the initial reticulate tectum; growth of columellae under the tectum and initial sporopollenin accumulation on them; the appearance of the endexine lamellae, first in fragments, then in long laminae; the cessation of the glyocalyx growth and its detachment from the plasma membrane, resulting in the appearance of gaps; massive accumulation of sporopollenin on the tectum, columellae, and endexine, and the appearance of the foot layer at the young post-tetrad stage, accompanied by establishment of caveae in sites of the former gaps; and final massive sporopollenin accumulation. This sequence of developmental events in all probability corresponds to the sequence of self-assembling micellar mesophases. This gives (together with earlier findings and experimental modeling of exine) strong evidence that the genome and self-assembly share control of exine formation. In this sense, self-assembly itself can be seen as an inherent mechanism of evolution.

Keywords Sporoderm development · Pattern formation · Micelle self-assembly

Introduction

Understanding morphogenesis in plants is profoundly important. In developmental biology, pollen development provides an ideal model system for the understanding of differentiation. Plant development involves a complex interplay of physical, chemical, and biological factors (Sampathkumar et al. 2014).

Tompson's (1917) ideas on self-assembly of complex biotic and abiotic patterns in nature were applied by Wodehouse (1934) to pattern determination in pollen. The

sporopolleninous spore and pollen walls have a complex sub-structural organization, established, as we believe following Heslop-Harrison (1972), through time by both biological (genomic) processes and purely physico-chemical process of self-assembly, working in tandem. Despite its great importance for plant reproductive biology, much remains to be understood about how the vast diversity of pollen walls arises during development.

Pollen of *Ambrosia* has been the focus of many studies both concerned with its morphology and evolution (see, for example, Stix 1960; Skvarla and Larsen 1965; Punt and Hoen 2009; Blackmore et al. 2009) and its importance as an airborne allergen and significant cause of hay fever (Wodehouse 1928; Makra et al. 2005; Martin et al. 2009; Thio et al. 2009; McLauchlan et al. 2011). A light microscopy study of tapetum development (and concomitant microspore stages) in *Ambrosia trifida* has been published by Lersten and Curtis (1990). Being an anemophilous plant producing vast quantities of pollen has also made *A. trifida* an experimental plant in which to study the optical properties of the exine (Atkin et al. 2011).

Handling Editor: Peter Nick

✉ Nina Gabarayeva
1906ng@mail.ru

¹ Komarov Botanical Institute, Russian Academy of Sciences, Popov St. 2, St. Petersburg, Russia 197376

² Moscow State University, Leninski Gory, 1, Moscow, Russia 119991

³ Royal Botanic Garden Edinburgh, 20a Inverleith Row, Edinburgh EH3 5LR, UK

The complex, thick-walled pollen grains of Asteraceae have also attracted interest from the perspective of development and pattern formation, especially the tetrad stage (see Blackmore and Barnes 1987; Blackmore and Claughner 1987; Blackmore 1990; Blackmore et al. 2009, 2010). It was shown by many ontogenetic palynologists that the tetrad stage is a key one in the determination of the exine pattern (Gabarayeva 2000; Blackmore et al. 2007, 2010; Vinckier and Smets 2005; Taylor and Osborn 2006; Taylor et al. 2013).

Our goal was to further understand the underlying developmental mechanisms of the assembly of pollen wall. Accordingly, our main aims were (1) to determine the complete sequence of processes leading to the emergence of the complex exine construction, (2) to compare our findings with those of earlier ontogenetic studies of Asteraceae and other taxa, and (3) to determine whether our hypothesis (Gabarayeva and Hemsley 2006; Hemsley and Gabarayeva 2007) on the role of micelle self-assembly processes in spore and pollen wall development also applies to *Ambrosia* pollen development. Micelles are supramolecular aggregates of long-chained diphilic molecules of surface-active substances which form spontaneously in solutions at specific concentrations, exceeding the so-called “critical micelle concentration.” The mentioned hypothesis suggests that many of the developmental events in exine formation proceed as a cascading sequence of self-assembling micelle mesophases and their aggregates in the colloidal system of the periplasmic space. The latter contains surface-active glycoproteins and lipopolysaccharides of the glycocalyx (Rowley 1973; Rowley and Dahl 1977; Pettitt 1979) at concentrations which increase during the tetrad period—the necessary condition for the appearance of sequential micellar mesophases (Fridrichsberg 1995). Later in development, lipoid SP precursors and monomers (see a review in Gabarayeva et al. 2016) are added to the periplasmic space (the essential arena of early sporoderm development), making the supporting medium hydrophobic. All these substances form colloidal solutions in the aqueous medium of the periplasmic space. Being colloid systems of surface-active substances in the course of development, exines are subjects of self-assembly. From the thermodynamics point of view, the energy necessary for aggregation of colloidal particles is less than the energy necessary for their dissociation. Hence, the formation of aggregates (micellar mesophases) is energy favorable, and the whole colloidal system will have a propensity to aggregation of colloidal particles (Hemsley and Griffiths 2000).

A growing body of evidence from a wide range of taxa documents a common sequence of micellar phases (Gabarayeva and Grigorjeva 2017; Grigorjeva and Gabarayeva 2018) and a universal importance of self-assembly in nature (e.g., Ingber 2003a, 2003b). We suggest that though the genome determines the exact chemical composition of all the substances and their concentrations,

necessary for exine development in the microspore periplasmic space, the reminder of the constructive process is determined by physico-chemical self-assembly.

We also intend to trace tapetum development and the mode of formation of the caveae which are associated with anemophily, causing an overall reduction in density which enables the pollen to float in air (Blackmore et al. 1984).

Material and methods

Plants of *Ambrosia trifida* were grown from seeds in the greenhouse of Lomonosov Moscow State University. For TEM, about 60 flower buds were collected to provide a complete sequence of stages. The material was fixed for 2 h at 20 °C in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) with added tannic acid, de-aerated under vacuum. After post-fixation in 2% osmium tetroxide for 10 h at 4 °C followed by acetone dehydration, the samples were embedded in the following mixture: Araldite = 19.0 ml (22.0 g), DDSA = 21.0 ml (21.0 g), and DMP-30 = 0.5 ml (0.6 g).

For TEM, ultrathin silver sections 60–70 nm in thickness were contrasted with 1% ethanol solution of uranyl acetate and 0.2% lead citrate. Sections were examined with a Hitachi H-600 transmission electron microscope.

Results

The tetrad stage

Post-meiotic and early tetrad stage

Prior to meiosis, a callose special cell wall forms around each microsporocyte. After meiosis and simultaneous cytokinesis, the tetrads are formed (Fig. 1a). During the post-meiotic stage, further callose deposition forms an individual special cell wall (SCW) around each of the four microspores (Fig. 1b). Higher magnification reveals spherical units on the plasma membrane (Fig. 1c, arrowheads) and shows that the spherical units have denticulate surfaces (Fig. 1d, arrowheads). Slightly later, many spherical units (arrowheads) with electron-lucent cores and some elongated (rod-like, double arrowheads) units appear in the periplasmic space (Fig. 1e, f).

Young tetrad stage

Later, the glycocalyx material accumulates at the plasma membrane surface surrounding each microspore, increasing the periplasmic space (Fig. 2a). Clusters of spherical units arranged into columns and oriented perpendicular to the plasma membrane (Fig. 2b).

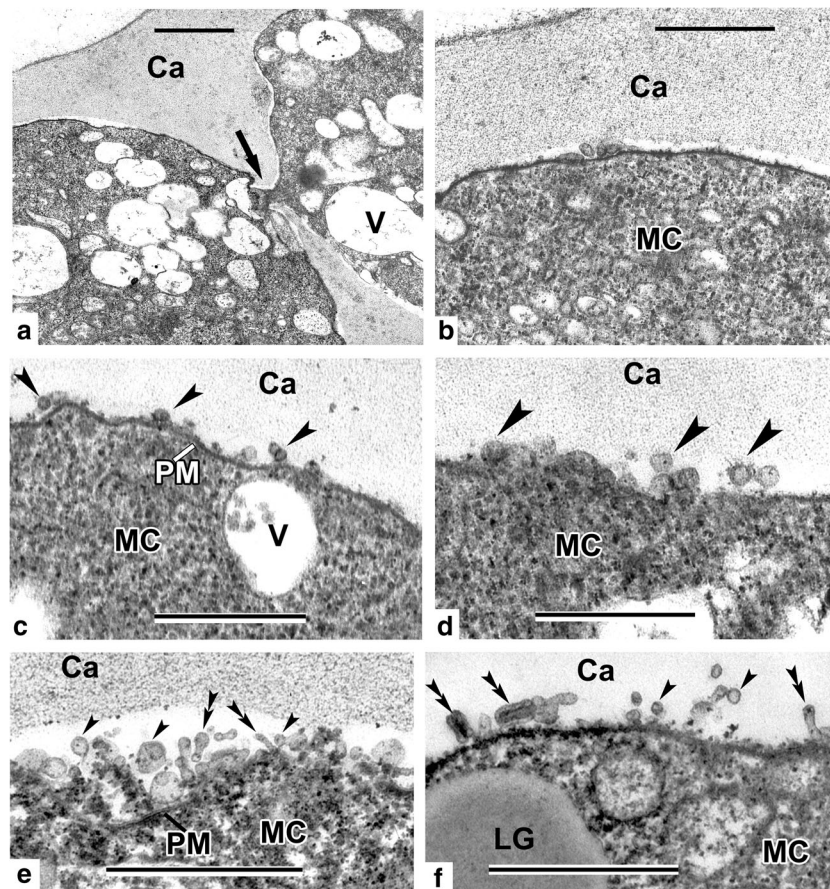


Fig. 1 Post-meiotic and early tetrad stages in *Ambrosia trifida*. **a** Two daughter microspores of a tetrad in the process of post-meiotic cytokinesis. Thick callose envelope surrounds the tetrad. In the course of cytokinesis, the tetrad partitions form by furrowing (**a**, arrow). Many large vacuoles are seen in the cytoplasm. **b** Early tetrad stage. The microspore cytoplasm is dense, and large vacuoles have disappeared. **c** A border of a microspore at higher magnification. Spherical units are observed on the

plasma membrane (arrowheads). **d** Spherical units on the plasma membrane at higher magnification (arrowheads). The right one has a denticulated surface. **e**, **f** Clusters of spherical (arrowheads) and rod-like (double arrowheads) units are located in the periplasmic space between callose and the plasma membrane. Ca callose, LG lipid globule, MC microspore cytoplasm, PM plasma membrane, V vacuole. Bars at 1 μm in **a**, **b** and at 0.5 μm in **c**, **d**

A low magnification image shows slightly later tetrads, one of which is lying close to the tapetum (Fig. 3a). Higher magnification reveals the structure of the glycocalyx which consists of spherical units tightly packed into radial columns (Fig. 3b, c). In the site of developing apertures (Fig. 3c, asterisk), the plasma membrane is completely lacking the glycocalyx and remains in close contact with the SCW.

Mid and late tetrad stages (Fig. 5c)

Later, portions of two tetrads at low magnification show the boundary between two tetrads, tightly pressed to each other (Fig. 4a). The parietal secretory tapetum of binucleate cells is very active (Fig. 4b, c). An adjacent tetrad is tightly pressed to the border of the tapetum. A portion of a microspore sectioned tangentially shows the reticulate pattern of the glycocalyx (Fig. 4b, arrow). Higher magnification shows many

RER cisternae in the tapetal cytoplasm, accumulating secreted material within the dilations and discharging it into anther locus (Fig. 4b). The microspore glycocalyx hexagonal pattern, seen in the tangential section in Figs. 4a and 5b, clearly appears in radial sections as an interrupted layer of clusters and gaps (Fig. 5a, c, arrowheads). At the beginning of the late tetrad stage, the initial primexine starts to be differentiated: the protectum, which has a hexagonal pattern, appears in sections as an interrupted layer (Fig. 5d, white arrows), supported by procolumellae (Fig. 5f, black arrowheads). The latter are evidently based on rod-like units, oriented perpendicularly to the plasma membrane (Fig. 5d, arrowheads). Simultaneously, small fragments of the first endexine lamella appear at the plasma membrane (Fig. 5d, black arrows). The glycocalyx is detached from the plasma membrane, so that a gap forms between it and the plasma membrane (Fig. 5d, asterisks).

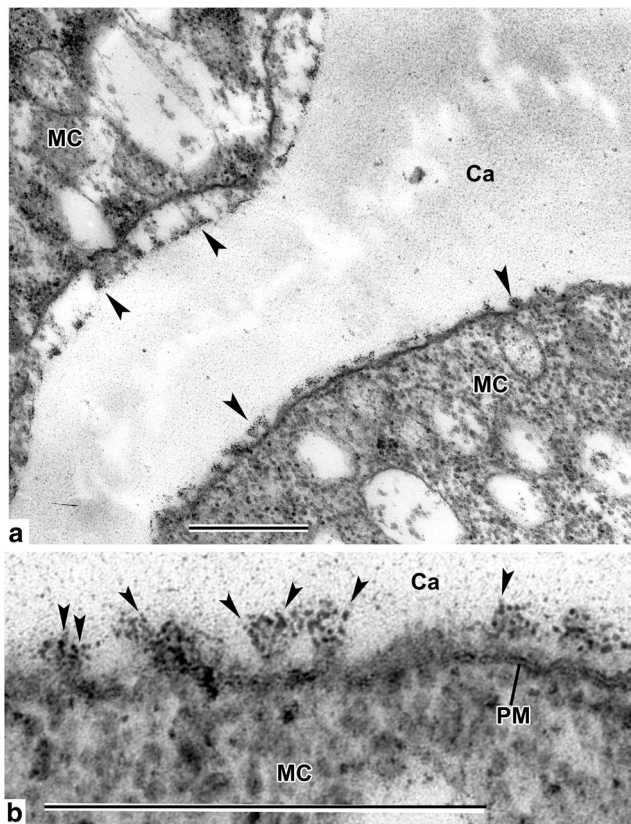


Fig. 2 Young tetrad stage in *Ambrosia trifida*. **a** First signs of the glycocalyx (arrowheads) on the microspore plasma membrane. **b** A border of a microspore at higher magnification. Tripartite structure of the plasma membrane (PM) and tufts of spherical units, arranged into columns (arrowheads), are discernible. Ca callose, MC microspore cytoplasm. Bars at 0.5 μm

Late tetrad stage (Fig. 5d)

Overviews of the anther loculi show that from the before meiosis to the end of the tetrad period, the secretory parietal tapetal cells are intact, each with a continuous cell wall, and keep their parietal position (Fig. 6a–d).

Free microspore period

Young free microspore stage (stage 6) After disintegration of callose, the pattern of the primexine becomes more distinct (Fig. 7a, PEx). The fragments of the endexine lamella become longer and appear with central white lines (Fig. 7a, arrows). A distinct gap, corresponding to the future cavea, is clearly visible between the ectexine and endexine (Fig. 7a, asterisk). At the sites of the apertures, the initial endexine lamellae appear as thin fibrils (Fig. 7b, arrowheads). During the free microspore period, the parietal tapetum reorganizes into the plasmodial tapetum.

Middle free microspore stage In interapertural regions, the endexine lamellae are pressed closely to each other (Fig. 8a, arrow).

An initial cavea forms at the site of the gap between the prime-ectexine and the endexine (Fig. 8a, asterisks). The endexine lamellae (Fig. 8b, arrows) dissipate inside the increased volume of the dilated periplasmic space. In the developing ectexine, young columellae, covered by portions of the tectum (Fig. 8c, arrowheads), alternate with initial glycocalyx units, consisting of radially oriented columns of spherical units (Fig. 8c, white arrows). The endexine lamellae are seen by their white lines (Fig. 8, black arrow).

Late free microspore stage The anther loculus is filled with microspores, surrounded by the tapetum, which has reorganized into a plasmodium (Fig. 9a). The ectexine with spines (Fig. 9a, Ect) is separated from the endexine (arrows) by vast caveae (Fig. 9a, Cav). A large striated structure occupies the central part of the anther loculus filled with the periplasmodial tapetum (Fig. 9b, arrowheads). Higher magnification shows that the foot layer (Fig. 9c, FL) appears on the endexine surface. The endexine consists of several lamellae, discernible by their white lines (Fig. 9c, End).

Somewhat later, vast caveae separate the ectexine and the foot layer, such that the endexine is only attached to the ectexine via the foot layer at a few places, mainly bordering the margins of the ecto-apertures (Fig. 10a–c, arrows). Different planes of sections through microspores show circular images of the cross-sectioned columellae, spines, and tectum with internal foraminae (Fig. 10c, arrowheads). The foot layer is thin (Fig. 10d, asterisk) in comparison with the white-lined endexine.

In the interapertural site, small granules appear on the plasma membrane (Fig. 11a, arrowheads?). At the margins of apertures, columellae are firmly attached to the foot layer and granules appear between the endexine lamellae (Fig. 11b). At the apertural dilation of the periplasmic space, the endexine lamellae are dissipated into fragments of different forms inside the supporting liquid—from rod-like to spherical units (Fig. 11c, arrows). This contrasts with the interapertural regions (Fig. 11d) where all the lamellae are fused together and recognizable only by their white lines. The laminate form of the endexine lamellae is clear from Fig. 11e.

Discussion

As is the case for all Asteraceae, the architecture of the exine in *Ambrosia trifida* is more complex than in most of flowering plants, though its cross section is “one-storeyed”—unlike the “two-storeyed” construction of *Tanacetum vulgare* (Gabarayeva et al. 2016) and *Echinops* sp. (Gabarayeva et al. 2018). Therefore, the developmental sequence which gives rise to this structure is also elaborate, as summarized in Fig. 12.

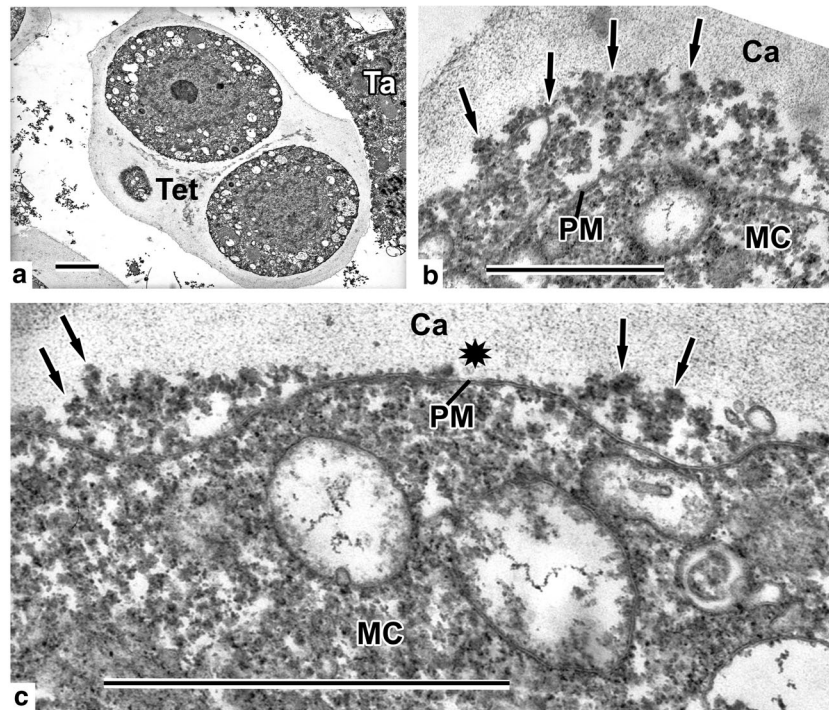


Fig. 3 Young tetrad stage in *Ambrosia trifida* in progress. **a** A tetrad (Tet) lying near the tapetum. **b, c** Borders of microspores. The glycocalyx consists of radially oriented chains of spherical units (arrows) on the plasma membrane. The site of the future aperture lacks the glycocalyx (**c**, asterisk). **d** Oblique section through the border of a microspore (the

plasma membrane is not seen). Radially oriented elongated units, shown in **b, c**, being cross sectioned (arrows), appear as circle units with dark cores and light halos. Ca callose, MC microspore cytoplasm, PM plasma membrane, Ta tapetum, Bars at 2 μm in **a** and at 0.5 μm in **b, c**

The sequence of developmental stages interpreted as micellar self-assembly in the periplasmic space: comparison with previous ontogenetic studies on Asteraceae and other species

The first developmental stages in *Ambrosia trifida* microspore wall development (Fig. 12a–d) have the same two initial micellar mesophases (Fig. 12a'–d', see legends for details) as observed in all the species we have studied (Gabarayeva et al. 2009a, b, 2010a, b, 2011a, b, 2013a, b, 2014, 2016, 2017, 2018; Gabarayeva and Grigorjeva 2010, 2011, 2012, 2014, 2017; Grigorjeva and Gabarayeva 2015, 2018), including *Tanacetum vulgare* and *Echinops exaltatus*. Spherical units (which we interpret as spherical micelles) and their aggregates are also clearly visible in Horner and Pearson's (1978, Figs. 21–25) excellent micrographs of *Helianthus annuus*. These units are probably the reason for the presence of internal foraminae in the ectexine, being formed when the initially normal micelles invert, exposing their hydrophilic cores in the lipid-containing medium present in late development.

At the mid-tetrad stage in *Ambrosia*, a reticulate pattern of the glycocalyx on the microspore surface (representing the future tectum), seen in sections as

clusters (Fig. 12e), corresponds most probably to the hexagonal (middle) mesophase. The reticulum is probably formed by clusters of normal cylindrical micelles (described by Rowley as tufts in Rowley 1990) alternating with clusters of reverse cylindrical micelles. Sheldon and Dickinson (1983) suggested protein self-organization in the process of pre-patterning of the plasma membrane, supposing that a reticulate pattern might be caused by hydrophilic-hydrophobic mixtures, analogous to distribution of oil droplets on the surface of water (Wodehouse 1934). Our observations support this concept which has played a part in the development of ideas concerning self-assembly (Gabarayeva 1993) and the micellar hypothesis (Gabarayeva and Hemsley 2006; Hemsley and Gabarayeva 2007). It is probable that in the case of *Ambrosia* such a mosaic, reticulate pre-pattern exists on the plasma membrane which defines the alternation of clusters of normal and reverse cylindrical micelles (Fig. 12e', e''). The subsequent accumulation of sporopollenin precursors, mainly on clusters of reverse micelles, apparently gives rise to the reticulate pattern of the tectum. The mid-tetrad stage glycocalyx of *Echinops* and *Tanacetum* is based on similar hexagonal micellar mesophases, but in these taxa, the cylindrical micelles form a uniform layer.

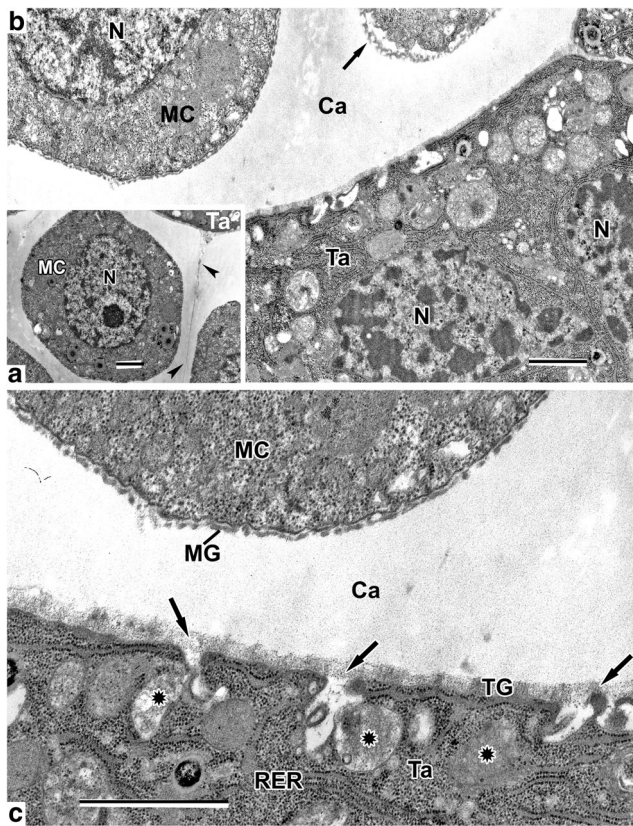


Fig. 4 Middle tetrad stage in *Ambrosia trifida*. Parietal secretory tapetum and an adjacent tetrad of microspores. **a** Two tetrads, tightly pressed to each other and to the tapetum in the anther locusus. A border between them (arrowheads) is the remnants of spore mother cell envelope. **b** A border of the tapetum with two nuclei and fragments of two tetrad microspores. Tangentially sectioned portion of the microspore shows a reticulate pattern of the glycocalyx (arrow). **c** Magnified portion of **a** to show details of a microspore and tapetum. Central section through the microspore reveals the discontinuous glycocalyx (MG) and the continuous tapetal glycocalyx (TG). Well-developed rough endoplasmic reticulum (RER) in the tapetal cell synthesizes a substance, accumulates it in dilations (asterisks), and discharges into the anther locusus (arrows). MC microspore cytoplasm, N nucleus, Ta tapetum. Bars at 1 μ m

In *Farfugium japonicum* (Compositae: Senecioneae), Takahashi (1989) found the same sequence of phases: initial discrete globular components, then rods, then string-like probacula. This represents the familiar sequence of micellar mesophases (string-like probacula are a typical transitional mesophase between spherical and cylindrical micelles). Comparable processes were documented in *Gerbera jamesonii* (Compositae: Mutiseae) by Southworth (1983) who observed dense probacula to be separated by a clear zone from matrix fibrils. Southworth (1983), Fig. 6 corresponds closely to cylindrical unit micelles with dark-contrasted core and weak-contrasted halo, separated by fibrillary material, and

compares closely to those in *Tanacetum* (Gabarayeva et al. 2016, Fig. 4), in *Echinops* (Gabarayeva et al. 2018, Fig. 5), and *Ambrosia* (Figs. 3 and 5).

The initial columellae (Fig. 12f), which appear beneath the tectum, are also based on reverse cylindrical micelles or their clusters (Fig. 12f'). Simultaneously, during the late tetrad stage, fragments of the first white-line centered lamella appear at the plasma membrane (Fig. 12f), based evidently on laminate micelles (Fig. 12f'). The latter increase in number (Fig. 12g') and give rise to several white-lined endexine lamellae which, after sporopollenin accumulation and compression, form the endexine layer (Fig. 12g) in the free microspore period. Finally, the foot layer appears the last on the outer surface of the endexine (Fig. 12g). At dilations of the aperture sites, lamellae of the endexine diverge and dissipate into rods and spherical units/micelles (Fig. 12a', h, h'); i.e., the sequence of micelle mesophases at aperture sites returns to the initial mesophase: spherical micelles.

The so-called “intermixed endexine” observed in *Ambrosia* (Fig. 11a–c) was also found in a number of species and is especially apparent in ontogenetic studies. Larson (1966), in an early TEM study of the mature pollen wall in *Passiflora caerulea*, concluded that in this species, the ectexine is intermixed with the endexine. A later developmental study of *Passiflora racemosa* (Gabarayeva et al. 2013a) showed that granulate parts of the endexine alternate with lamellate regions, the latter appear as strings of beads rather than as typical lamellae. The endexine of *Illicium floridanum* (Gabarayeva and Grigorjeva 2003) and *Swida alba* (Gabarayeva and Grigorjeva 2011) includes granules and lamellae which are intermixed. In the *Chamaedorea microspadix* endexine (Gabarayeva and Grigorjeva 2010), granules, situated under lamellae during the course of development, are first arranged into strings (one form of micelles) and later fuse into lamellae. In *Plantago major*, lamellae of the endexine-1 are intermixed with granules of the endexine-2 (Gabarayeva et al. 2017).

Additional, highly intricate and complex forms of micellar structures not only appear in the periplasmic space during the course of exine development but also are often observed in the microspore and tapetum cytoplasm and in the anther locusus. The typical nematic liquid crystal (where hydrophobic and hydrophilic layers alternate each other) is observed in the periplasmic tapetum (Fig. 9b), as it was seen earlier in the tapetum of *Persea americana* (Gabarayeva et al. 2010a). The appearance of such structures is not surprising: these formations appear through the interplay of purely

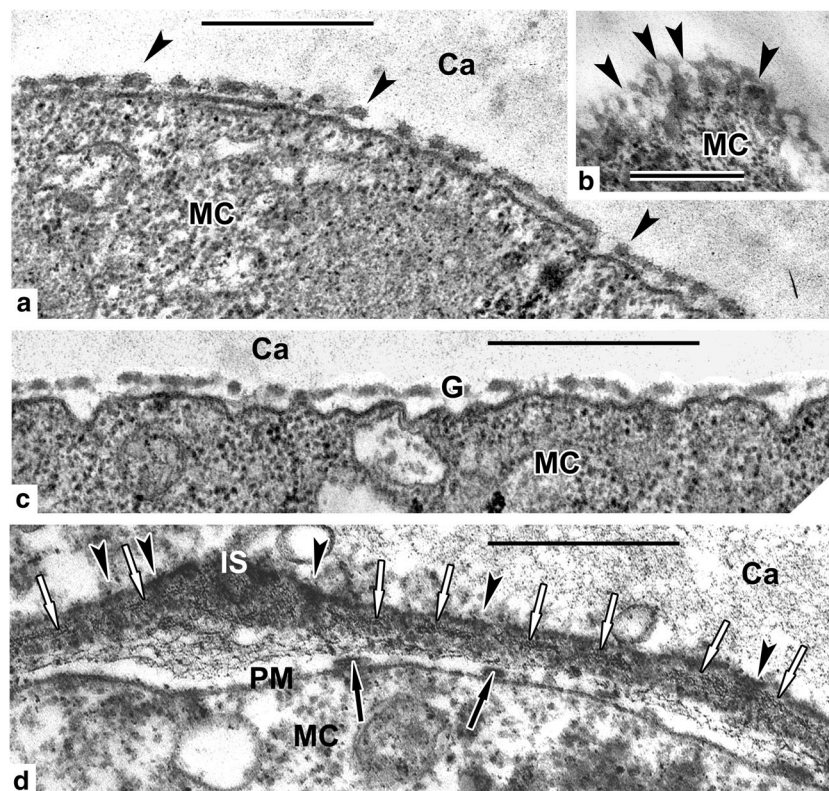


Fig. 5 Middle tetrad stage (**a–c**) and transition to the late tetrad stage (**d**) in *Ambrosia trifida*. **a–c** The reticulate pattern of the glycocalyx (seen in Fig. 4a, arrow) appears at radial sections as an interrupted layer of clusters and gaps (arrowheads in **a**, **G** in **c**). Tangential section shows clusters as roundish units (**b**, arrowheads). **d** Transition to the late tetrad stage. Initial primexine in the process of the differentiation at the late tetrad stage. The interrupted layer (as plates) inside the glycocalyx (white

arrows) is the initial tectum, at the ends of which initial columellae, oriented perpendicularly to the plasma membrane, which appear to consist of lipid substance (black arrowheads). Simultaneously, small fragments of the first endexine lamella appear at the plasma membrane (black arrows). Note the gap between the plasma membrane and the glycocalyx (asterisk). Bars at 0.5 μm in **a–d**

physical forces acting in any medium in which hydrophilic and hydrophobic surface-active substances meet each other. Liquid crystals were observed in *A. trifida* in the tapetal cytoplasm when meiosis proceeds in microspore mother cells (Lersten and Curtis 1990): the authors described them simply as *crystals* appearing as white areas in optically anisotropic systems. Under a polarizing microscope, the birefringence of liquid crystals gives rise to a variety of beautiful, colored patterns. The German botanist Reinitzer (1888) discovered the phenomenon of iridescent colors more than a century ago. Sitte (1981) pointed out that there are many well-studied examples of self-assembly as a structure-generating principle. Liquid crystal systems with rod-like molecules, which retain a degree of positional order (their molecules are lined up perpendicularly or tilted on the long axis) and are well-ordered orientationally, are called smectic phases. Nematic liquid crystals have regular orientation (preferential alignment along one spatial direction), but not positional order (their rod-like

molecules are not lined up perpendicularly or tilted on the long axis—see for classification, e.g., Hemsley and Gabarayeva 2007, Plate III, Fig. 11).

Blackmore and co-authors investigated pollen development in Compositae using a SEM freeze-fracture technique, providing a different perspective on the same developmental events. Nevertheless, with some significant differences, the results obtained confirm the same sequence of events. In *Cosmos bipinnatus*, Blackmore and Barnes (1985) described the deposition of a glycocalyx or primexine which displays a uniform granularity. Similar developmental sequences were described in several members of the Compositae: Cichorieae including *Tragopogon porrifolius* (Blackmore and Barnes 1987), *Catananche caerulea* (Blackmore and Barnes 1988), and *Scorzonera hispanica* (Barnes and Blackmore 1986).

It should be emphasized that the chemical composition of substances, their concentrations (specified by the

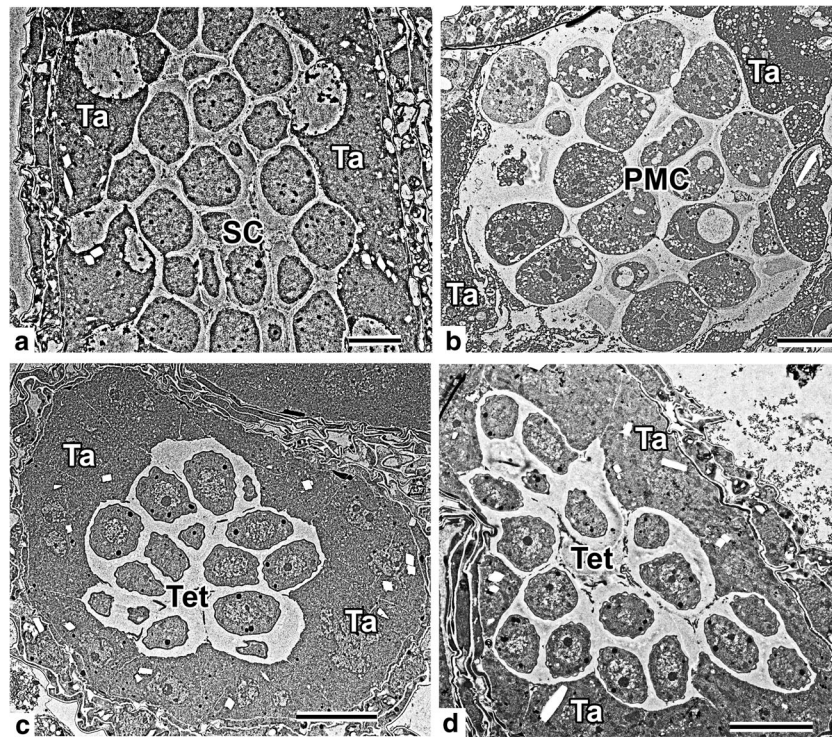


Fig. 6 Low-magnification images of the anther loculi at pre-tetrad and tetrad stages. **a** Sporogenous cells (SC), surrounded by tapetal cells (Ta). **b** Pollen mother cells (PMC) with initial callose depositions. Some are connected with cytotimic channels. The parietal secretory tapetum

surrounds the PMC. **c** Mid tetrad stage. The tetrads (Tet) are surrounded by parietal secretory tapetum. **d** Late tetrad stage. The microspores with initial columellae and spines are surrounded by the parietal secretory tapetal cells which still preserve their envelopes. Bars at 10 μ m

genome), and the volume of the periplasmic space are very changeable, and corresponding micellar mesophases can shift from one to another in either direction. We consider that the developmental programs which finely tune this variability are the basis for the establishment of the complex exine architecture seen in Asteraceae. Strong support for the micellar hypothesis was provided by the experimental modeling of mature exine-like structures (Hemsley et al. 1996, 1998; Hemsley 1998; Griffiths and Hemsley 2001) and of a number of simulations of exines at different developmental stages (Gabarayeva and Grigorjeva 2013, 2016, 2017), carried out in vitro where physico-chemical regularities of colloidal systems (hydrophobic relationships) were the variable factor.

Development of caveae

The mature ectexine of Asteraceae includes both caveate (with a large space between the internal tectum and the foot layer, sometimes traversed by a few slender columellae) and acaveate (in which larger columellae connect the internal tectum and the foot layer) forms. In a

review of the terminology and function of caveate pollen, Blackmore et al. (1984) suggested that such spaces or gaps were potentially present during the tetrad stage in all Asteraceae. At maturity, caveae would remain only in those taxa which do not develop an inner layer of columellae. Their presence is strongly correlated with anemophily in Asteraceae such as *Ambrosia* and *Artemisia* (Rowley and Dahl 1977).

The development of caveate pollen has been studied in *Cosmos bipinnatus* (Dickinson and Potter 1976), *Helianthus annuus* (Horner and Pearson 1978), *Leontodon autumnalis* (El-Ghazaly 1982), and *Scorzonera hispanica* and *Scorzonera humilis* (Blackmore and Claughner 1987). In *Ambrosia*, starting as gaps at the late tetrad stage, caveae increase in volume during the free microspore period. The appearance of gaps at the late microspore stage is apparently connected with cessation of deposition of new glycocalyx material on the plasma membrane, resulting in its detachment from the plasma-lemma surface. The same events were reported in *Helianthus annuus* (Horner and Pearson 1978, Figs. 24 and 25). We observed the same phenomenon in many other species under our studies, e.g., in *Acer tataricum*

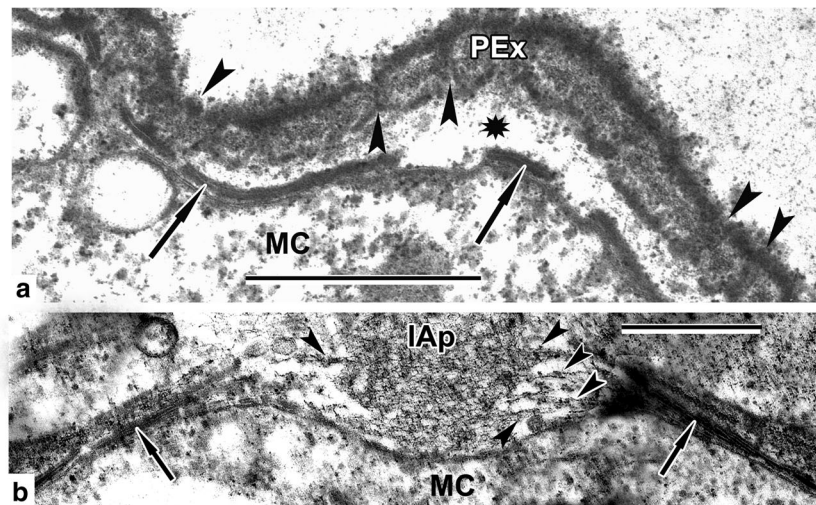


Fig. 7 Young free microspore stages in *Ambrosia trifida*. **a**, **b** Columellate-TECTATE pattern of the primexine gradually appears more distinct. Young columellae, covered by protectum, are discernible (**a**, arrowheads). Callose disintegrated. Fragments of the primordial endexine lamella (**a**, arrows) with typical white lines become longer. Note that

the gap between the prime-ectexine and the developing endexine is increased (**a**, asterisk). **b** Initial aperture (IAp) in the form of a bulb-like dilation is the site where several endexine lamellae (arrows) appear as thin fibrils (arrowheads). MC microspore cytoplasm, PEX primexine, Bars at 0.5 μm

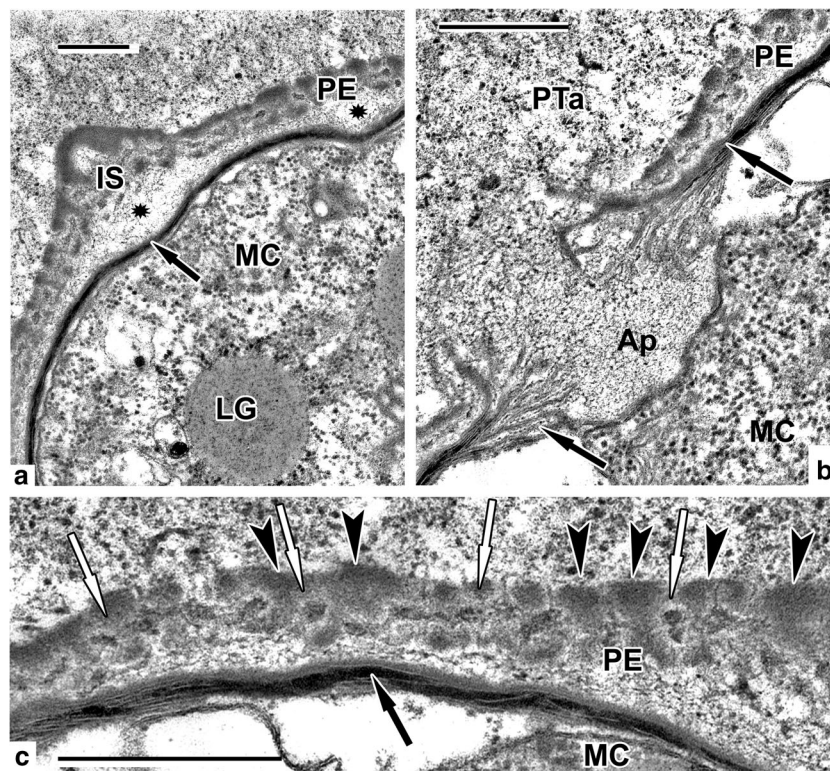


Fig. 8 Middle free microspore stage in *Ambrosia trifida*. **a** In the interapertural regions, the endexine lamellae are pressed to each other (arrow). The gap between the ectexine and the endexine is well discernible (asterisks). **b** An aperture site shows that the endexine lamellae (arrows) dissipate in the volume of the dilated periplasmic space. **c** In the prim-ectexine (PE), young columellae, covered by portions

of the tectum (arrowheads), alternate with initial glycocalyx units, consisting of radially oriented columns of spherical units (white arrows). The endexine lamellae are seen by their white lines (black arrow). Ap aperture site, IS initial spine, LG lipid globule, MC microspore cytoplasm, PTA periplasmic tapetum. Bars at 0.5 μm

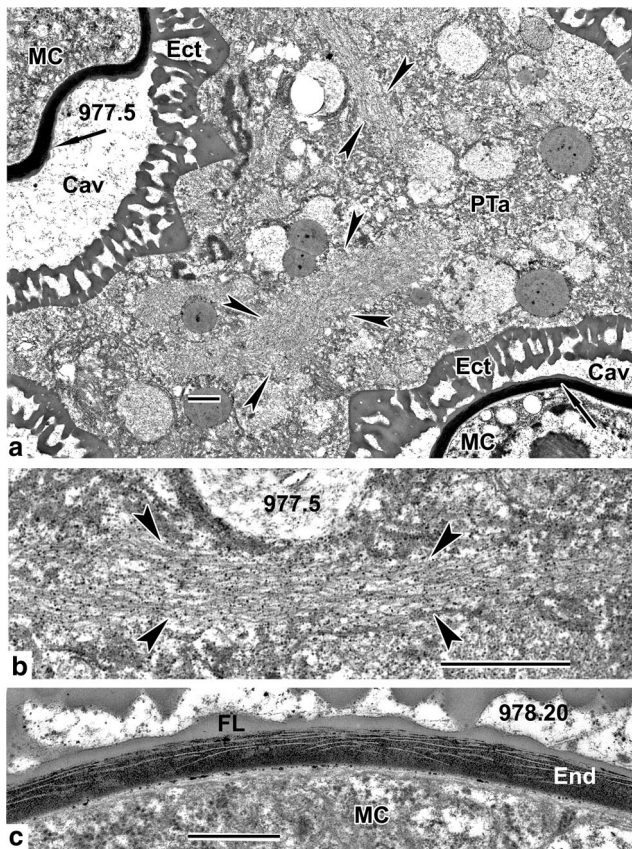


Fig. 9 Late free microspore stage in *Ambrosia trifida*. **a** Vast caveae (Cav), appearing on the sites of gaps, are present between the ectexine and endexine. An overview of the anther loculus with microspores, filled with plasmodial tapetum (PTa). Ectexine (Ect) with spines is separated from the endexine (arrows) by vast gaps (Gap). Some large striated structure occupies the central part of the anther loculus (restricted by arrowheads). **b** Magnified portion of striate structure, shown in **a**, reveals typical nematic liquid crystals in the periplasmodial tapetum (arrowheads). **c** The foot layer (FL) appears on the endexine (End); the latter consists of several lamellae, discernible by their white lines. Bars at 1 μm in **a** and at 0.5 μm in **b**, **c**

pollen wall development where the early start of the detachment of the glycocalyx from the plasma membrane meant that after earlier determination of procolumellae position alongside the microspore surface, the contact of the glycocalyx with the plasma membrane is not necessary any more (Gabarayeva et al. 2010b).

Acaveate pollen development has been studied in *Gerbera jamesonii* (Southworth 1983), *Artemisia vulgaris* (Rowley and Dahl 1977), *Tanacetum vulgare* (Gabarayeva et al. 2016), and *Echinops exaltatus* (Grigorjeva and Gabarayeva 2018). In *Artemisia* (Rowley et al. 1981), as well in *Echinops* and *Tanacetum*, gaps appear at the end of the tetrad period, but later, columellae are inserted within these gaps.

Hence, the appearance of gaps between columellae in acaveate pollen is also connected with growth cessation of the first glycocalyx generation and its detachment from the plasma membrane. The following type of exines depends on further processes: gaps could be preserved in the mature pollen becoming caveae (the case of *Ambrosia*), and they could be traversed by the later formed columellae (the case of *Tanacetum* and *Echinops*) or just disappear, being filled with the newly formed exine structures (the case of *Acer* and many other species). Therefore, the difference between the caveate and acaveate pollen is not a clear-cut.

Aperture formation

It is well known that in the site of developing apertures, the plasma membrane is completely lacking in glycocalyx and remains in close contact with the SCW. We suggested in our previous study on *Echinops* that the overall symmetry is imparted to the microspores during meiosis and their surfaces are differentiated or patterned into structural different areas (apertures and interapertural exine). The isolation and investigation of the INP1 gene in *Arabidopsis* by Dobritsa and Coerper (2012) demonstrated that aperture development is the result of the definition of distinct cellular domains by the protein INAPERTURATE POLLEN1. In the brilliant recent paper (Dobritsa et al. 2018), the authors have shown that in developing pollen, *Arabidopsis* INAPERTURATE POLLEN1 (INP1) protein acts as a marker for the pre-aperture domains. INP1 assembly occurs after meiotic cytokinesis at the interface between the plasma membrane and the overlying callose wall. Callose deposition is necessary for the formation of INP1-decorated domains on the membrane surface. These data suggest that INP1 is a late-acting factor involved in keeping specific membrane domains next to the callose wall to prevent formation of exine at these sites.

Tapetum

Two major classes of tapetum are recognized in the anthers of angiosperms: the secretory tapetum (also called glandular, parietal, or cellular) with persistent cell walls and the plasmodial tapetum (invasive or amoeboid), the cells walls of which break down during microspore development. The two major classes can themselves be subdivided on the basis of various criteria (Pacini et al. 1985; Pacini and Franchi 1991; Kamelina 1988; Barnes and Blackmore 1992; Hesse and Hess 1993).

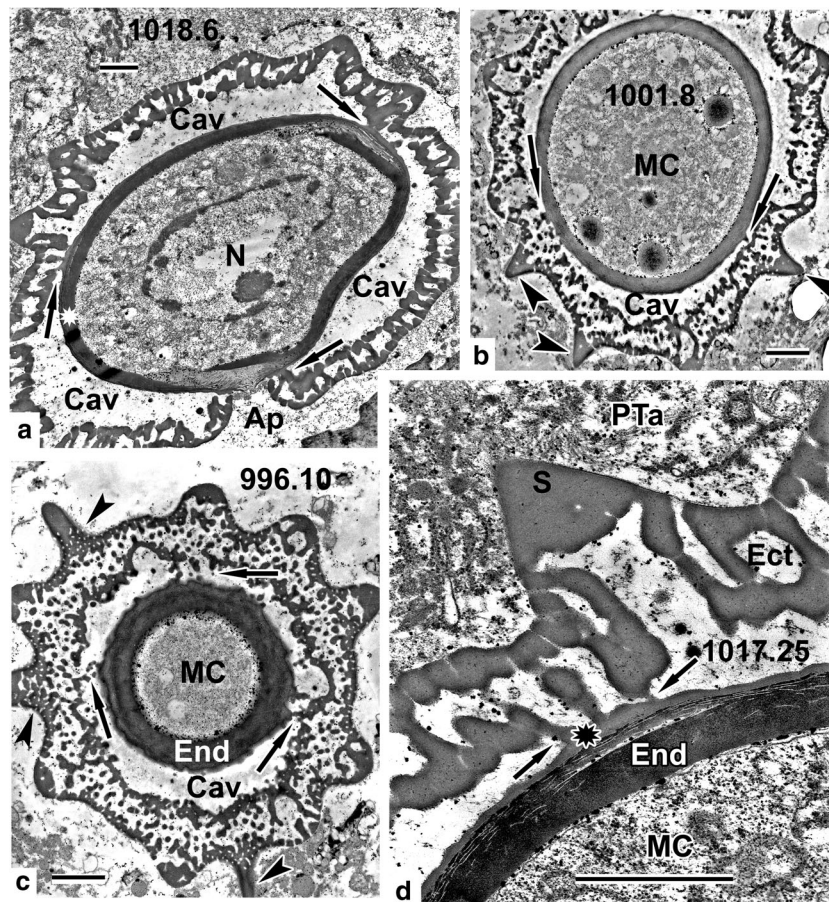


Fig. 10 Late free microspore stage in *Ambrosia trifida*. **a** A median section through a microspore to show huge caveae between the ectexine and endexine. The whole ectexine is attached to the endexine via the foot layer only at several points (arrows). The endexine is rather thick (asterisk). **b**, **c** Not a central (**b**) and a crust (**c**) sections through microspores show circular images of the cross-sectioned columellae, spines, and tectum with foraminae (arrowheads). The ectexine almost

“hangs in a thin air”. **d** Magnified fragments of the interapertural region. The foot layer is thin (asterisk) in comparison with the white-lined endexine (End). Very thin connections between columellae and the foot layer (arrows). Plasmodial tapetum (PTa) surrounds microspores. Ap aperture site, Cav cavea, Ect ectexine, MC microspore cytoplasm, N nucleus, S spine. Bars at 1 μ m

The more data accumulate on the behavior of tapeta at different stages of development, the more blurred the borders between the main types become. Tapetum manifests a considerable range of modifications (Pacini and Juniper 1983; Pacini and Franchi 1991). Pacini and Keijer (1989) reported about a special tapetum type—intrusive non-periplasmodial—in *Cichorium intybus* (Asteraceae) whereas Varotto et al. (1996) termed the tapetum of *Cichorium intybus* ameboid, though in the tetrad period, it maintains both its parietal position and its plasma membranes. A number of authors interpret tapetum as a dynamic system which is capable to return, at least once, from cellular to plasmodial and back to cellular state (Willemse 1971; Walles and Rowley 1982; Rowley and Walles 1985; El-Ghazaly and Nilsson 1991), and even do this thrice (cyclic-invasive type) in *Nymphaea colorata*

(Rowley et al. 1992). Our previous (Gabarayeva 1997, 2000) and other authors' (Taylor et al. 2012, 2013, 2015) data underscore the developmental lability in pollen wall formation—pollen characters and tapetum behavior.

Our data on *Ambrosia* show that the tapetum is clearly parietal and preserves its position and cellular envelopes until the late free microspore stage. At the latter stage, it undergoes reorganization into a plasmodium, engulfing microspores. Until the late free microspore stage, the tapetal cells have distinct radial, inner (locular), and outer tangential walls. These data coincide with an earlier LM study on *Ambrosia trifida* tapetum development by Lersten and Curtis (1990); the authors, however, named this type of tapetum as invasive—in spite of the fact that typical invasive (amoeboid) tapetum reorganizes into a plasmodium at the early tetrad stage (Pacini and Juniper

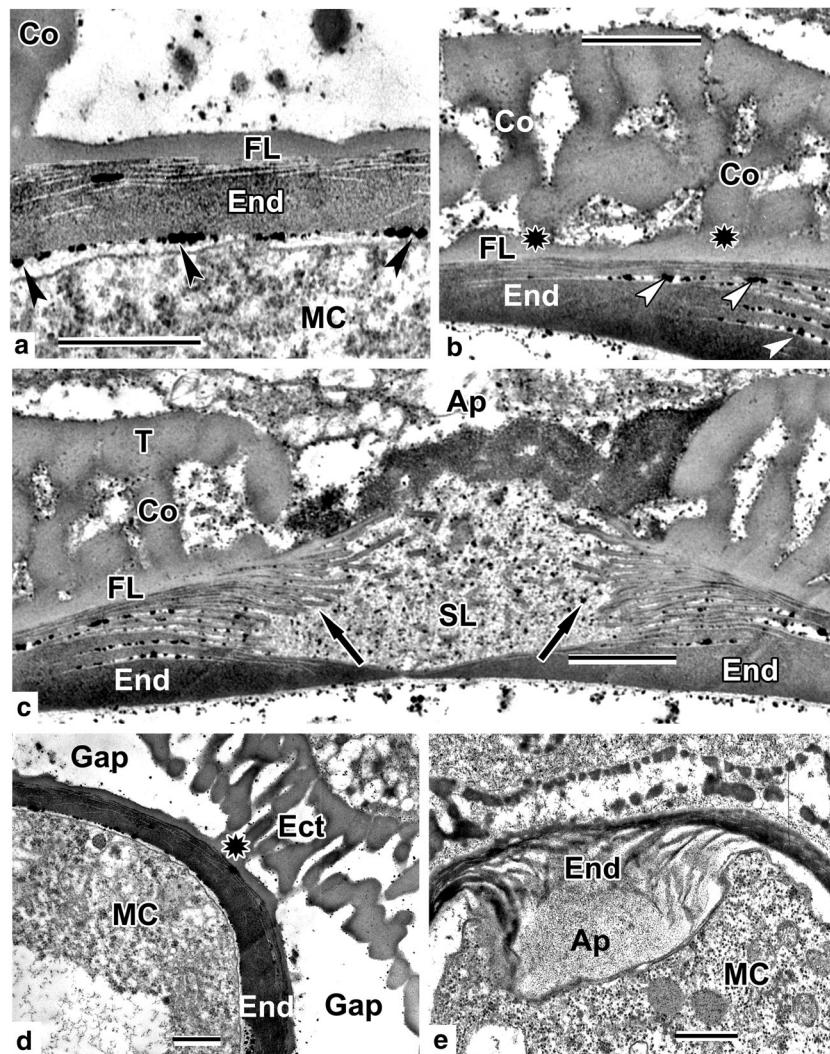


Fig. 11 Late free microspore stage in *Ambrosia trifida*. **a** Interapertural site. The appearance of small granules on the plasma membrane (*arrowheads*). **b** A margin of an aperture. At margins, columellae (Co) are safely attached to the foot layer/endexine complex (*asterisks*). Granules are evident between the endexine lamellae (*arrowheads*). **c** At the aperture dilation of the periplasmic space, the endexine lamellae are dissipated (*arrows*) in supporting liquid (SL) into fragments of different

forms—unlike the interapertural regions (**d**) where all lamellae are soldered with each other and recognizable by their white lines only. *Asterisk* in **d** marks the site of connection between columellae and the foot layer-endexine. **e** This oblique section through an aperture site clearly demonstrates a laminate nature of the endexine lamellae. Note the interrupted tectum at the aperture margin. Ap aperture site, Ect ectexine, End endexine, FL foot layer, MC microspore cytoplasm, T tectum. Bars at 0.5 μm

1983). Taking into consideration all the citing examples, the current terminological system of tapetal types is self-contradictory. This is why we just report that the tapetum in *Ambrosia* is the secretory parietal (cellular) during the tetrad stage and at the young free microspore stage, with reorganization into a plasmodium at the middle or late free microspore stage.

Conclusions

1. Tracing of the complete sequence of processes leading to emergence of the complex exine in *Ambrosia trifida* has shown that the tetrad stage is crucial for

the pattern determination because the most important preparatory events, defining the future exine structure, proceed within it.

2. The analysis of the ontogenetic sequence in *A. trifida* with that of other Asteraceae and remote taxa has shown the same sequence of the main stages.
3. The comparison of the sequence of developmental stages in *A. trifida* with the sequence of micellar self-assembly mesophases has revealed morphotemporal similarity of both sequences, emphasizing by this the probable role of micelle self-assembly processes in sporoderm development. The same regularities have been observed earlier in other Asteraceae and in many remote taxa.

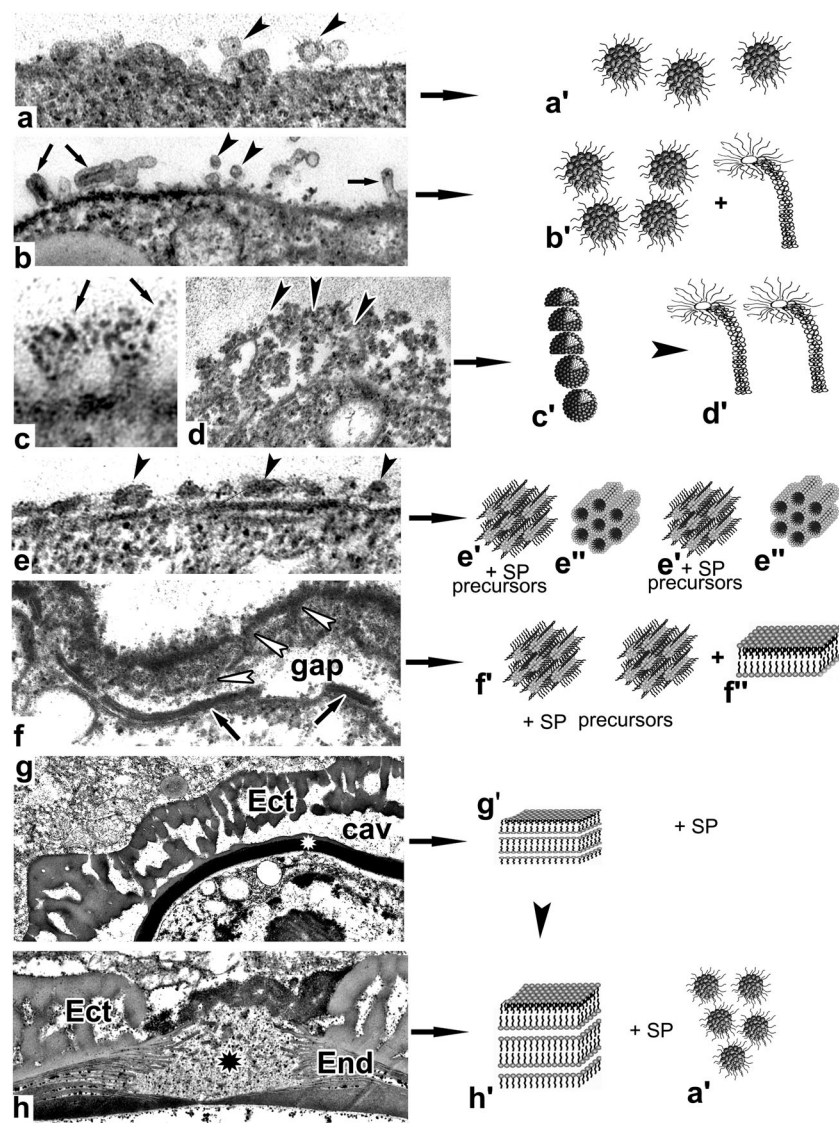


Fig. 12 Semi-schematic summary of the main developmental stages of the exine in *Ambrosia trifida* (left column) and an interpretation of the micellar processes involved (right column). The tetrad period. **a** Part of Fig. 1d. Spherical units (arrowheads) on the microspore plasma membrane at early tetrad stage: these units are the first micellar mesophase (**a'**) of the self-assembling sequence—reverse spherical micelles (the right arrowhead in Fig. 1d shows a typical reverse micelle with denticulate surface, corresponding to protruding hydrophobic tails of surfactant molecules). **b** Part of Fig. 1f. Separate spherical units on the plasma membrane gradually rearrange into columns (arrowheads) and rod-like (arrows) micelles (**b'**). **c** Part of Fig. 2b. Clusters of spherical micelles, arranged into columns (transitional mesophase) on the plasma membrane, are transitional mesophase—columns of normal spherical micelles (**c'**). **d** Part of Fig. 3b. The microspore glycocalyx (arrowheads) comprises cylindrical (rod-like) reverse micelles or their clusters (**d'**). **e** Part of Fig. 5a. The hexagonal glycocalyx pattern (shown in radial

section) as the middle micelle mesophase with alternation of clusters of tightly packed reverse micelles (**e'**) and clusters of normal micelles (**e''**). Apparently sporopollenin precursors accumulate on clusters of reverse micelles, with their hydrophobic surfaces. **f** Free microspore period. Part of Fig. 7c. Young free microspore stage. The appearance of young columellae (arrowheads) as the result of further accumulation of sporopollenin precursors on clusters of reverse micelles (**f'**). Simultaneously, fragments of laminar micelle (of the next micellar mesophase, **f''**) accumulate sporopollenin, giving rise to the first endexine lamella (**f**, arrows). **g** Late free microspore stage. Part of Fig. 9a. Mature exine of a pollen grain. The endexine (asterisk) is based on several laminar micelles (**g'**) which, after sporopollenin accumulation, are soldered with each other. **h** An aperture site. The endexine lamellae, based on laminar micelles (**h'**), are dissipated in the region of oncus into spherical units (asterisk) which are spherical micelles (**a'**), having accumulated sporopollenin

4. The caveae development in *A. trifida* and in other caveate species is connected with growth cessation of the first glycocalyx generation and its detachment from the plasma membrane.

5. Tracing the tapetum development has shown that it is clearly parietal and preserves its position and envelopes until the late free microspore stage, with subsequent reorganization into a plasmodium.

Acknowledgements The work was carried out in the framework of the institutional research project “Morphology, ultrastructure and development of sporoderm in higher plants” of the Komarov Botanical Institute of the Russian Academy of Sciences (no. AAAA-A18-118032190134-3) on the equipment of the Centre for Collective Use (“Cellular and molecular technology study of plants and fungi”) of the Komarov Botanical Institute (St. Petersburg). We also thank our anonymous reviewers for their valuable advice and suggestions.

Author contributions Conceptualization: N.G. and S.B.

Methodology: S.P., E.S., O.V., and V.G.

Investigation: N.G.

Writing of the original draft, review, and editing: N.G. and S.B.

Funding acquisition: N.G.

Resources: N.G.

Supervision: N.G. and S.B.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Atkin S, Barrier S, Cui Z, Fletcher PDI, Mackenzie G, Panel V, Sol V, Zhang X (2011) UV and visible light screening by individual sporopollenin exines derived from *Lycopodium clavatum* (club moss) and *Ambrosia trifida* (giant ragweed). *J Photochem Photobiol B Biol* 102:209–217. <https://doi.org/10.1016/j.jphotobiol.2010.12.005>
- Barnes SH, Blackmore S (1986) Some functional features in pollen development. In: Blackmore S, Ferguson IK (eds) *Pollen and spores: form and function*. Academic, London, pp 71–80
- Barnes SH, Blackmore S (1992) Ultrastructural organization of two tapetal types in angiosperms. *Arch Histol Cytol* 55(suppl):217–224
- Blackmore S (1990) Sporoderm homologies and morphogenesis in land plants, with a discussion on *Echinops sphaerocephala* (Compositae). *Plant Syst Evol* 5:1–12
- Blackmore S, Barnes SH (1985) *Cosmos* pollen ontogeny: a scanning electron microscope study. *Protoplasma* 126:91–99
- Blackmore S, Barnes SH (1987) Pollen wall morphogenesis in *Tragopogon porrifolius* L. (Compositae: Lactuceae) and its taxonomic significance. *Rev Palaeobot Palynol* 52:233–246. [https://doi.org/10.1016/0034-6667\(87\)90056-X](https://doi.org/10.1016/0034-6667(87)90056-X)
- Blackmore S, Barnes SH (1988) Pollen ontogeny in *Catananche caerulea* L. (Compositae: Lactuceae). I. Premeiotic phase to establishment of tetrads. *Ann Bot (Oxford)* 62:605–614
- Blackmore S, Claugh D (1987) Observations on the substructural organization of the exine in *Fagus sylvatica* L. (Fagaceae) and *Scorzonera hispanica* L. (Compositae: Lactuceae). *Rev Palaeobot Palynol* 5:175–184
- Blackmore S, van Helvoort HAM, Punt W (1984) On the terminology, origins and functions of caveate pollen in compositae. *Rev Palaeobot Palynol* 43:293–301
- Blackmore S, Wortley AH, Skvarla JJ, Rowley JR (2007) Pollen wall development in flowering plants. *New Phytol* 174:483–498
- Blackmore S, Wortley AH, Skvarla JJ, Robinson H (2009) Evolution of pollen in the compositae. In: Funk VA, Susanna A, Stuessy T, Bayer R (eds) *Systematics, evolution and biogeography of the Compositae*. IAPT, Vienna, pp 101–130
- Blackmore S, Wortley AH, Skvarla JJ, Gabarayeva NI (2010) Developmental origins of structural diversity in pollen walls of Compositae. *Plant Syst Evol* 284:17–32. <https://doi.org/10.1007/s00606-009-0232-2>
- Dickinson HG, Potter U (1976) The development of patterning in the alveolar sexine of *Cosmos bipinnatus*. *New Phytol* 76:543–550
- Dobritsa AA, Coerper D (2012) The novel plant protein INAPERTURATE POLLEN1 marks distinct cellular domains and controls formation of apertures in the *Arabidopsis* pollen exine. *Plant Cell* 24:4452–4464. <https://doi.org/10.1105/tpc.112.101220>
- Dobritsa AA, Kirkpatrick AB, Reeder SH, Li P, Owen HA (2018) Pollen aperture factor INP1 acts late in aperture formation by excluding specific membrane domains from exine deposition. *Plant Physiol* 176:326–339. <https://doi.org/10.1104/pp.17.007201>
- El-Ghazaly G (1982) Ontogeny of pollen wall of *Leontodon autumnalis* (Hypochoeridinae, Compositae). *Grana* 21:103–113
- El-Ghazaly G, Nilsson S (1991) Development of tapetum and orbicules of *Catharanthus roseus* (Apocynaceae). In: Blackmore S, Barnes SH (eds) *Pollen and spores*. Syst ass special vol 41. Clarendon, Oxford, pp 317–329
- Fridrichsberg DA (1995) Colloidal chemistry. Chemistry, St. Petersburg
- Gabayeva NI (1993) Hypothetical ways of exine pattern determination. *Grana* 33(suppl 2):54–59. <https://doi.org/10.1080/00173139309428980>
- Gabayeva NI (1997) Ultrastructural foundations of sporoderm development in Magnoliidae. Diss. Dsc., Komarov Bot. Inst. (In Russian)
- Gabayeva NI (2000) Principles and recurrent themes in sporoderm development. In: Harley MM, Morton CM, Blackmore S (eds) *Pollen and spores: morphology and biology*. Royal Botanic Gardens, Kew, pp 1–17
- Gabayeva NI, Grigorjeva VV (2003) Comparative study of the pollen wall development in *Illicium floridanum* (Illiciaceae) and *Schisandra chinensis* (Schisandraceae). *Taiwania* 48:147–167
- Gabayeva NI, Grigorjeva VV (2010) Sporoderm ontogeny in *Chamaedorea microspadix* (Arecaceae). Self-assembly as the underlying cause of development. *Grana* 49:91–114. <https://doi.org/10.1080/00173131003650920>
- Gabayeva NI, Grigorjeva VV (2011) Sporoderm development in *Swida alba* (Cornaceae), interpreted as a self-assembling colloidal system. *Grana* 50:81–101. <https://doi.org/10.1080/00173134.2011.580448>
- Gabayeva NI, Grigorjeva V (2012) Sporoderm development and substructure in *Magnolia sieboldii* and other Magnoliaceae: an interpretation. *Grana* 51:119–147. <https://doi.org/10.1080/00173134.2012.688863>
- Gabayeva NI, Grigorjeva VV (2013) Experimental modelling of exine-like structures. *Grana* 52:241–257. <https://doi.org/10.1080/00173134.2013.818165>
- Gabayeva NI, Grigorjeva VV (2014) Sporoderm and tapetum development in *Eupomatia laurina* (Eupomatiaceae). An interpretation. *Protoplasma* 251:1321–1345. <https://doi.org/10.1007/s00709-014-0631-2>
- Gabayeva NI, Grigorjeva VV (2016) Simulation of exine patterns by self-assembly. *Plant Syst Evol* 302:1135–1156. <https://doi.org/10.1007/s00606-016-1322-6>
- Gabayeva NI, Grigorjeva VV (2017) Self-assembly as the underlying mechanism for exine development in *Larix decidua* D. C. *Planta* 246:471–493. <https://doi.org/10.1007/s00425-017-2702-z>
- Gabayeva NI, Hemsley AR (2006) Merging concepts: the role of self-assembly in the development of pollen wall structure. *Rev Palaeobot Palynol* 138:121–139. <https://doi.org/10.1016/j.revpalbo.2005.12.001>
- Gabayeva NI, Grigorjeva VV, Rowley JR, Hemsley AR (2009a) Sporoderm development in *Trevesia burckii* (Araliaceae). I. Tetrad period: further evidence for the participation of self-assembly processes. *Rev Palaeobot Palynol* 156:211–232. <https://doi.org/10.1016/j.revpalbo.2008.12.001>
- Gabayeva NI, Grigorjeva VV, Rowley JR, Hemsley AR (2009b) Sporoderm development in *Trevesia burckii* (Araliaceae). II. Post-tetrad period: further evidence for the participation of self-assembly processes. *Rev Palaeobot Palynol* 156:233–247. <https://doi.org/10.1016/j.revpalbo.2009.01.004>

- Gabarayeva NI, Grigorjeva VV, Rowley JR (2010a) A new look at sporoderm ontogeny in *Persea americana*. Micelles and the hidden side of development. *Ann Bot (Oxford)* 105:939–955. <https://doi.org/10.1093/aob/mcq075>
- Gabarayeva NI, Grigorjeva VV, Rowley JR (2010b) Sporoderm development in *Acer tataricum* (Aceraceae). An interpretation. *Protoplasma* 247:65–81. <https://doi.org/10.1007/s00709-010-0141-9>
- Gabarayeva N, Grigorjeva V, Polevova S (2011a) Exine and tapetum development in *Symphytum officinale* (Boraginaceae). Exine substructure and its interpretation. *Plant Syst Evol* 296:101–120. <https://doi.org/10.1007/s00606-011-0479-2>
- Gabarayeva NI, Grigorjeva VV, Marquez G (2011b) Ultrastructure and development during meiosis and the tetrad period of sporogenesis in the leptosporangiate fern *Alsophila setosa* (Cyatheaceae) compared with corresponding stages in *Psilotum nudum* (Psilotaceae). *Grana* 50:235–262. <https://doi.org/10.1080/00173134.2011.631759>
- Gabarayeva N, Grigorjeva V, Kosenko Y (2013a) I Primexine development in *Passiflora racemosa* Brot. Overlooked aspects of development. *Plant Syst Evol* 299:1013–1035. <https://doi.org/10.1007/s00606-013-0757-2>
- Gabarayeva N, Grigorjeva V, Kosenko Y (2013b) II. Exine development in *Passiflora racemosa* Brot.: post-tetrad period. Overlooked aspects of development. *Plant Syst Evol* 299:1037–1055. <https://doi.org/10.1007/s00606-013-0756-3>
- Gabarayeva N, Grigorjeva V, Polevova S (2014) Sporoderm and tapetum ontogeny in *Juniperus communis* (Cupressaceae). Connective structures between tapetum and microspores. *Rev Palaeobot Palynol* 206: 23–44
- Gabarayeva NI, Grigorjeva VV, Blackmore S (2016) Pollen wall substructure and development in *Tanacetum vulgare* (Compositae: Anthemideae): revisiting hypotheses on pattern formation in complex cell walls. *Int J Plant Sci* 177:347–370. <https://doi.org/10.1086/684946>
- Gabarayeva N, Grigorjeva V, Polevova S, Hemsley AR (2017) Pollen wall and tapetum development in *Plantago major* L. (Plantaginaceae): assisting self-assembly. *Grana* 56:81–111. <https://doi.org/10.1080/00173134.2016.1159729>
- Gabarayeva NI, Polevova SV, Grigorjeva VV, Blackmore S (2018) Assembling the thickest plant cell wall: exine development in *Echinops* (Asteraceae, Cynareae). *Planta* 248(2):323–346
- Griffiths PC, Hemsley AR (2001) Raspberries and muffins—mimicking biological pattern formation. *Colloids Surf B: Biointerfaces* 25:163–170. [https://doi.org/10.1016/S0927-7765\(01\)00316-2](https://doi.org/10.1016/S0927-7765(01)00316-2)
- Grigorjeva V, Gabarayeva N (2015) The development of sporoderm, tapetum and Ubisch bodies in *Dianthus deltoides* (Caryophyllaceae): self-assembly in action. *Rev Palaeobot Palynol* 219:1–27. <https://doi.org/10.1016/j.revpalbo.2015.03.005>
- Grigorjeva VV, Gabarayeva N (2018) Pollen wall ontogeny in *Polemonium caeruleum* (Polemoniaceae) and suggested underlying mechanisms of development. *Protoplasma* 255:109–128. <https://doi.org/10.1007/s00709-017-1121-0>
- Hemsley AR (1998) Non-linear variation in simulated complex pattern development. *J Theor Biol* 192:73–79
- Hemsley AR, Gabarayeva NI (2007) Exine development: the importance of looking through colloidal chemistry “window”. *Plant Syst Evol* 263:25–49. <https://doi.org/10.1007/s00606-006-0465-2>
- Hemsley AR, Griffiths PC (2000) Architecture in the microcosm: biocolloids, self-assembly and pattern formation. *Philos Trans R Soc Lond A* 358:547–564
- Hemsley AR, Jenkins PD, Collinson ME, Vincent B (1996) Experimental modelling of exine self-assembly. *Bot J Linn Soc* 121:177–187. <https://doi.org/10.1111/j.1095-8339.1996.tb00752.x>
- Hemsley AR, Vincent B, Collinson ME, Griffiths PC (1998) Simulated self-assembly of spore exines. *Ann Bot (Oxford)* 82:105–109
- Heslop-Harrison J (1972) Pattern in plant cell wall morphogenesis in miniature. *Proc R Inst G B* 45:335–351
- Hesse M, Hess MW (1993) Recent trends in tapetum research. A cytological and methodological review. *Plant Syst Evol* 7:127–145
- Horner HT, Pearson CB (1978) Pollen wall and aperture development in *Helianthus annuus* (Compositae: Heliantheae). *Am J Bot* 65: 293–309
- Ingber DE (2003a) Tensegrity I. Cell structure and hierarchical systems biology. *J Cell Sci* 116:1157–1173. <https://doi.org/10.1242/jcs.00359>
- Ingber DE (2003b) Tensegrity II. How structural networks influence cellular information processing networks. *J Cell Sci* 116:1397–1408. <https://doi.org/10.1242/jcs.00360>
- Kamelina OP (1988) Types of the tapetum in angiosperms. *Ann Sci Univ Reims ARERS* 23:71–73
- Larson DA (1966) On the significance on the detailed structure of *Passiflora caerulea* exines. *Bot Gaz* 127:40–48
- Lersten NR, Curtis JD (1990) Invasive tapetum and tricelled pollen in (Asteraceae, tribe Heliantheae). *Plant Syst Evol* 169:237–243
- Makra L, Juhász M, Béczi R, Borsos E (2005) The history and impacts of airborne *Ambrosia* (Asteraceae) pollen in Hungary. *Grana* 44:57–64. <https://doi.org/10.1080/00173130510010558>
- Martin MD, Chamecki M, Brush GS, Meneveau C, Parlange MB (2009) Pollen clumping and wind pollination in an invasive angiosperm. *Am J Bot* 96:1703–1711. <https://doi.org/10.3732/ajb.0800407>
- McLauchlan KK, Barnes CS, Craine JS (2011) Interannual variability of pollen productivity and transport in mid-North America from 1997 to 2009. *Aerobiologia* 27:181–189. <https://doi.org/10.1007/s10453-010-9186-7>
- Pacini E, Franchi GG (1991) Diversification and evolution of the tapetum. In: Blackmore S, Barnes SH (eds) *Pollen and spores: patterns of diversification*. Clarendon, Oxford, pp 301–316
- Pacini E, Juniper BE (1983) The ultrastructure of the formation and development of the amoeboid tapetum in *Arum italicum* Miller. *Protoplasma* 117:116–129. <https://doi.org/10.1007/BF01288350>
- Pacini E, Keijer CJ (1989) Ontogeny of intruding non-periplasmal tapetum in the wild chicory, *Cichorium intybus* (Compositae). *Plant Syst Evol* 67:149–164
- Pacini E, Franchi GG, Hesse M (1985) The tapetum: its form, function and possible phylogeny in Embryophyta. *Plant Syst Evol* 149:155–185
- Pettitt JM (1979) Ultrastructure and cytochemistry of spore wall morphogenesis. In: Dyer AF (ed) *The experimental biology of ferns*. Academic, London, pp 211–252
- Punt W, Hoen PP (2009) The Northwest European Pollen Flora, 70 Asteraceae—Asteroideae. *Rev Palaeobot Palynol* 157:22–183
- Reinitzer F (1888) Zur Kenntnis des Cholesterins. *Monatshefte* 9:421–441
- Rowley JR (1973) Formation of pollen exine bacules and microchannels on a glycolyx. *Grana* 13:129–138
- Rowley JR (1990) The fundamental structure of the pollen exine. *Plant Syst Evol* 5:13–29
- Rowley JR, Dahl AO (1977) Pollen development in *Artemisia vulgaris* with special reference to glycolyx material. *Pollen Spores* 19: 169–284
- Rowley JR, Walles B (1985) Cell differentiation in microsporangia of *Pinus sylvestris*. III. Late pachytene. *Nord J Bot* 5:255–271. <https://doi.org/10.1111/j.1756-1051.1985.tb01655.x>
- Rowley JR, Dahl AO, Rowley JS (1981) Substructure in exines of *Artemisia vulgaris* (Asteraceae). *Rev Palaeobot Palynol* 35:1–38
- Rowley JR, Gabarayeva NI, Walles B (1992) Cyclic invasion of tapetal cells into loculi during microspore development in *Nymphaea colorata* (Nymphaeaceae). *Am J Bot* 79:801–808
- Sampathkumar A, Yan A, Krupinski P, Meyerowitz EM (2014) Physical forces regulate plant development and morphogenesis. *Curr Biol* 24: 475–483. <https://doi.org/10.1016/j.cub.2014.03.014>
- Sheldon JM, Dickinson HG (1983) Determination of patterning in the pollen wall of *Lilium henryi*. *J Cell Sci* 63:191–208
- Sitte P (1981) Role of lipid self-assembly in subcellular morphogenesis. In: Kiermayer O (ed) *Cytomorphogenesis in plants*. Cell biology

- monographs, 8th edn. Springer, Wien, pp 401–421. https://doi.org/10.1007/978-3-7091-8602-2_15
- Skvarla JJ, Larsen DA (1965) An electron microscopic study of pollen morphology in the Compositae with special reference to the *Ambrosiinae*. Grana 6:210–269
- Southworth D (1983) Exine development in *Gerbera jamesonii* (Asteraceae: Mutisieae). Am J Bot 70:1038–1047. <https://doi.org/10.1002/j.1537-2197.1983.tb07904.x>
- Stix E (1960) Pollenmorphologische Untersuchungen an Compositen. Grana 2:41–104
- Takahashi M (1989) Development of the echinate pollen wall in *Farfugium japonicum* (Compositae: Senecioneae). Bot Mag Tokyo 102:219–239
- Taylor ML, Osborn JM (2006) Pollen ontogeny in *Brasenia* (Cabombaceae, Nymphaeales). Am J Bot 93:344–356
- Taylor ML, Hudson PJ, Rigg JM, Strandquist JN, Green JS, Thiemann TC, Osborn JM (2012) Tapetum structure and ontogeny in *Victoria* (Nymphaeaceae). Grana 51:107–118
- Taylor ML, Hudson PJ, Rigg JM, Strandquist JN, Green JS, Thiemann TC, Osborn JM (2013) Pollen ontogeny in *Victoria* (Nymphaeales). Int J Plant Sci 174:1259–1276. <https://doi.org/10.1086/673246>
- Taylor ML, Cooper RL, Schneider EL, Osborn JM (2015) Pollen structure and development in Nymphaeales: insights into character evolution in an ancient angiosperm lineage. Am J Bot 102:1685–1702. <https://doi.org/10.3732/ajb.1500249>
- Thio BJR, Lee JH, Carson Meredith J (2009) Characterization of ragweed pollen adhesion to polyamides and polystyrene using atomic force microscopy. Environ Sci Technol 43:4308–4313. <https://doi.org/10.1021/es803422s>
- Tompson DA (1917) On growth and form. Cambridge University Press, Cambridge
- Varotto S, Parrini P, Mariani P (1996) Pollen ontogeny in *Cichorium intybus* L. Grana 35:154–161. <https://doi.org/10.1080/00173139609429076>
- Vinckier S, Smets E (2005) A histological study of microsporogenesis in *Tarennia gracilipes* (Rubiaceae). Grana 44:30–44
- Walles B, Rowley JR (1982) Cell differentiation in microsporangia of *Pinus sylvestris* with special attention to the tapetum. I. The pre- and early-meiotic periods. Nord J Bot 2:53–70
- Willemse MTM (1971) Morphological and quantitative changes in the population of cell organelles during microsporogenesis of *Pinus sylvestris* L. II. Morphological changes from prometaphase I until the tetrad stage. Acta Bot Neerl 20:411–427. <https://doi.org/10.1111/j.1438-8677.1971.tb00727.x>
- Wodehouse RP (1928) Pollen grains in the identification and classification of plants. I. The *Ambrosiaceae*. Bull Torrey Bot Club 55:181–198
- Wodehouse RP (1934) An oil drop theory of pollen-grain pattern formation. Am J Bot 21:18–22