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Original article

Gamal El-Ghazaly[1]: Development of *Magnolia grandiflora* pollen wall

Gamal El-Ghazaly[1]: タイサンボク *Magnolia grandiflora* の花粉壁の発生過程

Abstract The main developmental stages of the pollen wall of *Magnolia grandiflora* were investigated. Microspore tetrads varied in their morphology. In late microspore tetrads during the callose period, pro-columellae condensed around protrusions of the plasmalemma. As a result of the accumulation of sporopollenin precursors on the protrusions of the plasmalemma, columellae became evident. At a late tetrad stage, the foot layer developed on a white-line-centered lamella. At free microspore stages, the units of the exine thickened, and on further development, white-line-centered lamellae appeared over the inner surface of the foot layer. These lamellae represented a transitory or rudimentary endexine. At the vacuolate stage, a characteristic vesicular-fibrillar layer developed under the rudimentary endexine. The intine developed at the young pollen grains stage and differentiated into two zones with different electron density. The possible function of endoplasmic reticulum and Golgi vesicles in the development of the vesicular-fibrillar layer and the intine is discussed.
Key words: *Magnolia*, microsporogenesis, ontogeny, pollen wall development, ultrastructure

要　旨　タイサンボクの花粉壁の発生過程を調べた。小胞子の四分子には形態に変異が認められた。カロース期にある後期の四分子において，原形質膜の突起を中心として前柱状体が形成された。スポロポレニン前駆物質が突起のまわりに濃集するにつれて柱状体が明瞭になった。四分子期の後期となると，底部層が中央に白線をもつラメラの上に発達した。小胞子が分離すると外壁の構成層が厚くなり，底部層の内側には中央に白線をもつラメラが複数出現した。このラメラは移行的あるいは痕跡的な内層に相当する。液胞が生じると痕跡的な内層の下には特徴的なフィブリル・小胞層が形成される。内壁は若い花粉粒の時期に形成され，電子濃度の異なる 2 層に分化する。フィブリル・小胞層および内壁の形成に際して，小胞体およびゴルジ体が果たしていると想定される役割について議論する。
キーワード：花粉壁，小胞子形成，個体発生，微細構造，モクレン属

Introduction

The Magnoliaceae is an important family for the phylogenetic understanding of angiosperms and has traditionally been assigned to the core group of ‘primitive’ families. The subclass Magnoliidae have been much studied in several disciplines, i.e., palaeobotany (Endress, 1986; Crepet & Nixon, 1998), pollen morphology (Muller, 1970; Walker, 1976), and sexual plant reproduction (Endress, 1983; Gottsberger, 1988). Pollen wall development and structure in primitive angiosperms have also been intensively studied (Dahl & Rowley, 1965; Walker & Skvarla, 1975; Walker, 1976; Zavada, 1984; Waha, 1987; Gabarayeva, 1987a, b, 1991). These studies agree to a great extent and have clarified the main features of pollen wall morphology and ontogeny in several primitive flowering plants. Some features are, however, interpreted differently and still require more detailed studies. In the present paper the development of the pollen wall of *Magnolia grandiflora* L. was studied to clarify those features under argument in the literature with considerable discrepancies in interpretation. The argument mainly concerns the development of columellae and their morphological characteristic, i.e., granular, radial rods or radial cylindrical columellae. The development and morphology of the endexine and intine are also still in dispute.

Material and methods

Fresh anthers of *Magnolia grandiflora* L. collected in Takamatsu, Japan were fixed in a mixture of 1% paraformaldehyde and 3% glutaraldehyde in sodium cacodylate buffer (pH 7.4) according to the procedure of Karnovsky (1965). After rinsing several times in distilled water, they were postfixed with 2% osmium tetroxide for 24 h at room temperature and subsequently dehydrated in a graded ethanol series.

Some of the fixed anthers in absolute ethanol for scanning electron microscopy (SEM) were frozen with liq-

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uid nitrogen and fractured on a TF-1 chamber. Mature pollen grains were acetolyzed according to the method of Erdtman (1960). After critical-point drying, the fractured anthers and the acetolyzed pollen grains were sputter coated with platinum-palladium at ca. 5–10 nm thickness using an Eiko SR-2 rotative ion sputtering apparatus and examined in a Hitachi S-800 field emission scanning electron microscope at an accelerating voltage of 8 kV.

Other fixed anthers for transmission electron microscopy (TEM) were embedded in Spurr (1969). Sections were stained with 1% aqueous uranyl acetate followed by lead citrate. Observations were made using a Zeiss EM 906 at the Botany Department, Stockholm University, Sweden.

Results

Sporogenous tissue

At the early prophase, the wall of male meiocytes was thin and electron-dense, and the plasmalemma was undulated (Fig. 1). The cytoplasm was characterized by the presence of several small vacuoles, plastids with small starch grains, and numerous mitochondria (Fig. 1). Tapetal cells were more electron-dense than the meiocytes and were packed with mitochondria and dictyosomes (Fig. 1). At a later stage, the wall of tapetal cells started to degenerate and appeared fibrillar. The wall of the sporogenous cells appeared more electron-dense and less compact than before (Fig. 2)

Microspore tetrads

During meiosis and the early stage of microsporogenesis, a special callosic envelope was synthesized. At the end of meiosis, the four microspores were embedded in the special callosic envelope (Fig. 3). The surface of the microspore plasmalemma was coated with a layer of fibrillar matrix (Figs. 4–6). Each microspore formed its own wall within the fibrillate coat of the plasmalemma.

In microspore tetrads during the callose period, the exine began as protrusions originated from the plasmalemma (Figs. 4, 7). These protrusions were the start of exine units that upon further development became columellae as well as part of the tectum and foot layer (Fig. 8). The patterned sexine (tectum, columellae, and foot layer) was formed within the primexine (glycocalyx).

Tectum, columellae, and foot layer

Pro-columellae appeared to condense around the protrusions of the plasmalemma with accumulation of sporopollenin precursors. During the late tetrad stages, the pro-columellae became discretely differentiated into electron-dense columellae (Fig. 8). The tectum developed on the distal ends of the columellae and appeared polygonal in oblique section. The columellar arcade contained fibrils. Formation of the foot layer took place at the bases of the developing columellae on a white-line-centered lamella that was developed at the distal surface of the plasmalemma. This may explain the variable thickness of the foot layer (Fig. 8). Towards the apertural region, the tectum and columellae gradually became thinner and shorter, respectively (Fig. 9).

Free microspores—Endexine

By dissolution of the special callosic wall, microspores were released and rapidly enlarged. Sporopollenin was accumulated progressively on the tectum, columellae, and foot layer (Fig. 10).

The endexine layer developed as a thin layer on a white-line-centered lamella at the proximal surface of the foot layer (Fig. 10). The endexine was more visible and varied in thickness at the aperture site (Fig. 11). A wide periplasmic zone developed between the plasmalemma and the inner surface of the microspore wall (Figs. 10–12). At this stage, several vacuoles were observed in the cytoplasm of microspores and contained small vesicles full of fibrils (Fig. 11).

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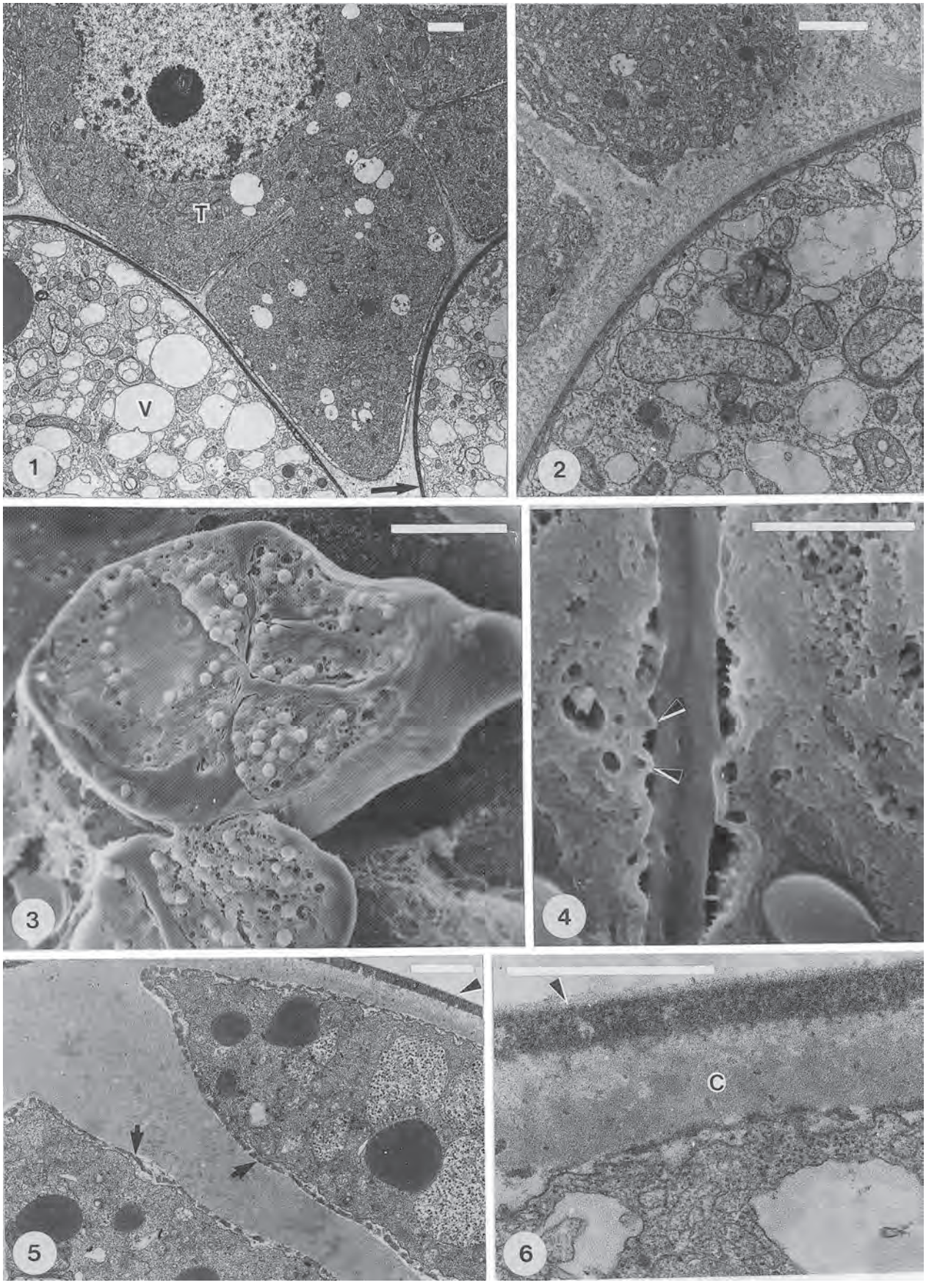
Figs. 1–2 Meiocyte stage. – **1:** Parts of two sporogenous cells surrounded by tapetal cells (T). Note the prominent nucleus and the dense cytoplasm of tapetal cells. The sporogenous cells contain many vesicles (V), and their wall is electron-dense (arrow). Scale bar = 1 μm. – **2:** Detail of a sporogenous cell and tapetal cells. The wall of tapetal cells is thick and fibrillar. The sporogenous cell is rich in mitochondria, and callose is beginning to accumulate between the plasmalemma and the wall. Scale bar = 1 μm. — **Figs. 3–6** Early tetrad stage. – **3**: Microspores within a special callosic envelope. Note that the callosic envelope is asymmetric in shape and varies in thickness. Scale bar = 1 μm. – **4:** Detail of two freeze-fractured microspores within the callosic envelope. The plasmalemma is protruded towards the callosic envelope (arrowheads). Scale bar = 1 μm. – **5:** Part of two microspores surrounded by the callosic envelope. The plasmalemma is coated with thin electron-dense matrix (arrows). The remnant of a sporogenous cell wall is darkly stained (arrowhead). Scale bar = 1 μm. – **6:** Detail of a microspore wall showing invaginations and the surface coating of the plasmalemma. Note the thick callosic envelope (C) and the loose electron-dense material of the sporogenous cell wall (arrowhead). Scale bar = 1 μm.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Vesicular-fibrillar layer

At the late microspore stage, the primexine (glycocalyx) matrix was still evident, and the wall thickened almost twice as that in the early free microspore stage. The fibril-filled vesicles in the microspore cytoplasm increased in number and density (Fig. 13). The plasmalemma and cytoplasm protruded across a periplasmic zone and contacted the exine (Fig. 13). These cytoplasmic protrusions were probably involved in the transfer of nutrients and other materials. With further development, vesicular-fibrillar material appeared in the periplasmic zone (Fig. 14). These vesicles were apparently attached to the plasmalemma, probably at those sites where cisternae of endoplasmic reticulum (ER) "contact" the plasmalemma (Fig. 14). The thickness of the foot layer and the endexine varied considerably (Fig. 14), as at the younger stage (Fig. 13).

By the end of this stage, a large vacuole was developed in the cytoplasm. The development of this vacuole presumably exerted a pressure on the wall of developing microspores. The fibrillar material of the vesicles became more electron-dense, and the vesicles became closely packed (Figs. 15–18). At this stage, the inner surface of the foot layer and the endexine also became undulated and variable in thickness (Fig. 17, 18). The exine at the aperture site was thin and usually folded, probably an artifact made during the preparation of microspores for TEM studies (Fig. 16).

Young pollen grain

At this stage mitosis took place, and the generative cell and the vegetative nucleus were produced. The plasmalemma appeared irregularly and highly convoluted. The layer of fibrillar vesicles was thick at the aperture site, and its lower portion was comparatively compact (Fig. 19). The cytoplasm was rich in endoplasmic reticulum, mitochondria, and both large and small lipoid globules. Cisternae of endoplasmic reticulum were attached to the plasmalemma at several sites (Fig. 19). The intine started to develop below the aperture site, continued to develop, and thickened both in non-apertural (Fig. 20) and apertural regions (Fig. 22). The intine enclosed several spheroid units with electron-dense material (Figs. 20, 22). The membranous boundary of the vesicular-fibrillar components was more apparent in this stage (Figs. 20–22). In many sites, the "vesicles" were attached to one another or joined to appear as an elongated section of a conduit (Figs. 20–22).

These components in the intermediate zone between the exine and the intine (Figs. 19–22) were probably moving between the exine and the cytoplasm and were not properly assigned to either endexine or intine. The spheroid inclusions within the intine were perhaps transitional material between the loculus, the exine, and the cytoplasm.

Mature pollen grain

The intine slightly became thiner and appeared more electron-dense than before (Fig. 23). The volume of the pollen grains increased, and the wall became thinner. The tectum varied in thickness, and the columellar arcade contained dispersed fibrils (Fig. 23). The cytoplasm contained elioplasts, droplets of lipids, and numerous mitochondria (Fig. 23). At certain sites, particularly close to the aperture, the pollen wall was folded and elevated (Figs. 30, 31). Each side of the folded wall consisted of a thick tectum, short columellae, a thin foot layer, and a rudimentary endexine (Fig. 23). Close to the aperture, the exine clearly became thinner. At the aperture site, the foot layer became very thin and variable in thickness. The endexine was hardly recognizable, separated from the foot layer by a white-line-centered lamella (Figs.

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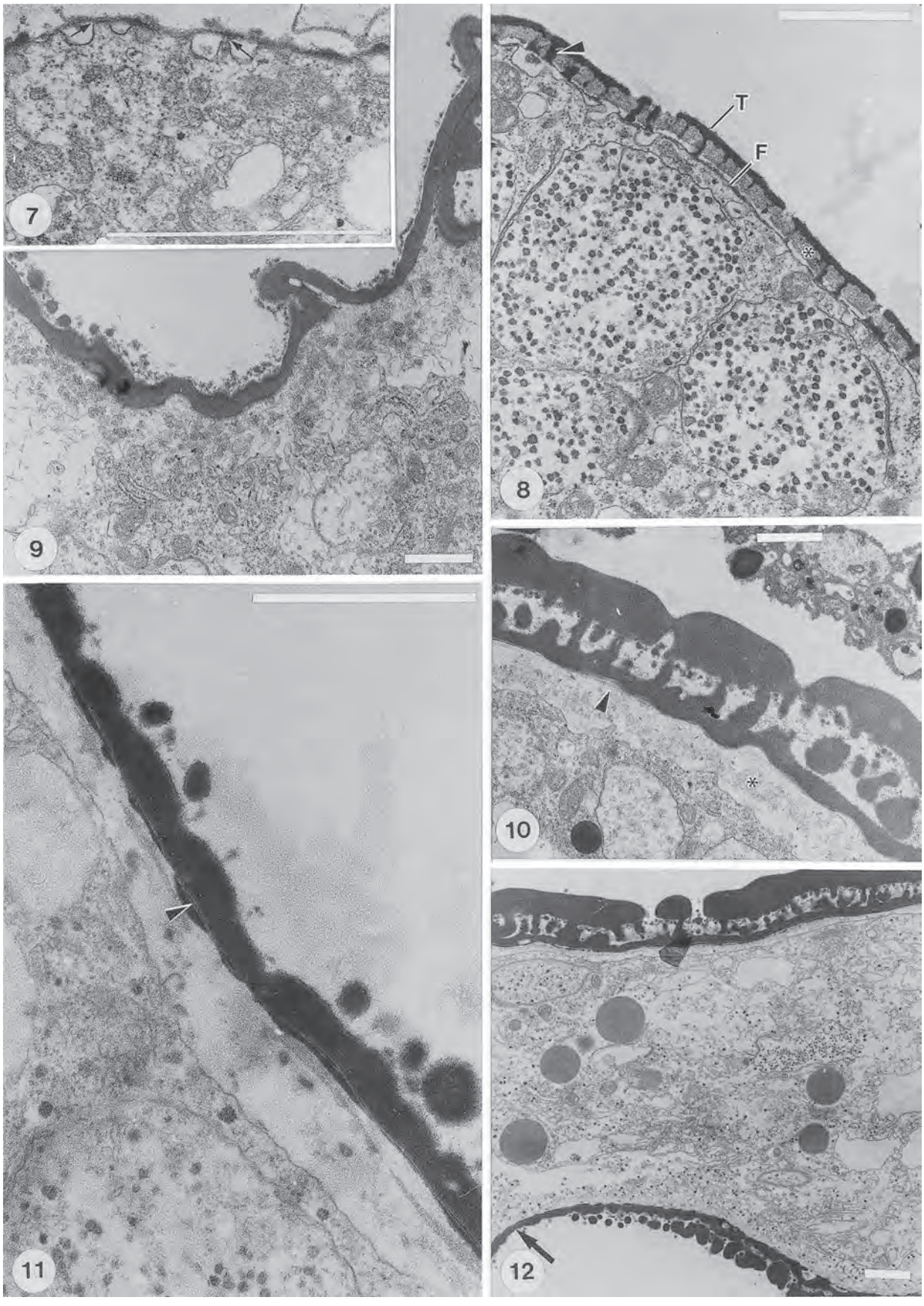
Figs. 7–9 Tetrad stage. – **7:** Detail of the microspore wall at the early tetrad stage showing protrusions of the plasmalemma (arrows). Scale bar = 2 μm. – **8:** Late tetrad microspore. Pro-columellae (arrowhead), a pro-tectum(T), and a foot layer (F) are clearly seen. The cytoplasm contains large vacuoles with many electron-dense lipoid droplets. Fibrillar material of the surface coating is prominent (asterisk). Scale bar = 1 μm. – **9:** Apertural site of a late tetrad microspore showing a comparatively thin exine. The tectum and columellae are absent, and the foot layer is thick. The wall at the aperture site appears folded, and the endexine is hardly recognizable at this magnification. Scale bar = 1 μm. — **Figs. 10–12** Early free microspore stage. – **10:** Section through a non-apertural wall. The tectum, columellae, and foot layer have thickened. The endexine appears as thin rudimentary fragments separated from the foot layer by a white-line-centered lamella (arrowhead). Note a periplasmic zone developed between the exine and the plasmalemma (asterisk). Scale bar = 1 μm. – **11:** Apertural site of a free microspore. The tectum is almost absent, and the foot layer is thick. The endexine appears irregularly thickened and is separated from the foot layer by a white-line-centered lamella (arrowhead). Scale bar = 1 μm. – **12:** Section through a non-apertural (top) and an apertural (bottom) walls. The exine is almost fully developed at the non-apertural region while it is much reduced in thickness at the apertural region. The exine gradually becomes thinner towards the center of the sulcus (arrow). Scale bar = 1 μm.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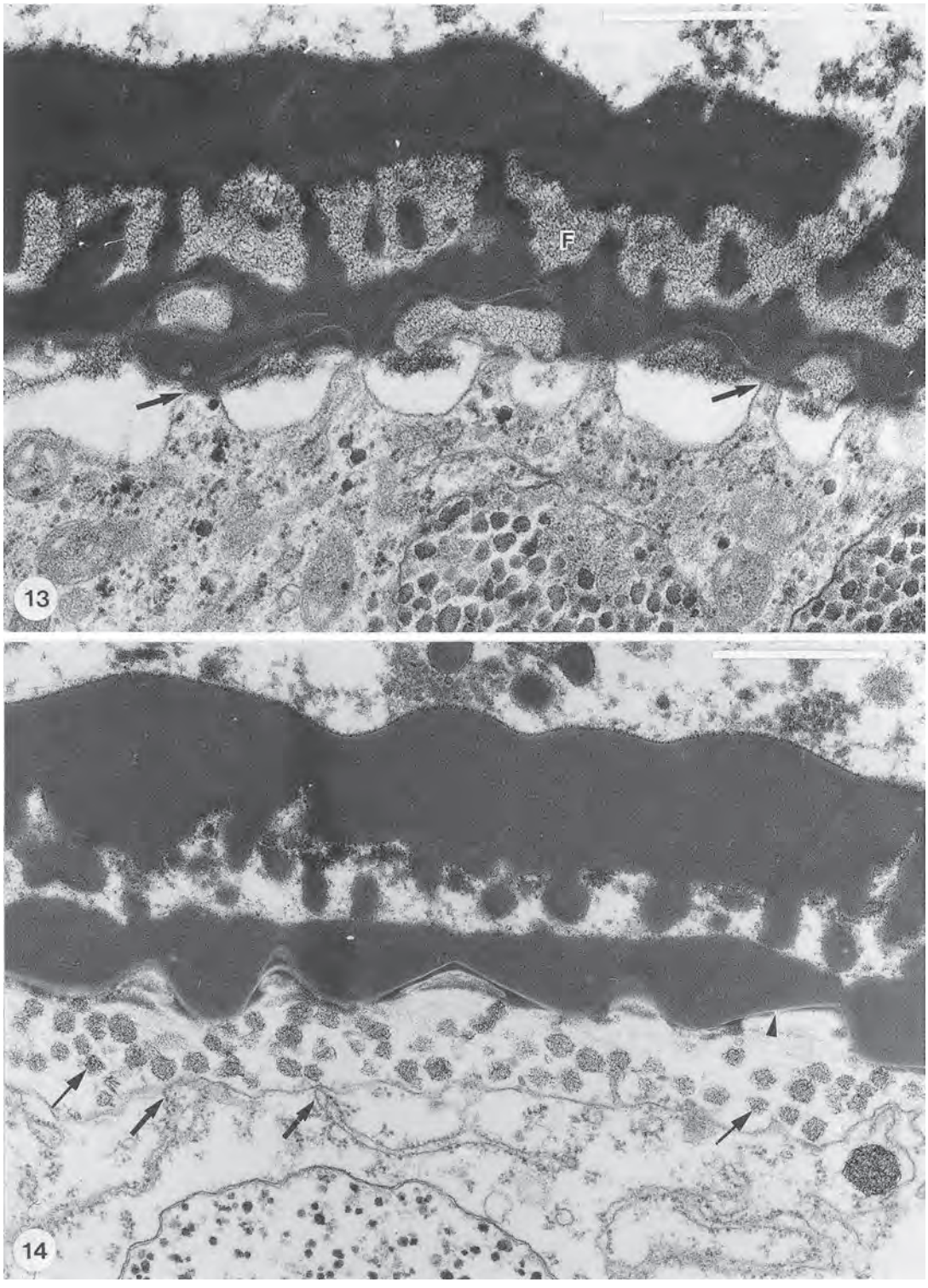
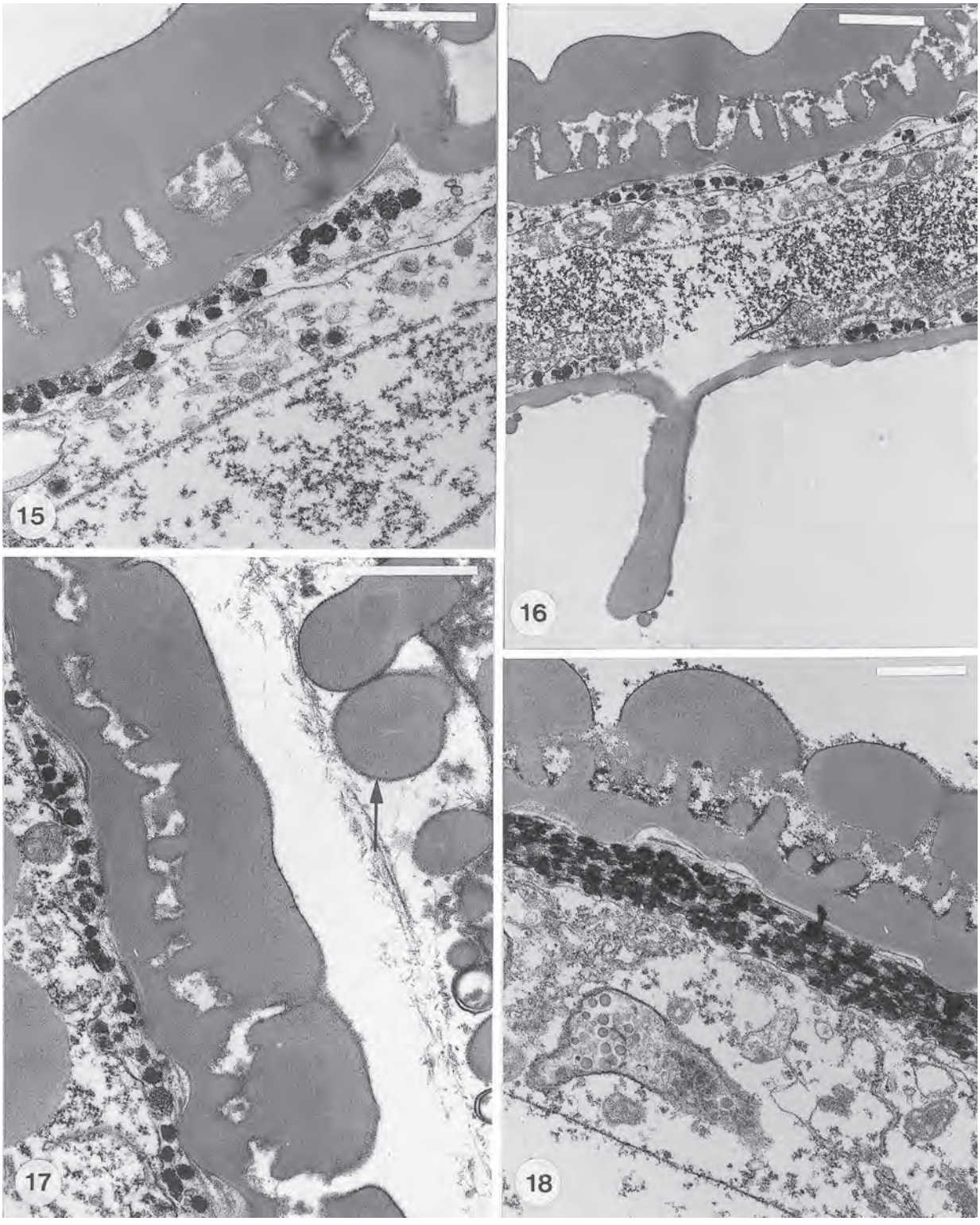


Figs. 13–14 Late free microspore stage. – **13:** Free microspore wall prior to vacuolation showing the exine that has thickened throughout. Protrusions of the plasmalemma are distinct and contact the exine (arrows). There is great variation in the thickness and shape of the foot layer (F) and endexine. The cytoplasm contains large vesicles filled with granules apparently made up of fibrillar material. Scale bar = 1 μm. – **14:** Free microspore wall at the early vacuolate stage. Note many coarse vesicular-fibrillar components (thin arrows) in the periplasmic zone between the plasmalemma and the endexine. The endexine is separated from the foot layer by a white-line-centered lamella (arrowhead). There are several cisternae of endoplasmic reticulum that appear in contact with the plasmalemma (thick arrows). Scale bar = 1 μm.



Figs. 15–18 Latest free microspore stage. – **15:** Late free microspore. Granular fibrillar material beneath the rudimentary endexine is more electron-dense than before. Scale bar = 1 μm. – **16:** Section showing the interapertural (upper) and apertural (lower) regions of the wall. The apertural membrane consists of a thin folded foot layer. The tectum and columellae are absent at the aperture site. Scale bar = 1 μm. – **17:** Orbicules attached to the tapetum (arrow). Fibrillar vesicles become denser than before (cf. Fig. 14). Scale bar = 1 μm. – **18:** In this section, the layer of fibrillar vesicles has thickened. The vesicles are radially arranged as irregular membranous tubules. Scale bar = 1 μm.

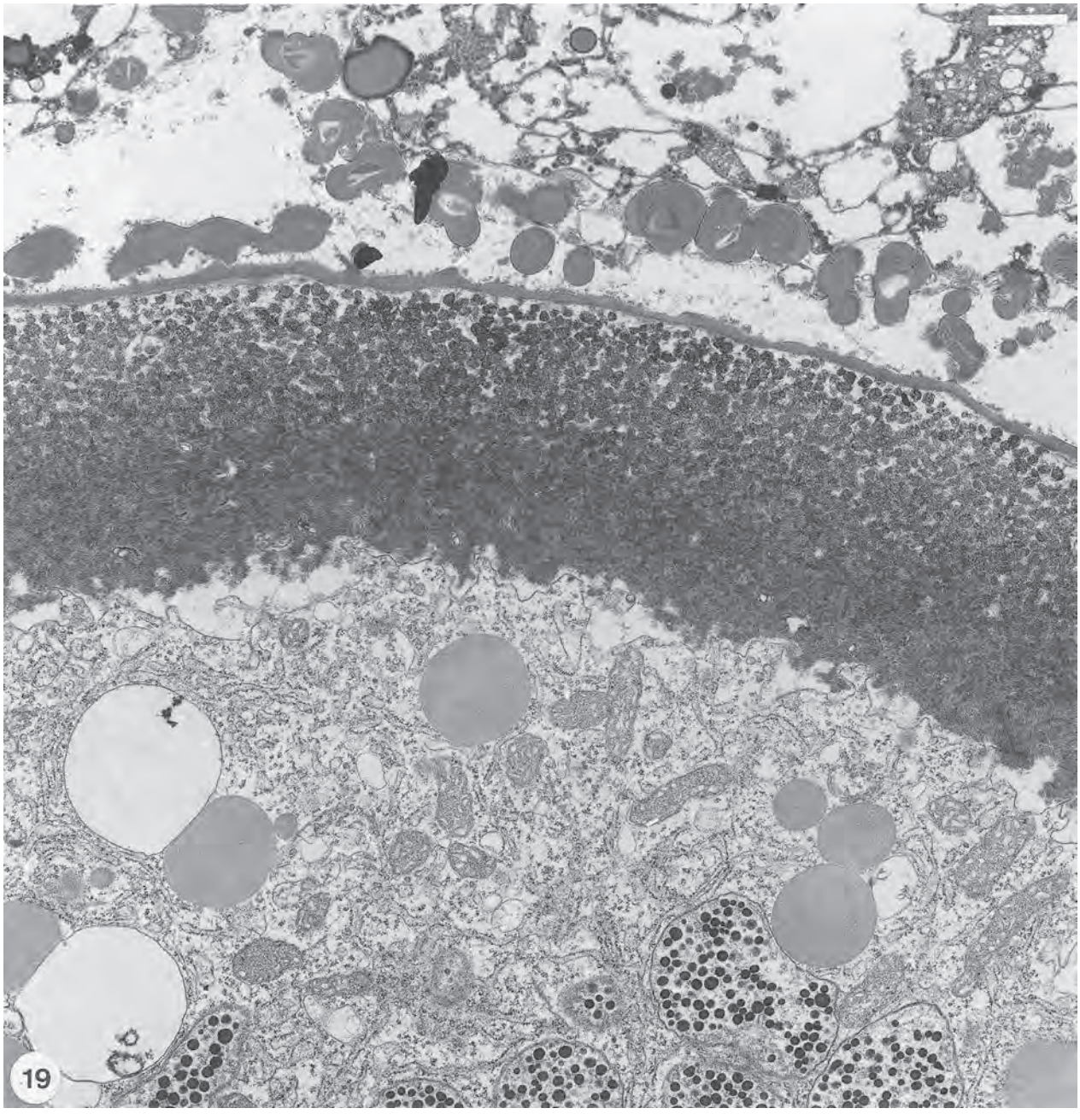


Fig. 19 Young pollen grain stage. Section in the apertural region. The foot layer is thin and the endexine is hard to detect. The layer of fibrillar vesicles is very thick, and its inner portion is compact and electron-dense. The plasmalemma is irregularly undulated. The intine is clearly seen between the invaginated plasmalemma and the proximal surface of the thick fibrillar layer. The cytoplasm contains several mitochondria and vesicles full of lipoid droplets. The tapetum is degenerating, and several orbicules are attached to the remnant of tapetal cells. Scale bar = 1 μm.

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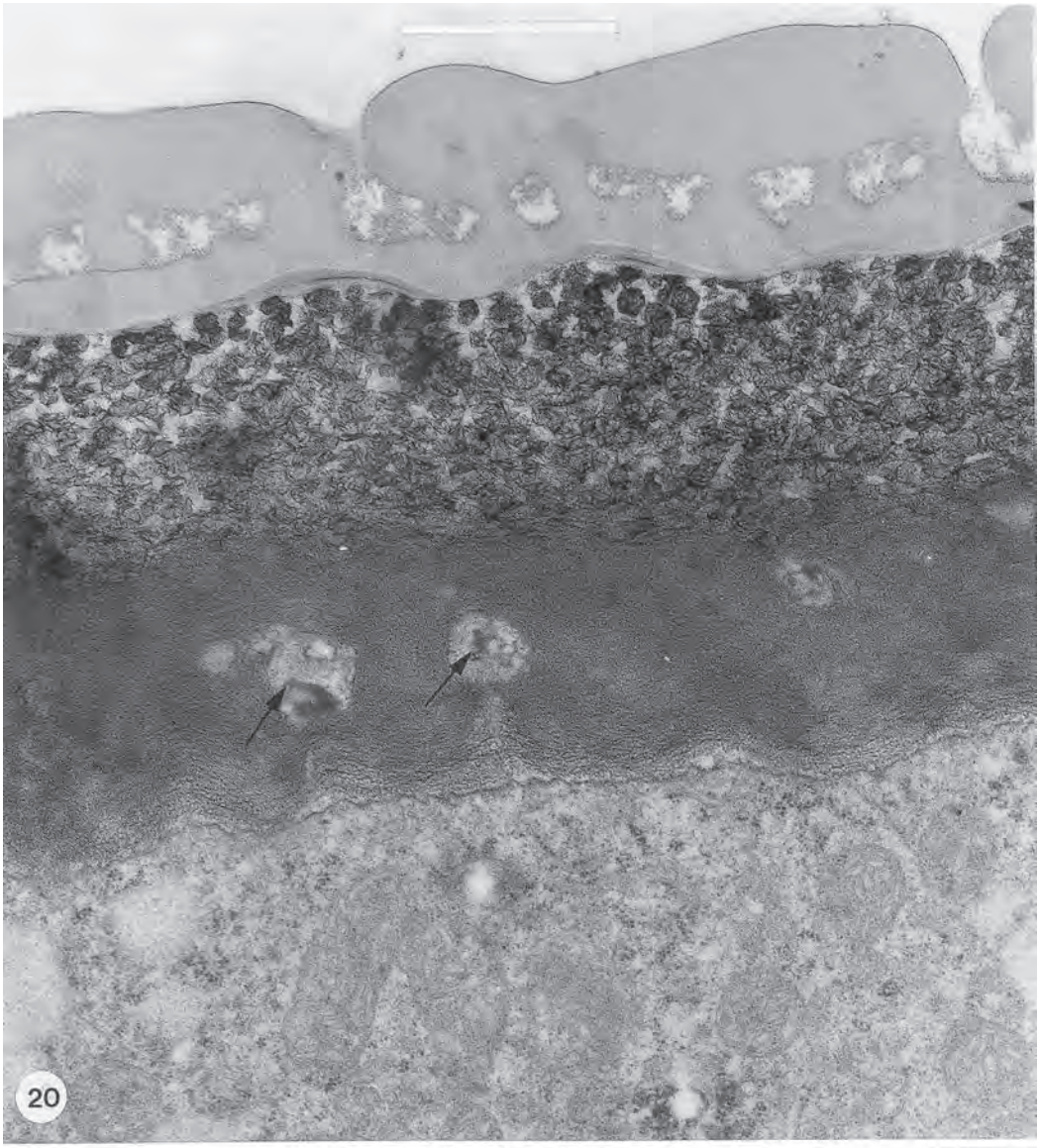
Figs. 20–21 Young pollen grain stage. – **20:** Section in the non-apetural region. The layer of vesicular-fibrillar material has thickened. The membranous and tubular boundary of the vesicular-fibrillar material is more evident than in the earlier stages. The intine is well developed and consists of a thick fibrillar layer with spherical inclusions (arrows). The cytoplasm contains many mitochondria. Scale bar = 1 μm. – **21:** Detail of the intermediate layer between the endexine and the intine. The irregular, tubular extensions that envelope the fibrillate material are clearly seen. Scale bar = 1 μm.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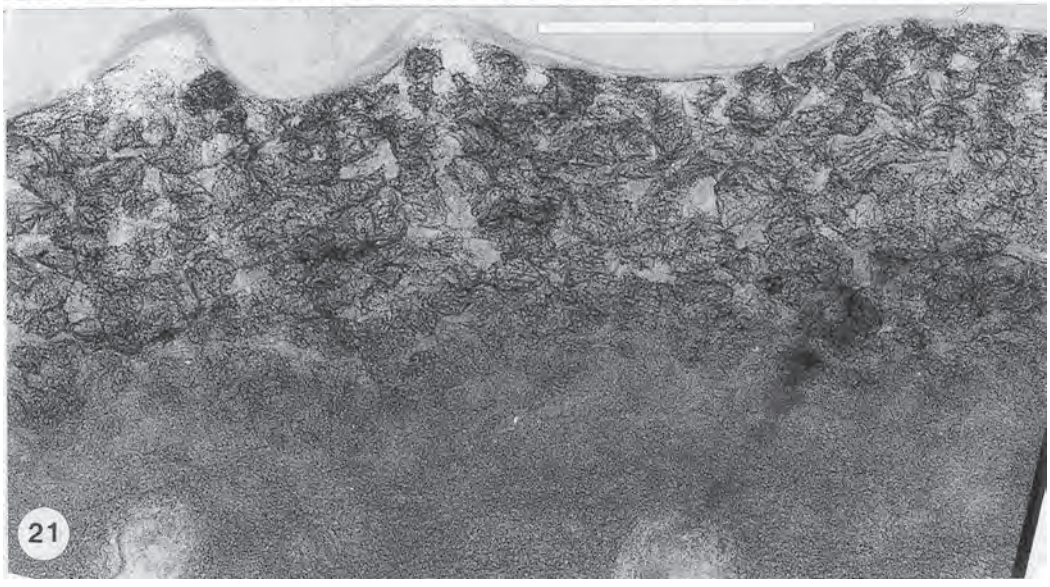
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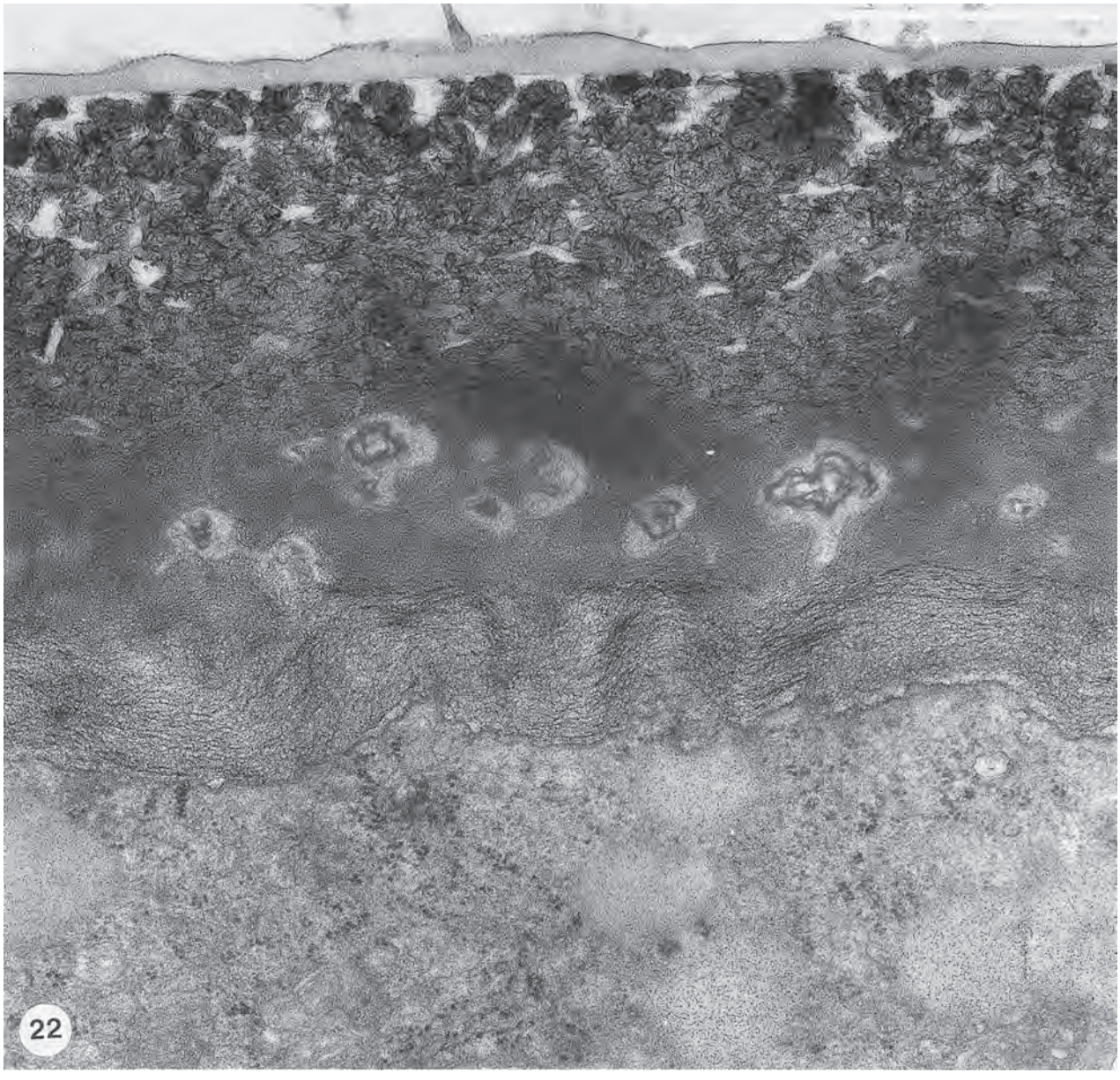


Fig. 22 Young pollen grain stage. Section in the apertural site. The foot layer is thin, and the vesicular-fibrillar layer and the intine are thicker than before. The intine is fibrillar and contains several spherical inclusions. Scale bar = 1 μm.

24, 26). The units of the vesicular-fibrillar layer and the intine were more compact and less obvious than in the previous stages (Fig. 25).

Pollen grains before being released from the anther

The volume of pollen grains decreased considerably just before they were released from the anther. The layer of the fibrillate vesicles and the intine became compact (Fig. 27). The proximal surface of the intine was undulated, followed by a translucent thin layer (Fig. 27). In the final stage of pollen development, the fibril-filled vesicles could no longer be distinguished (Fig. 28). The fibrillate nature of the intine was not as clear as in the previous stages of development (Figs. 28, 29).

Discussion

The main developmental stages of *Magnolia grandiflora* pollen grains can be summarized as sketches A–I of Fig. 32.

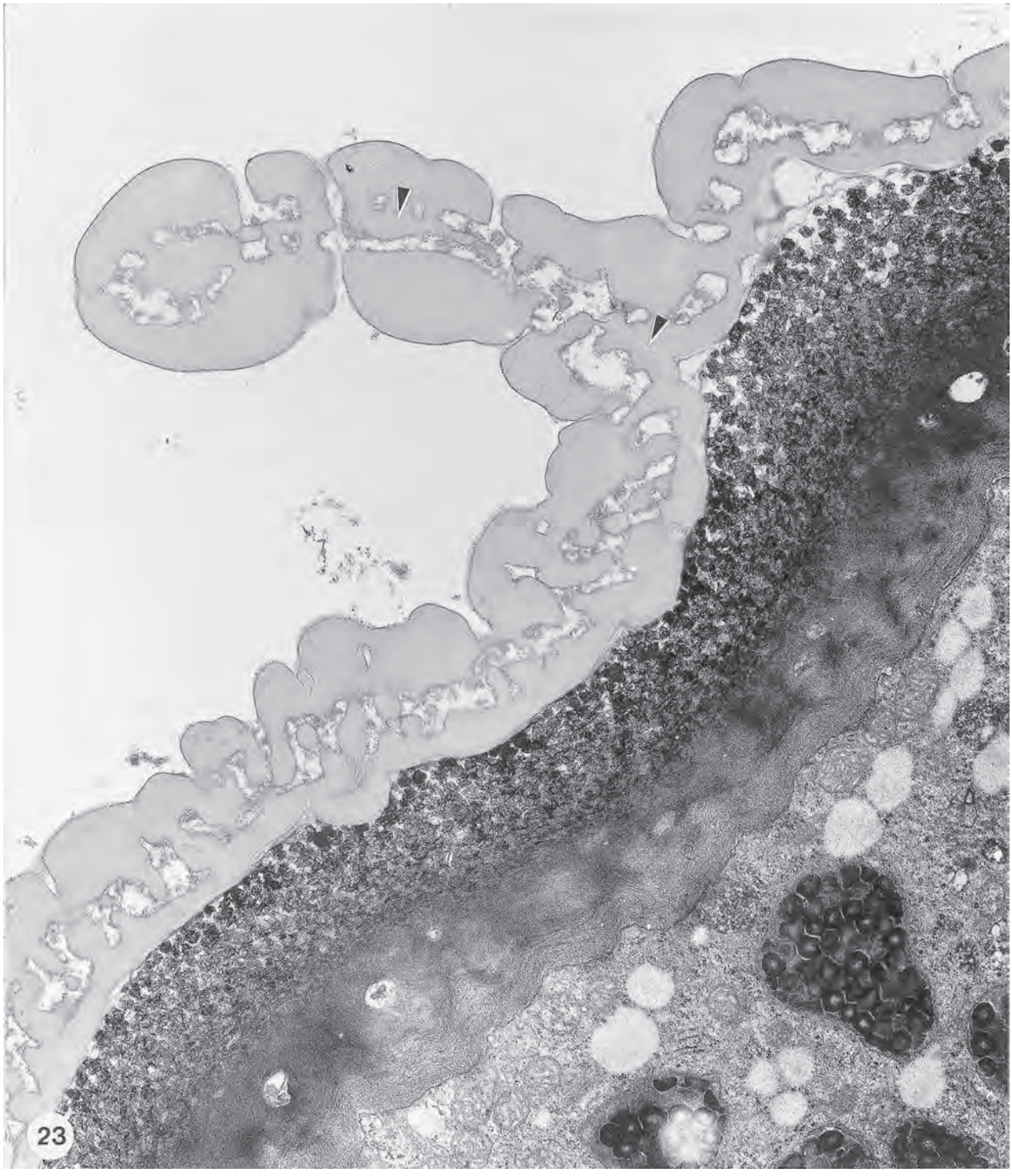
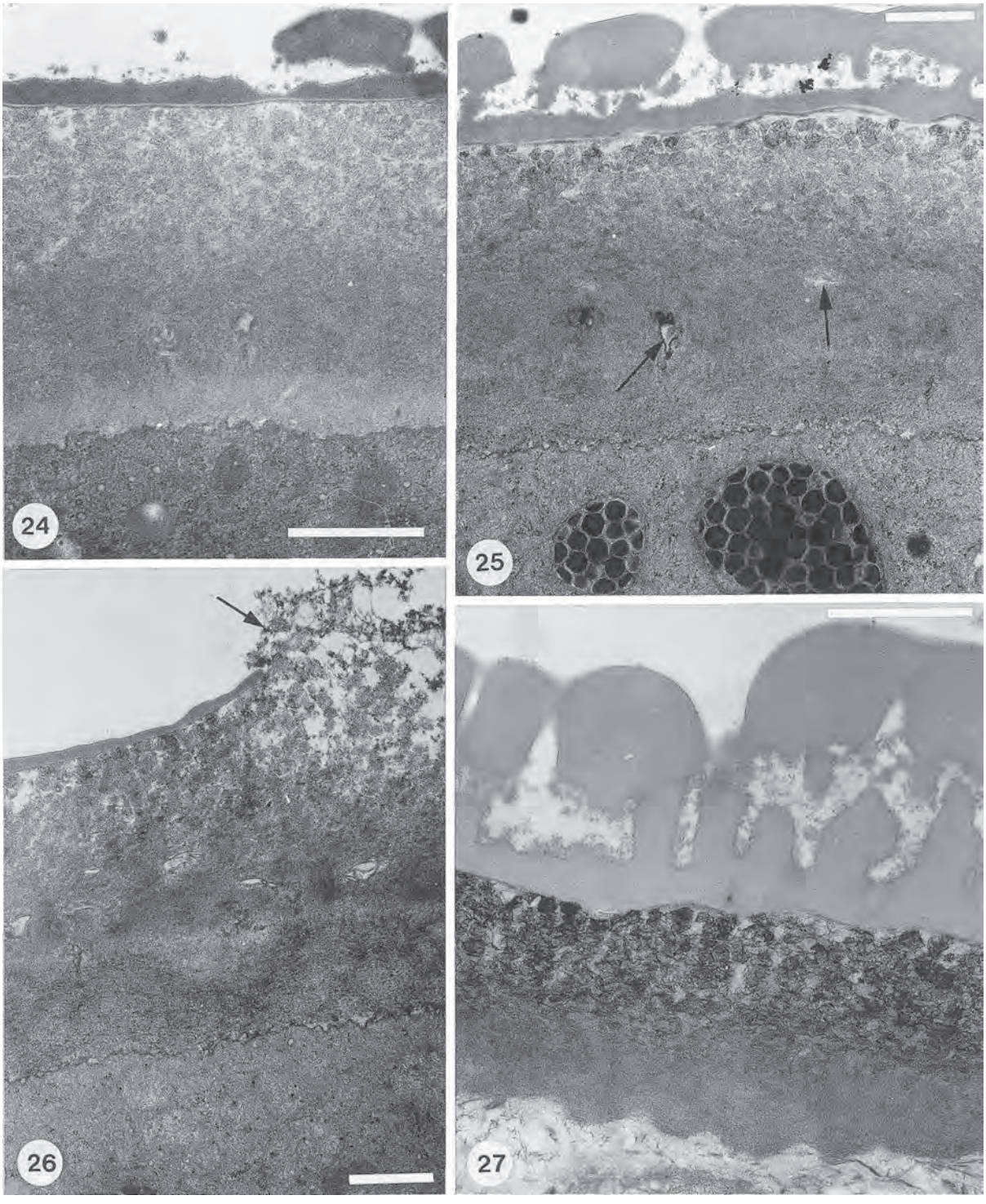
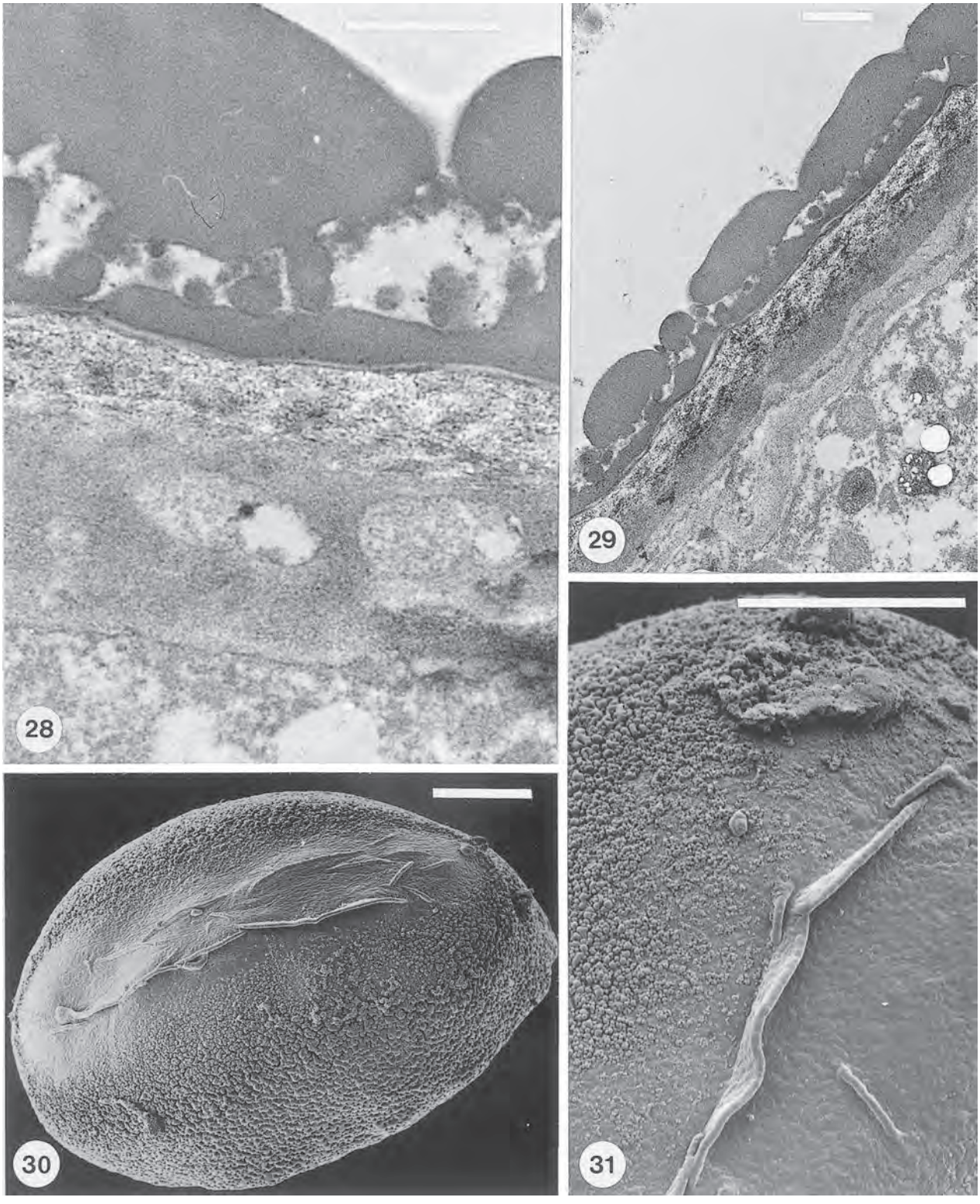


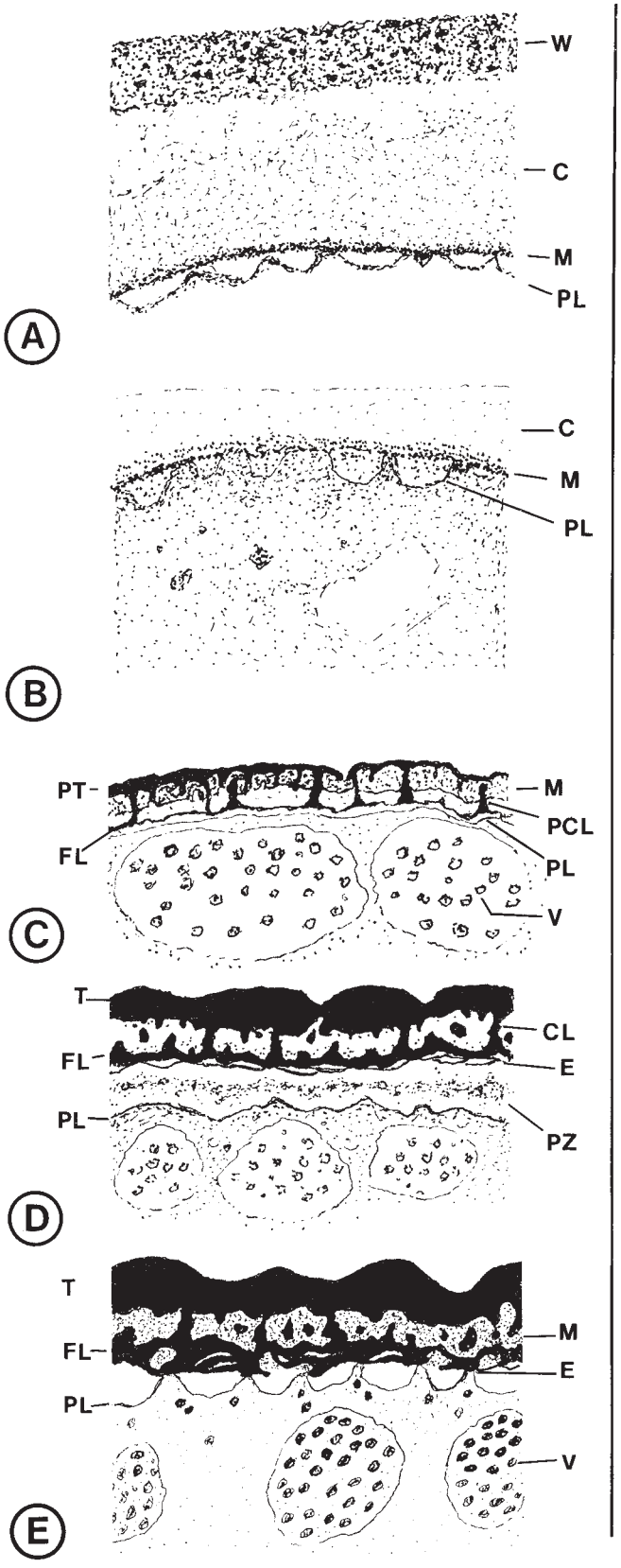
Fig. 23 Mature pollen grain stage. Section close to the aperture. The exine is generally thinner than that at the interapertural region of the wall. Part of the pollen wall is folded and elevated. Each side of the folded wall consists of a thick tectum, short and thin columellae, and a thin foot layer. The exines of the folded wall fuse at the base and are delimited from each other by a thin white-line-centered lamella (arrowheads). The cytoplasm is rich in mitochondria and lipoid globules.
Scale bar = 1 μm.



Figs. 24–27 Mature pollen grain stage. – **24:** Section in the apertural region. The foot layer is comparatively thin, and the endexine appears as a thin lamella, separated from the foot layer by a white-line-centered lamella. Scale bar = 1 μm. – **25:** Section close to the aperture. The exine is generally thinner than that at the interapertural region of the wall. The intine is well developed and has irregular channels (arrows). The cytoplasm is rich in mitochondria and elioplasts. Scale bar = 1 μm. – **26:** Section in the central region of an aperture. The exine consists only of a thin foot layer, which appears ruptured. Note the release of vesicular-fibrillar material through the opening of the sulcus (arrow). Scale bar = 1 μm. – **27:** Section in the interapertural region of a mature pollen grain before release from the anther. The exine is thick, and the intine is compact, followed by an irregular translucent layer. Scale bar = 1 μm.



Figs. 28–31 Mature pollen grain stage. – **28:** The fibrillar material of the vesicles and the intine are not as clear as in the previous stages. Scale bar = 1 μm. – **29:** The wall is stretched and becomes thinner. The vesicular-fibrillar layer and the intine are compact and dissociated. Scale bar = 1 μm. – **30:** A mature pollen grain with a sulcus and folds of the wall. The exine in the nonapertural region is coarsely and densely granulate. Scale bar = 10 μm. – **31:** Detail of the exine surface at the sulcus area. Note the elevated ridge at the sulcus area and the ornamentation of the exine around the sulcus. Scale bar = 10 μm.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Callose wall

In *Magnolia grandiflora*, the special callosic envelope of the tetrad stage is asymmetrical. The callose layer between the microspores is much thinner than the outer callosic envelope of each tetrad. This clear variation in callose thickness between the microspores and in the outer callosic envelope is a characteristic feature of *Magnolia*. The microspores in each tetrad are arranged mainly as a square tetrad (tetragonal tetrad), although a few cases of decussate arrangement are observed. Hayashi (1960) attributed the arrangement of tetrads in *Magnolia liliflora* to a modified simultaneous method and reported that the quadripartition was caused by furrowing.

Development of the wall—Tetrad stage

The wall development begins during the tetrad stage on the surface of the plasmalemma which is coated with a fibrillated material. Based on its composition including protein, acidic polysaccharides, and neutral polysaccharides, and its position, Rowley & Dahl (1977) termed this plasmalemma coating glycocalyx. Heslop-Harrison (1962) termed this coating primexine. At a later tetrad stage, the plasmalemma forms extensions, similar to those observed in *Echinodorus cordifolius* (El-Ghazaly & Rowley, 1999).

Pro-columellae then condense on the protrusions of the plasmalemma within the glycocalyx in patterned arrays and subsequently develop into mature wall elements with the accumulation of sporopollenin. This sequence seems to indicate that both the glycocalyx and the plasmalemma control the structural pattern of the exine. In the microspore surface coating of *Nymphaea mexicana* (Gabarayeva & El-Ghazaly, 1997), radially oriented substructural elements were observed from the early tetrad stage through the whole exine development. These elements, being the structural units of the microspore surface matrix, were associated with the accumulation of sporopollenin precursor.

In *Magnolia grandiflora*, columellae differentiated as cylindrical units in the exine. Nowicke et al. (1986) used plasma-ashing on pollen of many *Paeonia* species and found that rod-shaped substructures were evident in the exine, whereas they were not observed without ashing.

Blackmore & Claugher (1987) studied the exines of *Fagus* and *Scorzonera* by fast atom bombardment and found that their exines were composed of hollow tubes. In the early tetrad stages of these genera, when the tectum was first evident, the exine units showed a honeycomb pattern resulting from close packing and interdigitation of procolumellae. Occurrence of such a honeycomb arrangement has been observed in the early stages of microspore development in *Triticum aestivum* (Poaceae)(El-Ghazaly & Jensen, 1985, 1986a), *Echinodorus cordifolius* (Alismataceae)(El-Ghazaly & Rowley, 1999), and *Borago officinalis* (Boraginaceae)(Gabarayeva et al., 1998).

Granular and columellar exine

The columellae in *Magnolia grandiflora* are radially oriented, but appear granular in oblique section. Around the aperture, these columellae are quite short and thin and usually appear granular and rarely radial in sections.

The granular exine occurs both in gymnosperms and angiosperms and is hypothesized to have derived from 'atectate' exines (Doyle et al., 1975; Walker & Skvarla, 1975; Crane, 1985). The granular exine is recorded in various plant groups, such as Bennettitales, Gnetales, and many angiosperms, and has been hypothesized as a

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Fig. 32 Schematic drawings showing the different stages of pollen wall development in *Magnolia grandiflora*. — **A–C:** Microspore tetrad stage. – **A:** Plasmalemma (PL) with primexine (glycocalyx) matrix (M), enveloped by callose (C) and a sporogenous cell wall (W). – **B:** Note protrusions of the plasmalemma (PL) and presence of primexine matrix (M). – **C:** Development of a pro-tectum(PT), pro-columellae (PCL), and a foot layer (FL). Primexine matrix (M) is evedint. — **D–G:** Free microspore stage. – **D:** Early free microspores. The previously formed tectum (T), columellae (CL), and foot layer (F) have thickened. A periplasmic zone (PZ) and endexine lamellae (E) are visible. – **E:** Late free microspores. Further development of the tectum, the columellae, foot layer, and endexine. Primexine matrix (M) is still evident. Protrusions of the plasmalemma (PL) are in contact with the proximal surface of the endexine (E). The cytoplasm contains electron-dense vesicles (V). – **F:** Vacuolate microspores. A new vesicular-fibrillar layer (VF) is developed beneath the endexine (E). Cisternae of endoplasmic reticulum (ER) are in contact with the plasmalemma (PL). – **G:** Fibrillar vesicles (VF) are radially arranged and become compact. — **H:** Young pollen grain stage. Fibrillar vesicles (VF) are enclosed in irregular membranous extensions. The intine (I) is thick and fibrillar and contains comparatively large vesicles. — **I:** Mature pollen grain stage. The layers of the pollen wall become compact. The vesicular-fibrillar layer (VF) is not clearly visible as before. The fibrillar material of the intine (I) is pressed and varies in electron density.

primitive character within angiosperms (cf. Doyle, 1978).

As a myriad of exine-types have been imprecisely described as granular and spongy, one has to apply great discretion while grouping these types to draw meaningful phylogenetic inferences.

Foot layer and endexine

In *Magnolia grandiflora*, the plasmalemma plays an important role again in the development of the foot layer through the white-line-centered lamella that forms on the plasmalemma in the late tetrad period. In *Magnolia grandiflora* and many other species of *Lilium* (Heslop-Harrison, 1968), *Triticum* (El-Ghazaly & Jensen, 1986a), and *Nymphaea* (Gabarayeva & El-Ghazaly, 1997), the foot layer develops on this lamella. This trilamellated structure also contributes to the development of the endexine in *Magnolia grandiflora*. The endexine develops on the proximal surface of the foot layer at the young free microspore stage as a thin layer on the white-line-centered lamella. Doyle et al. (1975) and Walker (1976) suggested that the endexine disappeared in primitive species of angiosperms and reappeared in advanced ones. Our observation in *Echinodorus cordifolius* (El-Ghazaly & Rowley, 1999) and the present one in *Magnolia grandiflora* indicate that a rudimentary endexine, that can be easily overlooked, is present in primitive angiosperms. Existence of a lamellated endexine in extant gymnosperms and angiosperms has also been indicated by Taylor (1982) and Gabarayeva (1987a). The transitory endexine or endexine-like zones observed in *Magnolia grandiflora* pollen are also observed in *Triticum* (El-Ghazaly & Jensen, 1986a) and *Lilium* (Heslop-Harrison, 1968; Takahashi, 1995).

In *Magnolia grandiflora,* the foot layer is about three to four times thicker than the endexine, similar to *Triticum aestivum* (El-Ghazaly & Jensen, 1986a) and *Echinodorus cordifolius* (El-Ghazaly & Rowley, 1999). In other species, the foot layer is much thinner than the endexine, as in *Artemisia vulgaris* (Rowley & Dahl, 1977) and *Rondeletia odorata* (El-Ghazaly et al., 2001), or as thick as the endexine, as in *Caesalpinia japonica* (Takahashi, 1989). There is an excess of data on such variation of these two layers in different species. This kind of information may be useful phylogenetically.

Vesicular-fibrillar layer

In *Magnolia grandiflora,* the microspore wall gradually thickens from young free microspores to vacuolate microspores. At the beginning of the vacuolate microspore stage, a thick layer of fibrillate vesicles develops beneath the endexine. Cisternae of endoplasmic reticulum appear in close contact with the plasmalemma at this stage. It seems that these cisternae act in the synthesis and transport of these vesicles. A close examination of these vesicles indicates that they are irregular tubular rows filled with fibrils and appear as separate vesicles in sections. These fibrillate vesicles persist into the mature stage, but become dissociated before the pollen grains are released from the anther. Similar layers with vesicles of different sizes and shapes have been described in other species of *Triticum* (El-Ghazaly & Jensen, 1986b), *Betula* (El-Ghazaly & Grafström, 1995), *Nymphaea* (Gabarayeva & El-Ghazaly, 1997), and *Rondeletia* (El-Ghazaly et al., 2001).

This vesicular-fibrillate or "granular-fibrillate" layer has been identified as a part of the endexine or the intine (Gabarayeva, 1987a, b). The observation of the present study and others indicates that this layer belongs to neither the endexine nor the intine. At present, it is sufficient to describe its ultrastructural morphology without creating a new term.

The development of a large vacuole in the cytoplasm of late microspores presumably exerts pressure on the wall of developing pollen grains. This pressure makes the layers of the wall appressed against one another, and the endexine and the vesicular-fibrillar layer become thinner. In some cases, like in grasses, the rudimentary endexine becomes appressed against the inner surface of the foot layer and disappears (El-Ghazaly & Jensen, 1986a).

Intine

After mitosis and the development of young pollen grains, the intine in *Magnolia grandiflora* begins to develop. Generally, the intine is laid down after the exine (Erdtman, 1969). The development of the endexine and the intine may occur during the vacuolate period as shown in *Vicia* (Audran & Willemse, 1982) and *Lilium* (Heslop-Harrison & Dickinson, 1969).

In *Magnolia grandiflora*, the development of the intine coincides with the formation of long protrusions of the plasmalemma and the dominance of Golgi vesicles. It seems reasonable to assume that the material of the intine and the elongations of the plasmalemma were formed as a result of exocytosis of Golgi vesicles. The intine includes vesicles that are ca. 1 nm in diameter and appear circular in sections. They are probably formed from the invaginations of the plasmalemma. The vesicles contain fibrils, and the proximal surface of the plasmalemma is approached by several cisternae of endoplasmic reticulum. The implication is that the vesicles and the endoplasmic reticulum insert material through the plasma membrane, which somehow controls the for-

mation of the intine. In *Triticum aestivum* (El-Ghazaly & Jensen, 1986a, b), the intine development is associated with the plasma membrane. In some pollen types such as Ranunculaceae (Roland, 1971), Golgi vesicles multiply during the intine synthesis, while in others such as *Cosmos* (Knox & Heslop-Harrison, 1970), endoplasmic reticulum and polyribosomes do.

Aperture

The pollen grains of *Magnolia* are monosulcate. The units of the exine characteristically become short or thin around the aperture margin. The area of germination is covered with a thin foot layer. Granules of different sizes are observed in the area of the sulcus that occupies about 7% of the surface area of the pollen. These granules could be columellae in oblique section. Granular and columellar elements have been described in Magnoliaceae (Praglowski, 1974; Gabarayeva, 1987a). All apertures in fresh pollen have at least a thin covering of the exine (El-Ghazaly & Rowley, 1999; El-Ghazaly, 1982). This exine covering is self-evident in the pollen of many taxa, such as *Triticum* and *Echinodorus,* with an aperture membrane ornamented by granules or spinules.

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References

Audran, J. C. & Willemse, M. T. M. 1982. Wall development and its auto fluorescence of sterile and fertile *Vicia faba* L. pollen. Protoplasma **110**: 106–111.

Blackmore, S. & Claugher, D. 1987. Observations on the substructural organization of the exine in *Fagus sylvatica* L. (Fagaceae) and *Scorzonera hispanica* L. (Compositae: Lactuceae). Review of Palaeobotany and Palynology **53**: 175–184.

Crane, P. R. 1985. Phylogenetic analysis of seed plants and the origin of angiosperms. Annals of the Missouri Botanical Garden **72**: 716–793.

Crepet, W. L. & Nixon, K. C. 1998. Two new fossil flowers of magnoliid affinity from the late Cretaceous of New Jersey. American Journal of Botany **85**: 1273–1288.

Dahl, A. O. & Rowley, J. R. 1965. Pollen of *Degeneria vitiensis*. Journal of the Arnold Arboretum **50**: 1–35.

Doyle, J. A. 1978. Potentials and limitations of exine structure in studies of early angiosperm evolutions. Courier Forschungsinstitut Senckenberg **30**: 54–61.

Doyle, J. A., van Campo, M. & Lugardon, B. 1975. Observations on exine structure of *Eucommiidites* and Lower Cretaceous angiosperm pollen. Pollen et Spores **17**: 429–484.

El-Ghazaly, G. 1982. Ontogeny of pollen wall of *Leontodon autumnalis* (Hypochoeridinae, Compositae). Grana **21**: 103–113.

El-Ghazaly, G. & Grafström, E. 1995. Morphological and histochemical differentiation of the pollen wall of *Betula pendula* Roth. during dormancy up to anthesis. Protoplasma **187**: 88–102.

El-Ghazaly, G., Huysmans, S. & Smets, E. F. 2001. Pollen development of *Rondeletia odorata* (Rubiaceae). American Journal of Botany **88**: 14–30.

El-Ghazaly, G. & Jensen, W. A. 1985. Studies of the development of wheat (*Triticum aestivum*) pollen: III. Formation of microchannels in the exine. Pollen et Spores **27**: 5–14.

El-Ghazaly, G. & Jensen, W. A. 1986a. Studies of the development of wheat (*Triticum aestivum*) pollen. I. Formation of the pollen wall and Ubisch bodies. Grana **25**: 1–29.

El-Ghazaly, G. & Jensen, W. A. 1986b. Studies of the development of wheat (*Triticum aestivum*) pollen: formation of the pollen aperture. Canadian Journal of Botany **64**: 3141–3154.

El-Ghazaly, G. & Rowley, J. R. 1999. Microspore and tapetal development in *Echinodorus cordifolius* (Alismataceae). Nordic Journal of Botany **19**: 101–120.

Endress, P. K. 1983. Dispersal and distribution in some small archaic relic angiosperm families (Austrobaileyaceae, Eupomatiaceae, Himantandraceae, Idiospermoideae-Calycanthaceae). Sonderbande des Naturwissenschaftlichen Vereins in Hamburg **7**: 10–87.

Endress, P. K. 1986. Reproductive structures and phylogenetic significance of extant primitive angiosperms. Plant Systematics and Evolution **152**: 1–28.

Erdtman, G. 1960. The acetolysis method. Svensk Botanisk Tidskrift **54**: 561–564.

Erdtman, G. 1969. Handbook of Palynology. 486 pp. Munksgaard, Copenhagen.

Gabarayeva, N. I. 1987a. Ultrastructure and development of endexine lamellae in *Manglietia tenuipes* (Magnoliaceae) in connection with the question of endexine existence in primitive angiosperms. Botanicheskii Zhurnal (Leningrad) **72**: 1310–1317.

Gabarayeva, N. I. 1987b. Ultrastructure and development of pollen grain wall in *Manglietia tenuipes* (Magnoliaceae): the formation of intine in connection with the activity of cytoplasmic organelles. Botanicheskii Zhurnal (Leningrad) **72**: 1470–1477.

Gabarayeva, N. I. 1991. Ultrastructure and development of exine and orbicules in *Magnolia delavayi* (Magnoliaceae) during tetrad and at the beginning of post tetrad period. Botanicheskii Zhurnal (Leningrad) **76**: 10–19.

Gabarayeva, N. I. & El-Ghazaly, G. 1997. Sporoderm development in *Nymphaea mexicana* (Nymphaeaceae). Plant Systematics and Evolution **204**: 1–19.

Gabarayeva, N. I., Rowley, J. R. & Skvarla, J. J. 1998. Exine

development in *Borago* (Boraginaceae). 1. Microspore tetrad period. Taiwania **43**: 203–214.
Gottsberger, G. 1988. The reproductive biology of primitive angiosperms. Taxon **37**: 630–643.
Hayashi, Y. 1960. On the microsporogenesis and pollen morphology in the family Magnoliaceae. The Science Reports of the Tohoku University, Fourth Series (Biology) **26**: 45–59.
Heslop-Harrison, J. 1962. Origin of exine. Nature **195**: 1069–1071.
Heslop-Harrison, J. 1968. Tapetal origin of pollen-coat substances in *Lilium*. New Phytologist **67**: 779–786.
Heslop-Harrison, J. & Dickinson, H. G. 1969. Fine relationship of sporopollenin synthesis associated with tapetum and microspore in *Lilium*. Planta **84**: 199–214.
Karnovsky, M. J. 1965 A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. The Journal of Cell Biology **27**: 137–138.
Knox, R. B., Heslop-Harrison, J. 1970. Pollen wall proteins: Localization and enzymatic activity. Journal of Cell Science **6**: 1–27.
Muller, J. 1970. Palynological evidence on early differentiation of angiosperms. Biological Review **45**: 417–450.
Nowicke, J. W., Bittner, J. L. & Skvarla, J. J. 1986. *Paeonia*, exine subtructure and plasma ashing. "Pollen and Spores: form and function" (Blackmore, S. & Ferguson, I. K., eds.), 81–95. Linnean Society Symposium Series No. 12. Academic Press, London.
Praglowski, J. 1974. Magnoliaceae Juss. Taxonomy by J. E. Dandy. World Pollen and Spore Flora **3**: 1–45.
Roland, F. 1971. Characterization and extraction of the polysaccharides of the intine and of the generative cell wall in the pollen grains of some Ranunculaceae. Grana **11**: 101–106.
Rowley, J. R. & Dahl, A. O. 1977. Pollen development in *Artemisia vulgaris* with special reference to glycocalyx material. Pollen et Spores **19**: 169–284.
Spurr, A. R. 1969 A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research **26**: 31–43.
Takahashi, M. 1989. Pattern determination of the exine in *Caesalpinia japonica* (Leguminosae: Caesalpinioideae). American Journal of Botany **76**: 1615–1626.
Takahashi, M. 1995. Three-dimensional aspects of exine initiation and development in *Lilium longiflorum* (Liliaceae). American Journal of Botany **82**: 847–854.
Taylor, Th. N. 1982. Ultrastructural studies of paleozoic seed fern pollen: sporoderm development. Review of Palaeobotany and Palynology **37**: 29–53.
Waha, M. 1987. Sporoderm development of pollen tetrads in *Asimina triloba* (Annonaceae). Pollen et Spores **29**: 31–44.
Walker, J. W. 1976. Evolutionary significance of the exine in the pollen of primitive angiosperms. "The Evolutionary Significance of the Exine" (Ferguson, I. K. & Muller, J., eds.), 251–308. Linnean Society Symposium Series No. 1. Academic Press, London.
Walker, J. W. & Skvarla, J. J. 1975. Primitively columellaless pollen: a new concept in the evolutionary morphology of angiosperms. Science **187**: 445–447.
Zavada, M. 1984. Pollen wall development of *Austrobaileya maculata*. Botanical Gazette **145**: 11–21.

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