Taiwania, **44**(2): 212-229, 1999

Exine Development in *Borago* (Boraginaceae) 2. Free Microspore Stages.

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(Manuscript received 12 April, 1999; accepted 20 May, 1999)

ABSTRACT: At the beginning of the free microspore period, when the callosic special cell wall is digested, darkly contrasted particles appear distally on each of the columellae. They are 15 to 20 nm in diameter and are connected to each other like beads on a string. Several of such "strings" form a bouquet-like formation on the distal end of columellae. We consider these particles to be involved with sporopollenin accumulation and refer to them as sporopollenin acceptor particles (SAPs). SAPs appear to be associated with an increase in height of columellae and formation of gemmae. By the end of exine development columellar height has increased 2 to 3 times. In mature pollen gemmae have rounded ridges on their surface that are the same size as SAPs in the late microspore stage.

KEY WORDS: ***Borago*, Columellae, Exine, Gemmae, Microspores, Sporopollenin acceptor particles (SAPs).**

INTRODUCTION

Pollen of *Borago officinalis* has drawn attention from a very early period in studies of pollen morphology. Geoffroy included *Borago* pollen in his 1711 list of plants with interesting flowers. Mohl (1834) studied pollen, including *Borago*, during a period of significant improvement in microscopes and microtechnique. He made all his drawings of pollen himself, whereas it was the general rule during the period to have descriptions rendered by professional artists. His 1834 paper was unavailable and his translation published in France in 1835, which we have (Mohl 1835), has only three (without *Borago*) of the original six plates. Aldridge's (1842) work, which included *Borago*, was an early attempt to see if pollen grains could be of use in ideas about a "natural classification". Work with pollen of *Borago* directed toward classification includes books and papers by Erdtman (1952), Avetisian (1952, 1956), Stix (1964) and Bou (1968). The complexity of the exine and its apertures is well shown by the phase contrast microscopy of Stix (1964).

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Previous work showing *Borago* pollen by TEM and SEM has been published by Ben Saad-Liman and Nabli (1984). Work showing pollen of this taxon by LM and SEM has been published by Clarke (1977), Diez (1984, 1987), Quiroz-Garcia and Palacios-Chávez (1985). Ghorbel and Nabli (1998) have shown the pollen tube and its pathway in the pistal in *B. officinalis*

Our previous study on *Borago* sporoderm development (Gabarayeva *et al.*, 1998) considered exine initiation through the end of the tetrad period. One aim of this work was to trace sporoderm development from the early free microspore stage to a mature exine stage with special attention to columellar growth. Another aim, a reason for the study of development in *Borago*, was the gemmae and their unusual surface structure as was first shown in the work of Ben Saad-Liman (1984).

MATERIALS AND METHODS

Buds of *Borago officinalis* L. were collected from the Botany Department of Stockholm University. Intact anthers were immersed in one of three fixation mixtures (pH 7, 20°C, 24h) and immediately placed under vacuum (ca 10^{-2} torr) until bubbles were no longer released from the specimens and they had settled to the bottom of the vial. The fixation mixtures were then rotated for the rest of the periods of fixation, dehydration and acetone/epoxy infiltration.

All the three fixation mixtures were based upon Karnovsky's (1965) formulation of 2.5% glutaraldehyde and 7.5% paraformaldehyde in 0.06M phosphate buffer diluted 1:1 with distilled water, pH 7. They are noted in the illustration descriptions. We added cations both to the fixation mixture and to the subsequent exposure to osmium tetroxide (1% in water, 20C, 1h) secondary fixation: LN = 1% lanthanum nitrate, RR = 0.003% ruthenium red and AB = 1% Alcian blue. After acetone dehydration the anthers were embedded in Spurr's (1969) hard resin.

Sections were stained for TEM with a saturated solution of uranyl acetate in ethanol and 0.2% lead citrate (pH 12.2). The sections were examined with Hitachi H-600 and Zeiss EM-10A transmission electron microscopes. Unstained semithin (3-7 μm) sections were examined by phase contrast and differential interference contrast (Normarski) light microscopy as an aid to the selection of stages for TEM thin sectioning.

For SEM fresh pollen grains were dried using a critical point drying alternative, Peldri-II (Chissoe et al. 1990), sputter-coated with gold/palladium (60/40) and examined with a JEOL 880 SEM equipped with a lanthanum hexaboride gun.

RESULTS

Early free microspore stage

Our first figure (Fig. 1) gives a low magnification survey of aperture, endexine and ectexine condition in this stage. The foot layer is curved or wavy. Perhaps the most distinctive aspect of the survey micrograph in Figure 1 is the constriction (neck) in columellae as seen as an almost clear zone just below the distal surface. As a result, this

portion of columellae appears almost separated from the rest of the exine. The exine surface is "covered" by sporopollenin acceptor particles (SAPs) which have the aspect of fuzz at this low magnification, i.e., they are not individually discernible as they are in the following higher magnification figures (Figs. 2-5).

In the first stages of this report there are remnants of the special cell wall callose (Fig. 2). Fig. 2 was printed dark to show isolated remnants of the callose and that some of the SAPs have been displaced from columellae by the removal of callose, probably during specimen preparation. SAPs are abundant after callose is lost (Fig. 3). Also in the first stages of this report the foot layer is discontinuous (Figs. 3-5).

Sporopollenin Acceptor Particles (SAPs)

SAPs occur in apertural (Fig. 8) and interapertural regions (Figs. 3-5, 8, 9) and are often seen to be connected (Fig. 9). They are 10-15 nm in diameter and are darkly contrasted (Fig. 9A and B). Often an outer zone of low contrast is apparent around the dark core (e.g., Fig. 3 and 4). The SAPs are arranged on the distal part of columellae like a bouquet (Figs. 3-5).

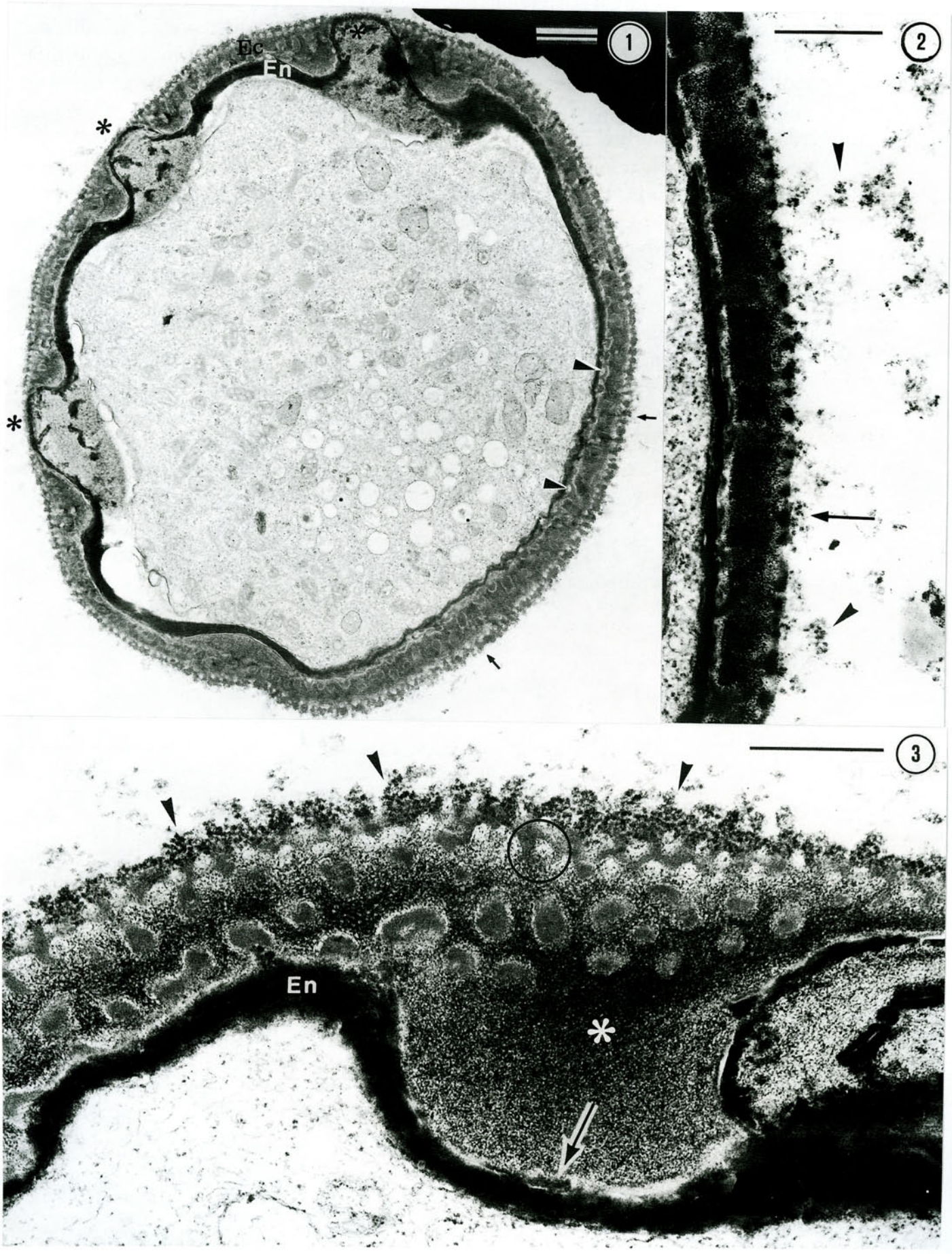
Development of the endexine and foot layer

After loss of the callose envelope the endexine thickens, especially in apertural regions. The foot layer also thickens; its increase begins as a ring zone around the base of columellae (Fig. 4). When columellae are cut obliquely these rings are seen as half loops (Fig. 4).

Columellar height increase and development of gemmae

The height of columellae begins to increase in stages shown in Figs. 10-12 as can be seen by comparing these micrographs with those in Fig. 9. In the subsequent stages represented by Figs. 13-17 the height of columellae is greatly increased.

Figs. 1-3. Early free microspore stage. Fig. 1. Columellae, foot layer, ectexine (Ec) and endexine (En) form when tetrad is enveloped by callose. The foot layer consists of curved sectors (arrowheads) in some planes of section. There is little or no foot layer between the equatorial part of the three apertures (asterisks). The constriction in columellae (Figs. 4 and 5) just below their distal surface causes the distal portion to appear almost separated from the rest of the ectexine. The exine surface is coated by particles (arrows; Figs. 2 and 3) that we equate with sites of sporopollenin accumulations. Fixation: LN. Bar=1 μm. Fig. 2. The section is from a microspore that was partially covered by callose of the special cell wall. Remnants of callose (arrowheads) in this section contain Sporopollenin Acceptor Particles (SAPs) (Figs. 3 and 4). These SAPs have apparently become detached from the columellae during specimen preparation. SAPs associated with the columellae are marked by an arrow. Fixation: LN. Bar=1 μm. Fig. 3. The section is slightly oblique and the central core zone of columellae is apparent as "spaces" (one is circled). The surface of the columellae is coated by small darkly contrasted SAPs (arrowheads). The foot layer (arrow), discontinuous at this stage, is under the glycocalyx thickening (asterisk) that borders a colpus. Fixation: LN. Bar=0.5 μm.


Ec
En
1
2
3
En

The SAPs are now in more-or-less circular gemmae-like arrangement (Figs. 11-17). In oblique sections these circular arrangements (Figs. 18 and 19) are bordered by SAPs that are close to one another (Fig. 18). They also appear in the stage represented by Fig. 18 to be "connected" by a short stock to the homogeneous appearing central portion of gemmae. In some sections of pollen near maturity there are components at or near the surface of gemmae that protrude perpendicularly (Fig. 23).

In mature pollen the gemmae have ridges on and around their circumference as seen in TEMs (Fig. 25) and SEMs (Figs. 27-29). In acetolysed material the ridges have a sharply acute angle in profile and a rounded concave valley between ridges. On fresh pollen grains dried for SEM using a critical point drying alternative, the ridges on gemmae were convexly rounded (Figs. 27-29).

Gemmae during late microspore and early pollen grain stages

Glycocalyx is prominent until after the late microspore stage; in pollen grain stages there is little or no glycocalyx (Figs. 20-24). Spacing of gemmae appears to be irregular (Figs. 20-22) and SEMs of mature pollen (Figs. 26-29) show this to be true three dimensionally. TEM images (Figs. 23 and 24) show striations on some gemmae and slender protruding structures on others depending upon whether gemmae are sectioned so as to be entirely or only partly within the plastic section. When sectioned, gemmae may show differential contrast between the surface and core (Fig. 23). In mature pollen, gemmae show a variety of images in TEM sections (Fig. 25). In SEMs of mature and acetolyzed grains the gemmae have rounded ridges with the same separation, curvature and height over the valleys as the SAPs in Fig. 18.

Apertures

Apertures in different stages of development are illustrated in Figs. 1, 3, 6-8, 10-14, and 20. The three apertures in Fig. 1 are located in the central region of the grain since endexine does not cross under the aperture zone. The apertures in Fig. 1 are bordered and underlain by components of the endexine. Sections crossing apertures poleward from the equatorial plane have endexine extending across the proximal part (arrow in Fig. 7; Figs. 10, 11, 13 and 14).

Figs. 4-8. Mid-microspore stages. Fig. 4. The foot layer is thickened as a flange around the base of columellae. When sections are cut obliquely the foot layer appears as a series of half loops (arrows). Because of the thinness of TEM sections it is rare for the foot layer half loop to be in the same section as its columella. In this section there is one foot layer half loop connected to a columella (asterisk). Fixation: LN. Bar=0.5 μm.
Fig. 5. This is an enlargement of the interapertural exine in Fig. 6. The columellae are sectioned longitudinally and thus the flanges of foot layer (arrowheads) at the base of columellae (large arrows) are parallel with the underlying endexine (En). SAPs are indicated by small arrows. Fixation: LN. Bar=100 nm. Fig. 6. The section is near the equatorial plane as is evident by the absence of endexine under the apertures (A) (Fig. 7). An oncoid thickening in a colpus is marked "O". In this relatively early development of apertures there is extensive thickening of glycocalyx (asterisks) embracing the colpus. SAPs (arrowheads) cover the apertural membrane. Fixation: LN. Bar=1 μm. Fig. 7. The colpi at the left and lower right are near the equatorial plane as indicated by the absence of endexine under the colpus. The aperture at the upper right has a thick layer of endexine (arrow) under the colpus and is located considerably poleward of the microspore's equator. SAPs (arrowheads) occur on distal surface apertures as well as on the rest of the exine. Fixation: LN. Bar=1 μm. Fig. 8. The micrograph shows the SAPs (arrowheads) on enlargements of two of the colpi in Fig. 7. The arrows indicate columellae and foot layer across the glycocalyx thickenings embracing apertures. Fixation: LN. Bar=100 nm.

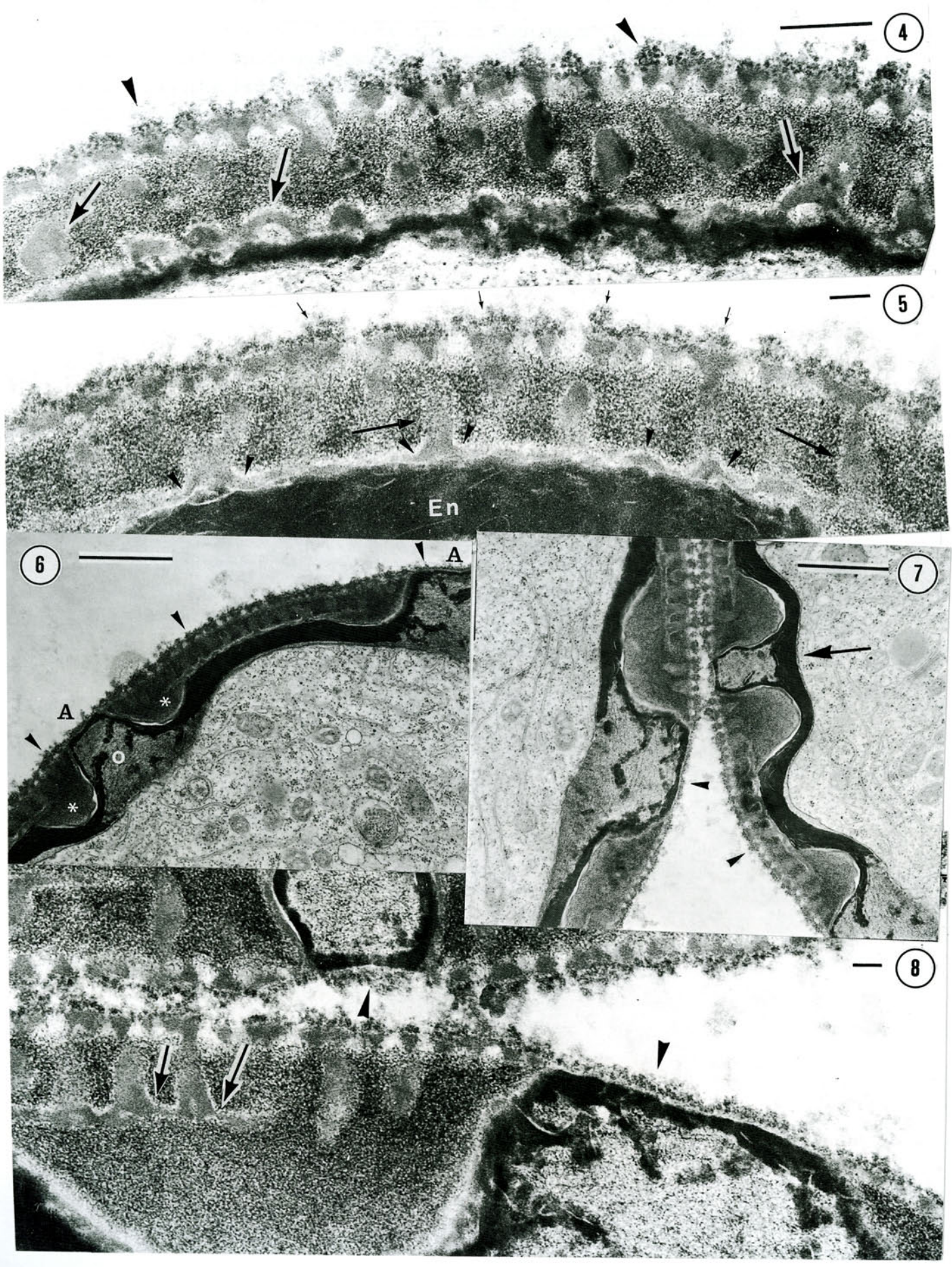

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Fig. 3 shows foot layer under the glycocalyx thickening bordering an aperture. Fig. 6 shows the glycocalyx at either side of an aperture and the oncus within the aperture. The foot layer also extends above the glycocalyx thickening in Fig. 3 but it is better illustrated in Fig. 8. The foot layer also is in contact with endexine bordering colpi (e.g., Figs. 11 and 12). SAPs are prominent on the distal surface of apertures (e.g., Figs. 7 and 8). As on columellae, gemmae form on the distal surface of apertures (Figs. 11-13 and 20).

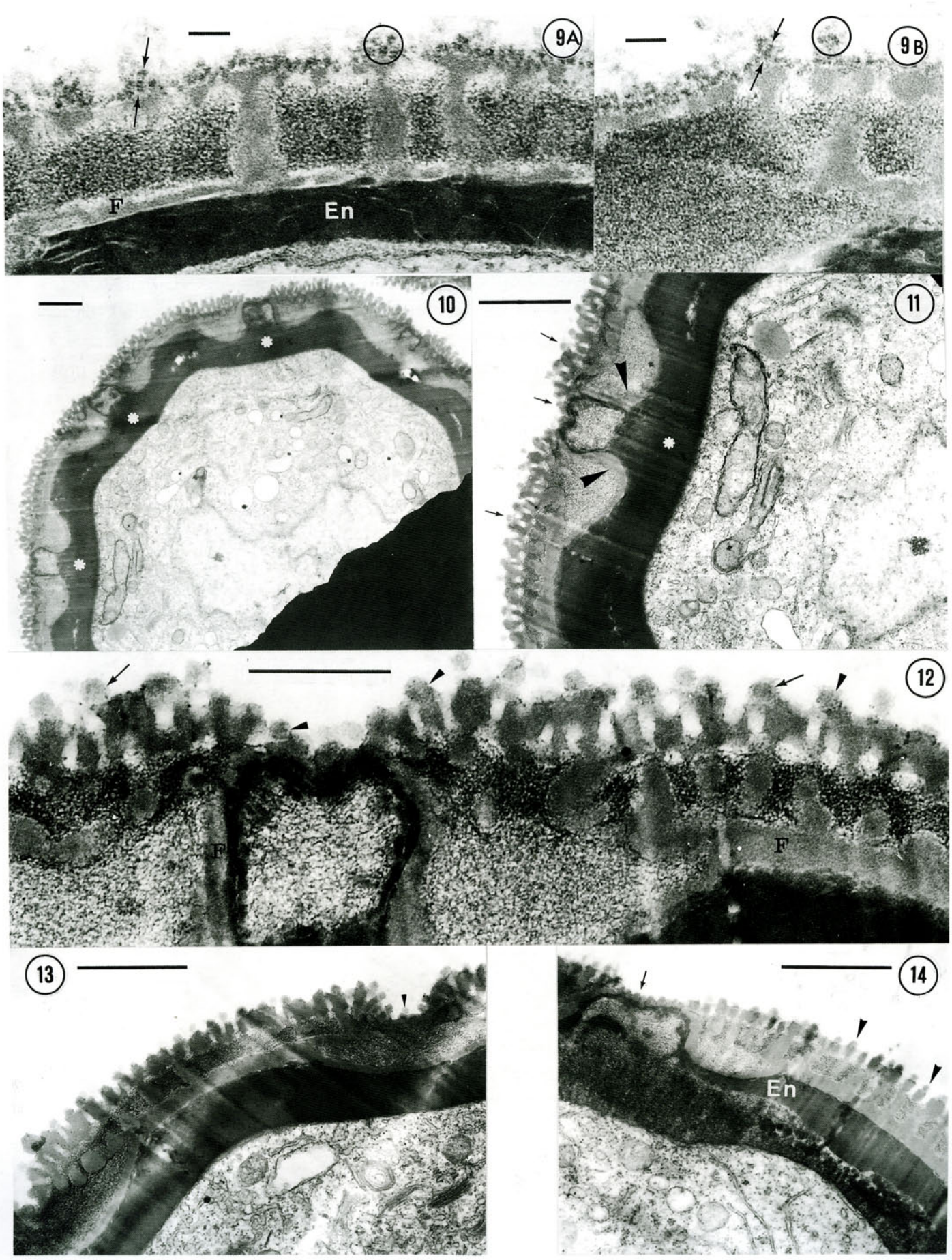
DISCUSSION

Sporopollenin acceptor particles (SAPs) and the increase in height of columellae and formation of gemmae

In *Borago* microspores and pollen, SAPs occur on the distal surface of columellae, on the surface of apertures and in gemmate configurations. We assume that these particles or chains of particles are involved in the accumulation of sporopollenin because gemmae form on apertural membranes and at the distal end of columellae.

Particles of similar size and contrast were associated with columellar growth in *Poinciana* (Leguminoseae; Skvarla and Rowley 1987) and exine formation in *Asimina* (Annonaceae; Waha 1987) and *Anaxagorea* (Annonaceae; Gabarayeva 1995). They are referred to as "sporopollenin precursor particles" by Waha (1987) and Gabarayeva (1995) and "sporopollenin receptors" by Skvarla and Rowley (1987). After incubation in thorium dioxide, which is dense to electrons and binds to negatively-charged groups, Rowley *et al.* (1995) found SAP-sized particles connected like beads coiled around ca 100 nm wide exine components of *Nuphar* (Nymphaeaceae).

Figs. 9-14 . Mid-microspore (Fig. 9), early pollen (Figs. 10-12) and late microspore (Figs. 13 and 14) stages. Figs. 9A and B. In some orientations SAPs are aligned in files (between arrows). The diameter of SAP files and their diameter in end views, where they are circular (circular frame), are equal (ca. 15-20 nm in diameter). Endexine (En). Fixation: LN. Bar=100 nm. Fig. 10. Early pollen grain stage. There is a great increase in height of columellae and thickness of foot layer and endexine. The section is poleward from the equatorial plane as is shown by thick endexine under the colpi (asterisks). Fixation: LN. Bar=1 μm. Fig. 11. Poleward from the equator there is a thick endexine (asterisk) underlying the aperture. The section is sufficiently oblique so that the spaces between columellae appear elliptical. The aperture surface has gemmae (arrows). The foot layer borders the endexine to either side of colpi (arrowheads) and distally on the colpus where the foot layer contacts the aperture membrane. Fixation: LN. Bar=1 μm. Fig. 12. A micrograph at higher magnification of the aperture region in Fig. 11 to show the surface of gemmae. Gemmae that are not sectioned (i.e., those that lie within the thin section) show 15-20 nm wide striations (arrowheads). Those that are sectioned (arrows) show a dark core and light 15-20 nm wide outer zone. The foot layer (F) contacts columellae at the distal surface of the colpus. Fixation: LN. Bar=0.5 μm. Fig. 13. Late microspore stage. The aperture is near the polar end of the colpus. Gemmae on the aperture membrane are marked by an arrowhead. Fixation: LN. Bar=1 μm. Fig. 14. An equatorial aperture zone. There is no endexine proximal to the equatorial portion of the aperture, typical of the equatorial zone. There are SAPs or gemmae (arrowheads) on each columella. There are gemmae (arrow) on the distal membrane of the aperture. Endexine (En). Fixation: LN. Bar=1 μm.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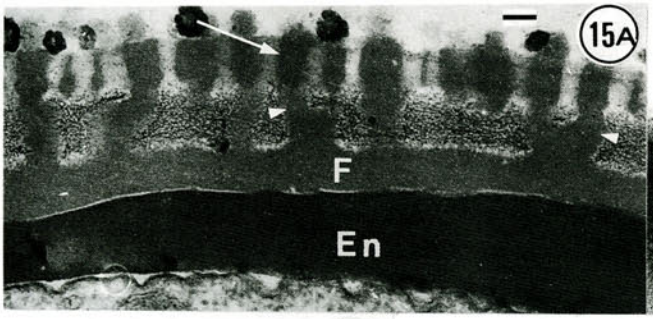
Summary of columellar development from our youngest stage through maturity

The stage in Fig. 30B has columellae of distinctly different heights. This variation is not attributable to different fixation mixtures or location on microspores such as proximity to apertures. The columellae in the next three older stages (Figs. 30 C-E) are less high and smaller in diameter than our earliest stage (Fig. 30A). These variations and reductions indicate the dynamic nature of the development of columellae. The stages represented by Figs. 30 C-F have a constriction in columellar diameter that is first near the distal surface. This constriction coincides with the height of the glycocalyx (the "primexine matrix" of Heslop-Harrison 1963). It would be logical to think of the distal part of columellae above the constriction as the tectum. Since there is great height increase in the portion of columellae distal to the constriction and the constriction is grown over between stages 30F and G any tectal development would seem transitory until mature stages. By pollen maturity the columellar height greatly exceeds stages showing the constriction and there is a well defined tectum located 0.2 to 0.5 μm above the former constriction. We propose that columellae grow distally, after the microspore tetrad period, and are surmounted at maturity by the gemmae illustrated in Figs. 23-29. The increase in height is as great as three times. It ought to be considered, however, that columellar growth may be both basal and distal. Since it is clear that the foot layer is inserted and thickened at the basal portion of columellae, it is reasonable that columellae may also grow basally.

Endexine and foot layer

In early free microspores the special cell callosic envelope is only partly digested. There is a definite rudimentary foot layer and the beginning of endexine lamellations. Initially the increase in foot layer thickness is located around the base of columellae. In oblique sections, which are common in TEM thin sections, the foot layer looks as though it consists of a series of half loops. These loops have an inside diameter that is the same as the diameter of columellae. Such half loops in the early foot layer were common in *Centrolepis* (Centrolepidaceae) microspores as reported by Rowley and Dunbar (1996). In their work on pollen of *Camellia* (Theaceae) Zavada and Wei (1993) termed the foot layer of *C. tsaii*, that is much like the foot layer of *Borago officinalis*, "foliated".

Figs. 15-19. Late microspore stages. Figs. 15A and B. The same late microspore stage as micrographs in Figs. 13 and 14. The massive nature of the columellae, foot layer and endexine is emphasized in Fig. 15A. Gemmae are well developed in Fig. 15A. In the thinner section in Fig. 15B SAPs are evident in gemmae (arrows) and on columellae. Fig. 15A shows that the portion of columellae above the constriction (arrow) and below it (arrowheads) are more-or-less equal in height at this stage. In Fig. 15A the black spots covering some gemmae are stain precipitate. Figs. 15A and 15B: columellae (C), glycocalyx (asterisk), foot layer (F) and endexine (En). Fixation: Fig. 15A: RR; Fig. 15B: LN. Bars= 100 nm. Figs. 16 and 17. The same late microspore stage as in the micrographs in Figs. 13 and 14. These figures emphasize the surface texture of the gemmae. Substructural loops (arrows; one is circular framed in Fig. 16). are present in gemmae. The massive character of columellae (C) and foot layer (F) is evident in Fig. 17. The primexine matrix is marked by an asterisk and endexine by "En" . Fixation: LN. Bars= 100 nm. Fig. 18. Tangential section of the exine. Some of the gemmae (arrowheads) are surrounded by darkly contrasted SAPs (arrows). The tectum is marked "T", the glycocalyx by an asterisk, and cross-sectioned columellae by "C". Fixation: RR. Bar= 100 nm. Fig. 19. A tangential section. Gemmae (arrowheads) are at the surface of the tectum (T). Many columellae (C) are circular; those that are elliptical in this oblique section indicate fused columellae that will probably be seen as branched in radial sections (e.g., Figs. 20-25. Fixation: LN. Bar= 1 μm.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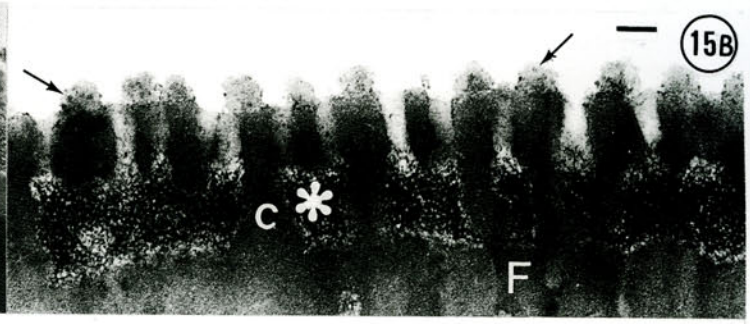
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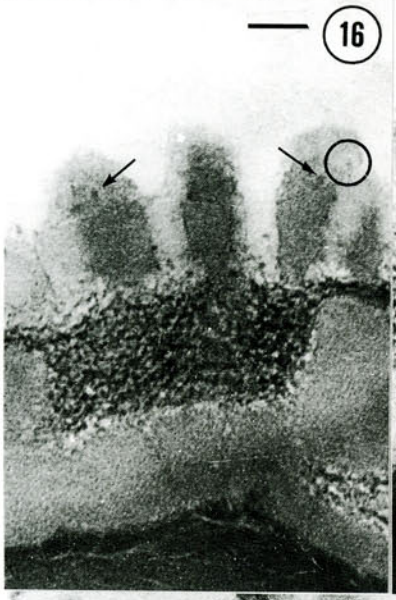
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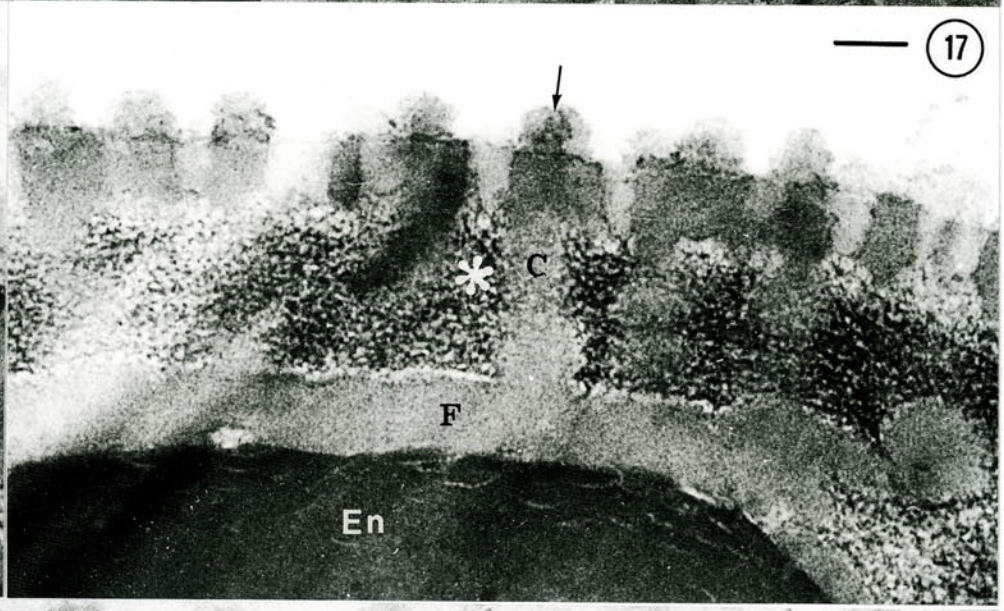
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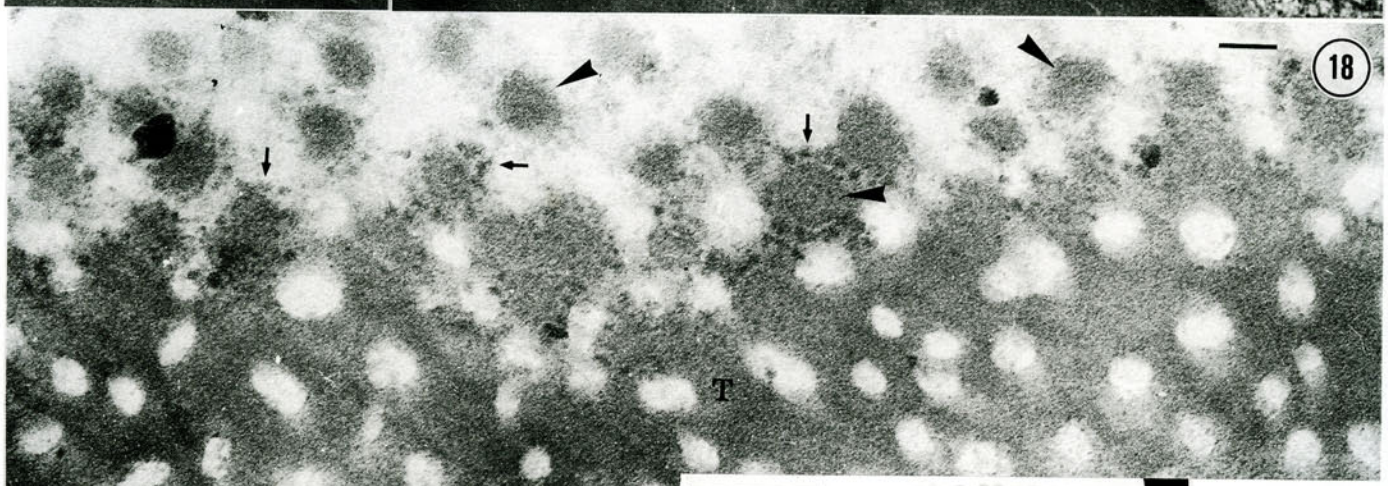
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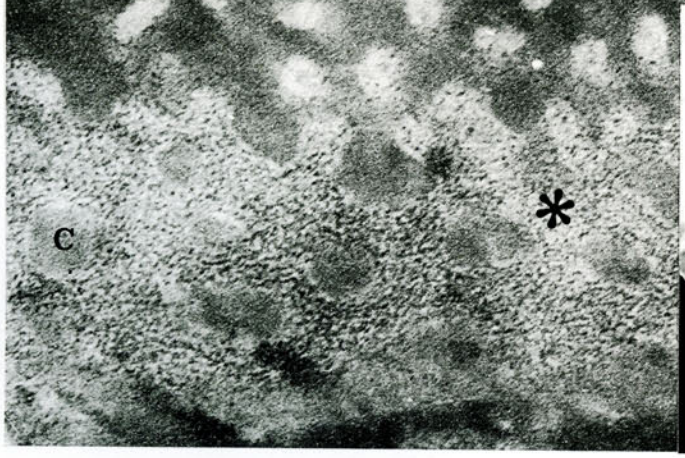
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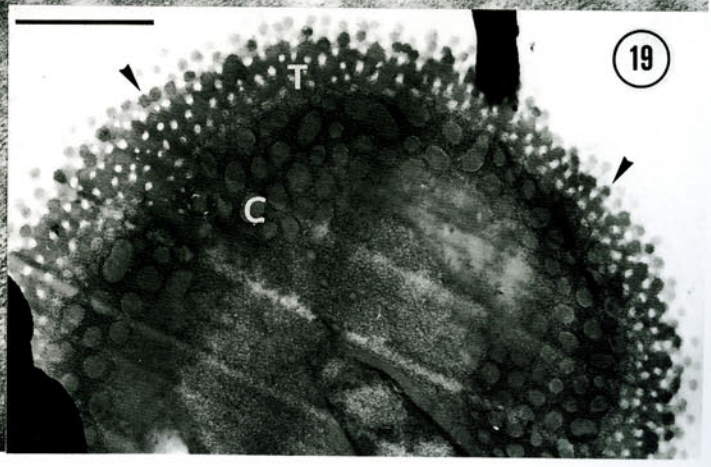
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The importance of foot layer growth is that it shows that formation of the endexine does not prevent development of the ectexine presumably from the plasmalemma, i.e., transport from the protoplast to the ectexine is not prevented.

Apertures

Descriptions of the complex apertures in mature pollen of this species are in general deferred to the detailed and well illustrated work of Ben Saad-Liman and Nabli (1984).

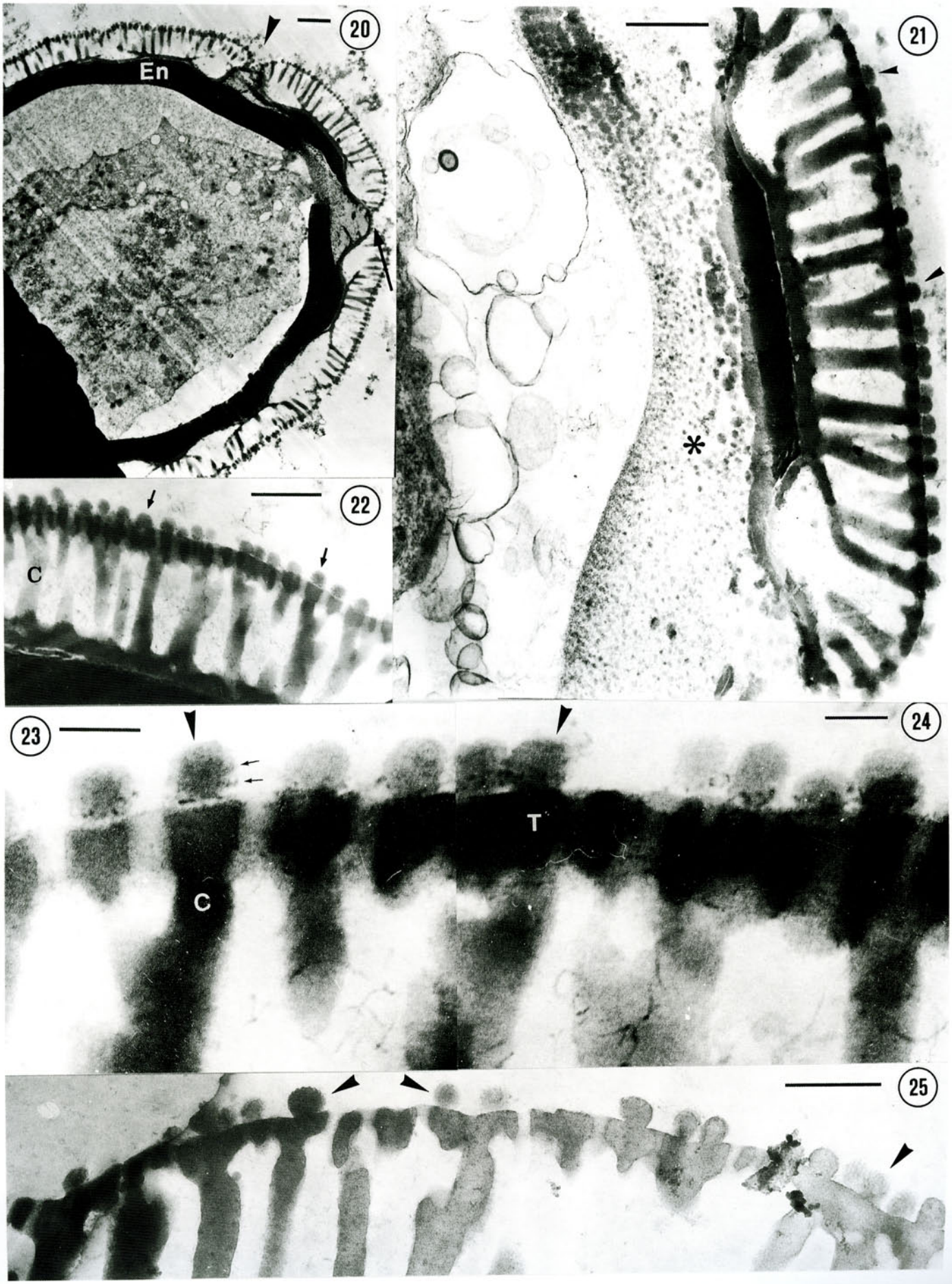
Commonly the pollen of *B. officinalis* has ten apertures that are linked equatorially by the endoapertures. This linking or overlapping of endoapertures is seen as a bright medial band in our SEM in Fig. 26 and more distinctly in photomicrographs of Ben Saad-Liman and Nabli (1984: Fig. 2 B and 6 D). They describe this band as an "equatorial girdle". In their Figs. 1 and 7 they use drawings to illustrate the position and relative size of colpi, precolpal cavities, mesoapertures and endoapertures. These features and interapertural sectors are illustrated by a succession of well selected SEMs and TEMs at a variety of magnifications, and one of the prize figures is an SEM of a transverse section through all ten apertures (Ben Saad-Liman and Nabli 1984).

We find that the precolpal cavities described on mature pollen (Ben Saad-Liman and Nabli 1984) form where there are glycocalyx thickenings to either side of apertures during the tetrad and free microspore stages (Fig. 6; Gabarayeva *et al.* 1998, Fig. 27). In addition we find that the foot layer is divided around these glycocalyx thickenings from the time of the foot layer formation in a late tetrad period and the early free microspore stage. Thus the future precolpal cavities are positioned within the foot layer.

Structure of gemmae

We have found, as the illustrations of Ben Saad-Liman and Nabli (1984) suggest, that each columella becomes surmounted by a gemma. In their Fig. 2G these gemmae are nearly spherical with a surface of similarly separated ridges. The ridges are like concavely sharpened knife-edges with concave valleys between them. The gemmate surface is similar to what we have recorded in acetolysed material (e.g., Fig. 28).

Figs. 20-25. Pollen grain stage (Figs. 20-24) and mature pollen (Fig. 25). Fig. 20. The section is near the equator for the colpus marked by an arrow and poleward for the colpus marked by an arrowhead. The rest of this section is poleward of the ends of apertures. Fixation: AB. Bar=1 μm. Fig. 21. A sector of the interaperturate exine between apertures on the equatorial plane. The granular zone below is called "equatorial endoapertural material" (asterisk) by Ben Saad-Liman and Nabli (1984). Fixation: RR. Bar=0.5 μm. Fig. 22. A section poleward from the ends of apertures. The height of columellae is ca. 0.8 μm at this stage while in the earlier stage represented by Fig. 17 the columellar height is only 0.2-0.3 μm. Gemmae (arrows). Fixation: RR. Bar=0.5 μm. Figs. 23 and 24. Micrograph of the same section as in Fig. 22. Tectum (T), columellae (C) and gemmae (arrowheads). During formation of gemmae SAPs in longitudinal sections (arrows) are arranged perpendicular to the center of incipient gemmae. Fixation: RR. Bars=100 nm. Fig. 25. Acetolyzed fresh mature pollen. The gemmae (arrowheads) show a variety of surface configurations. Bar=0.5 μm.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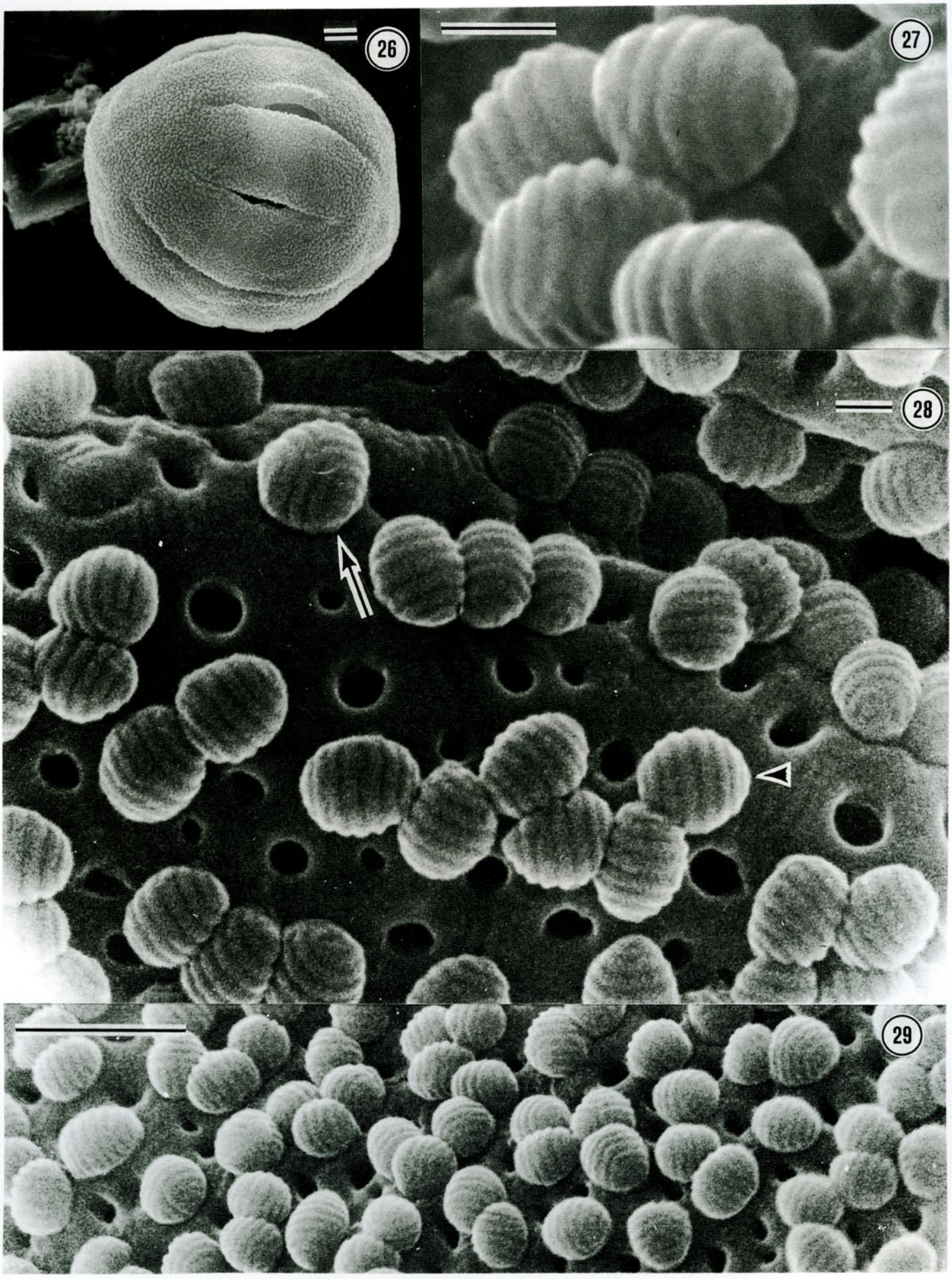
In fresh pollen the ridges in profile had the convex curvature expected from closely spaced SAPs (e.g., Fig. 18). If, however, SAPs are spheroidal then we would not expect ridges to have a smooth surface since there would be dips between spheres. Dips may be covered by secondarily accumulated sporopollenin. As part of an ongoing experiment with 4-methylmorpholine N-oxide monohydrate (MMNO), a potent solvent for polysaccharides, the smooth convex ridges were removed from mature pollen of *Borago* during exposure to MMNO. SAP-like structures remained and were on short stalks in profile and they were reacted with 5% phosphotungstic acid in 10% acetone, which is credited as a contrasting reaction for proteins. This could mean that SAPs include enzyme molecules as Gabarayeva (1993) suggested for sporopollenin accumulation in general.

In a SEM at 40,000X Ben Saad-Liman and Nabli (1984: Fig. 2E) illustrate what appears to be many sites (possibly raised) which they term "microsculptures" on each gemma. Their specimen could be one having a remnant of SAPs on gemmae. We included Fig. 2 to show that SAPs may be detached from the exine surface, most likely in this case by preparational damage to the callose partly covering the microspore.

CONCLUSIONS

1. First evidence for exine formation consists of striated or coiled processes that are similar in form and size (70-100 nm in diameter) to many reports for other taxa.
2. With the loss of the callose of the special cell wall there are SAPs on the distal surface of columellae. These are considered to be associated with sporopollenin accumulation because they occur on columellae during their increase in height. SAPs are involved in the formation of gemmae both on the distal end of columellae and on the apertural membrane.
3. Gemmae on mature pollen are spheroidal and have rounded ridges separated by valleys. The configuration of both the ridges and valleys could be produced by arrangement of SAPs in files. The ridges are rounded like the surface of spheres about the size of SAPs in, e.g., Fig. 18. There is some evidence that SAPs are not isolated particles but like strings of beads.
4. By maturity columellae of *Borago* have increased in height 2 to 3 times from our earliest recovery of them through pollen maturity. The way that columellae grow late in wall development remains unclear although there is some evidence that at different ontogenetic points SAPs and the plasmalemma are involved in this process.

Figs. 26-29. Scanning electron micrographs of mature pollen of *Borago officinalis*. Fig. 26. Four of the about ten apertures typical of the species are visible along with a bright equatorial band expressed by the endoapertures in this secondary image due to the relatively thinness of the exine in the endoaperture zone (please see Ben Saad-Liman and Nabli 1984, Fig. 3C). Bar=2 μm. Figs. 27-29. Micrographs emphasizing supratectal gemmae and tectal holes. The figures are at magnifications of 200,000X, 100,000X and 30,000X respectively. The SEMs show that gemmae vary in size and shape. In most cases the ridges around gemmae coil in one direction (arrowhead) on the spheroidal gemmae but in some cases there is a herringbone-like arrangement of "ridges" (arrow). Perforations in the tectum are variable in size; perforations in Fig. 28 range from 50 to 125 nm in diameter. Preparation: Fresh pollen to acetolysis. Bars: Figs. 27-28=100 nm; Fig. 29=0.5 μm.


26
27
28
29

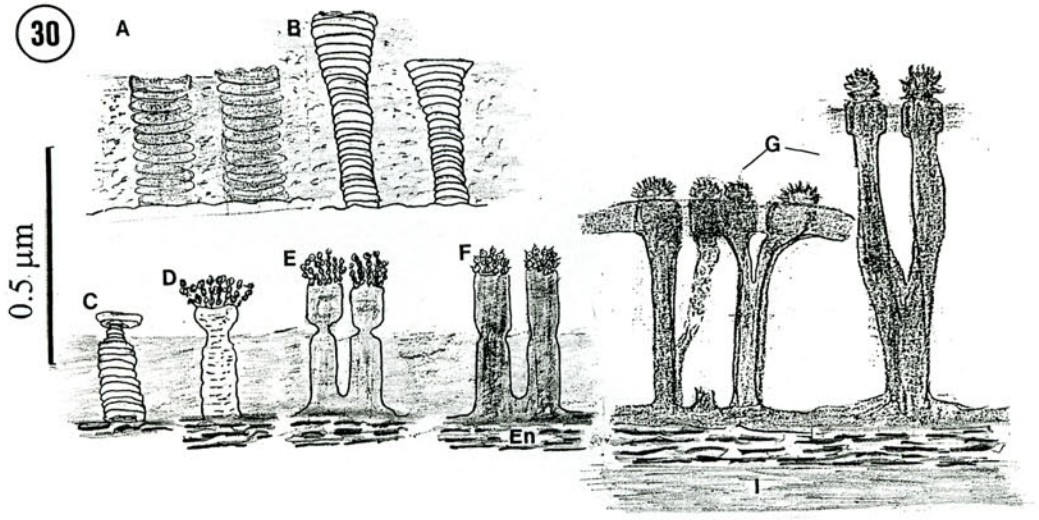


Fig. 30 A-G. Sketches based upon TEMs. Stage A depicts the youngest exine columellae observed. They have a larger overall diameter than all older stages. The stage represented by B has columellae that are variable in height and diameter. Both stages A and B have glycocalyx comparable to columellae in height and the columellae are taller than the older stages C and D. Stages C-G have both foot layer and endexine. Starting with stage C there is a constriction in columellae roughly coincident with glycocalyx height until pollen grain stages when the glycocalyx is lost (e.g., stage G). Stage C is enveloped by callose while the rest are not. SAPs become apparent at the time of callose digestion, i.e., between stages C and D. After chemical fixation SAPs are spread out as in a bouquet then become complexed into a more-or-less spheroidal gemmate structure centered on the growing columellae (E-F). Near maturity columellae have greatly increased in height although there is considerable variation in height (G). Columellar branching is common and in some TEM images, at least, the phenomenon appears to be the result of fusion of two or more columellae generally only basally (G). In any case the space between is also commonly somewhat filled in during microspore stages (E and F). Sketches A-C represent sections used for figures in Gabarayeva et al. (1998), i.e., A=Figs. 3-6; B=Figs. 15 and 16; C=Figs. 22 and 25. The remaining sketches are based upon sections represented here, i.e., D=Figs. 12-14; E=Figs. 15B, 16; F=Fig. 15A; G=Figs. 20-22.

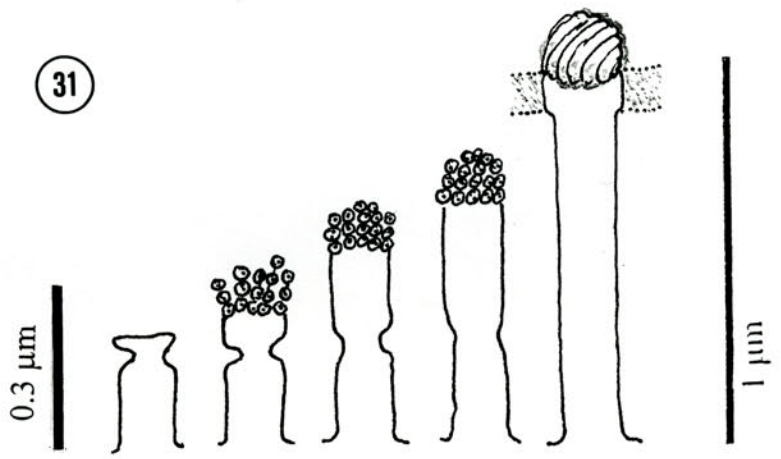


Fig. 31. Sketches depicting elaboration of endexine and development of a constriction (neck) near the distal surface of columellae. SAPs are evident at this surface. We propose that the columellae grow distally and are surmounted at maturity by the gemmae, illustrated in Figs. 23-29. The increase in height is as great as three times.

ACKNOWLEDGEMENTS

Nina Gabarayeva held a Visiting Scientist Fellowship from the Swedish Natural Science Research Council during the initial part of this work. Subsequently Dr. Gabarayeva was supported by a fellowship of an exchange program between The Swedish Academy of Science and The Russian Academy of Science. We gratefully acknowledge the technical assistance of Peter Cinnman (Komarov Botanical Institute, Russian Academy of Science) and of W.F. Chissoe (Samuel Roberts Noble Electron Microscopy Laboratory, University of Oklahoma). The reviewers suggestions and corrections have been of great value.

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玻璃苣屬(紫草科)之花粉外壁發育. 2.自由孢子時期

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（收稿日期：1999 年 4 月 12 日；接受日期：1999 年 5 月 20 日）

摘　　要

在自由孢子初期，當特殊的胼胝壁溶解時，每柱狀層遠軸面末端出現強對比的顆粒。它們直徑為 15 至 20nm，且像一串念珠互相連接在一起。數串念珠構成一個花束狀出現在柱狀層遠軸面末端。我們認為這些顆粒和孢粉寧質的堆積有關，且將它們歸類為孢粉寧質接受顆粒。孢粉寧質接受顆粒的出現伴隨著柱狀層的高度成長和棒狀物的形成。在花粉外壁發育末期柱狀層高度增長為 2 至 3 倍。在自由孢子晚期，成熟花粉棒狀物表面有圓型隆起且和孢粉寧質接受顆粒大小相同。

關鍵字：玻璃苣屬，柱狀層，花粉外壁，棒狀物，孢子，孢粉寧質接受顆粒。

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