Pollen Wall Development of *Xiphidium coeruleum* (Haemodoraceae) and its Systematic Implications

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ABSTRACT

The development of the pollen grain wall in *Xiphidium coeruleum* (Haemodoraceae) was studied using TEM and cytochemical staining techniques. Microsporocyte ontogeny initiates with the degradation of the cellulosic cell wall and subsequent deposition of a thick callosic cell wall. Following callose deposition, successive meiosis occurs, resulting in a tetragonal tetrad of microspores. during meiosis, the cell walls of the tapetum break down, releasing the syncytial periplasmodium. Irregular non-sporopollenous globular bodies are deposited in this peripheral periplasmodium, which is rich in ER, golgi bodies, vesicles, and characteristic starch plastids. Within the microspore cytoplasm, vesicles, golgi bodies, and plastids are plentiful during the early tetrad stage. At this time the plasma membrane of the microspore develops characteristic evaginations. An extracellular membrane, the 'white line', is secreted outside the microspore plasma membrane, followed by callose wall degradation. Bead-like deposits of exine or primexine are deposited at points along the 'white line' simultaneously on inner and outer surfaces and opposite the original plasma membrane evaginations. The bead-like exine deposits continue to grow during the release of the microspores and develop into laterally appressed, rod-shaped ektexinous elements having a tangentially oriented commissure, the vestige of the original 'white line'. The mature intine is two-layered, the outer exintine containing radially oriented vesicular structures, which are apparently derived from plasma membrane extensions. Exine development in *Xiphidium* is similar to 'nexine 1' development in *Lilium* and may have evolved from an ancestral tectate–columellate condition by the loss of the sexine. Wall development in members of the Zingiberales is strikingly similar to that reported here for the Haemodoraceae – evidence of a possible relationship between the two taxa.

Key words: *Xiphidium coeruleum*, Haemodoraceae, pollen, tapetum, development, exine.

INTRODUCTION

The Haemodoraceae has a unique pollen wall ultrastructure. Unlike many other monocotyledons, the exine in all investigated family members lacks a clear division into foot-layer, columellae, and tectum, consisting instead of a more homogeneous one-two-, or (rarely) three-layered structure comprised primarily of rod-shaped or tangentially oriented elements (Simpson, 1983). The great majority of genera and species in the Haemodoraceae possess the two-layered exine-wall type with a distinctive commissure separating inner and outer layers. A gradation of exine structural types can be noted among members of the family, implying an evolutionary sequence (see Simpson, 1983, 1990). These studies of exine wall structure have been of taxonomic value in definitively demonstrating the close relationship between the tribes Haemodoreae and Conostylideae (which had previously been classified in separate families by several authors; Simpson, 1983) and clarifying the interfamilial classification. Of all families proposed to be closely related to the Haemodoraceae, only members of the Pontederiaceae have a similar exine structure, some of these with a virtually identical one- or two-layered, non-tectate–columellate exine structure (Simpson, 1987; see also Simpson, 1985*a*, *b*). This evidence, along with data from anatomical and embryological studies, supports the hypothesis that the Haemodoraceae and Pontederiaceae are sister taxa (Simpson, 1987, 1990).

The purpose of the present study is to determine the developmental basis for the unusual pollen-wall structure in the Haemodoraceae. Of major importance are: (1) origin and development of the distinctive 'commissure', which separates inner and outer exine layers; and (2) development of the tapetum (reported as being plasmodial in *Xiphidium*; see Stenar, 1938) and its role in pollen-wall formation. Knowledge of exine development

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may allow a better understanding of interfamilial relationships in the Haemodoraceae, as it will provide a basis for future assessment of the proposed exine structural homology between the Haemodoraceae and Pontederiaceae. In addition, recent controversy as to the classification of monocotyledons, particularly the integrity of the superorder Bromeliiflorae (*sensu* Dahlgren and Rasmussen, 1983; see Walker, 1990), which includes the Haemodoraceae and Pontederiaceae, may well be clarified.

MATERIALS AND METHODS

Flowering material of *Xiphidium coeruleum* Aubl. was obtained from transplants of a Panamanian collection grown at the climatron of the Missouri Botanical Garden, St Louis, Missouri, USA (Antonio 1201). A developmental range of flower buds was dissected from helicoid cyme units of the inflorescence. Several anthers from each flower were cut transversely in a cold (4 °C) solution of 4% glutraldehyde in 0·1 M Sorensen's phosphate buffer. One anther from each flower was squashed in 0·1% toluidine blue stain and observed with light microscopy to determine the developmental stage. Preliminary observations indicated that the critical period of exine deposition occurred in the late tetrad stage; thus, this stage was preferentially selected for study. Anthers were fixed in cold glutaraldehyde for approx. 4 h, followed by several rinses in buffer and further fixation in 2% osmium tetroxide for 2 h. The material was then quickly rinsed twice in buffer, progressively dehydrated to 100% ethanol, and infiltrated (via a gradation series) with Spurr's resin (Spurr, 1969). The material was polymerized at 70 °C for approx. 10 h. Sections 0·5 μm thick were stained with 1% toluidine blue and mounted on a slide for light-microscope observations. Anthers of the proper developmental stage were ultrathin-sectioned (approx. 85 nm thick) with a Diatome diamond knife on a Reichert Ultracut-E ultramicrotome, mounted on uncoated hexagonal 200-mesh copper grids, and post-stained with uranyl acetate (saturated solution in 50% ethanol, 15 mins) and lead citrate (0·2% aq., 7 mins). To inhibit stain precipitation, grids were gently and quickly washed in a running stream of filtered, distilled water between and after post-staining changes. Observations were made on both a Zeiss EM 9S-2 and a Philips EM 410 transmission electron microscope.

For cytochemical tests (not illustrated) glutaraldehyde-fixed anthers of a range of critical developmental stages (as determined by TEM observations) were embedded in Historesin (LKB, Inc.) and sectioned with a glass knife at 2 μm thickness. Sections were transferred and dried on a microscope slide, stained for 2–5 mins, and observed with both light and epifluorescence microscopy using a Nikon Microphot-FX photomicroscope. Cytochemical observations included the following: (1) positive reaction (red staining) with periodic acid–Schiff (PAS) reagent to detect complex carbohydrates; (2) positive reaction (brown-black staining) with IKI (1% in distilled H_2O) to detect starch; (3) positive reaction (bright yellow fluorescence) with auramine O (0·1% in distilled H_2O) to detect ektexine; (4) positive reaction (bright while fluorescence) with cellufluor (Polysciences, Inc.; 0·1% in distilled H_2O) to detect cellulose; (5) positive reaction (blue staining) with alcian blue (1% in 3% acetic acid) to detect pectins; and (6) positive reaction (bright yellow fluorescence) with aniline blue (1% in distilled H_2O) to detect callose (see Berlyn and Miksche, 1976; Kress and Stone, 1982). A Nikon B-2A filter combination (dichroic mirror 510 nm, excitation filter 450–490 nm, barrier filter 520 nm) was used for detecting Auramine O fluorescence and a Nikon UV-SA filter combination (dichroic mirror 400 nm, excitation filter 330–380 nm, barrier filter 420 nm) was used for cellufluor and aniline blue fluorescence. In addition, to identify sporopollenin deposits, the technique of Dickinson and Bell (1973) was used. This technique involves placing an acetolysis mixture (nine parts acetic anhydride: one part sulphuric acid) on thick-sectioned material, covering with a coverslip, and heating; non-sporopollenous material dissolves, whereas sporopollenin is preserved.

RESULTS

Pre-meiotic microsporocytes contain extensive, regularly arranged smooth endoplasmic reticulum and are densely packed with numerous mitochondria and golgi bodies (Fig. 1A, B). The plasma membrane of the microsporocytes is characteristically pulled away from what appears to be a remnant of the initial cellulosic/pectic cell wall (Fig. 1A). Double membrane-bound plastids, containing internal lamellae and electron-transparent vesicles, are commonly distributed in the microspore cytoplasm (Fig. 1B). Fibrillar deposits are appressed to the microsporocyte plasma membrane (Fig. 1B); this fibrillar material resembles the later-formed callosic wall in staining properties; however, no callose was detected at this stage by cytochemical tests. Numerous, minute vesicles and occasional lamellar membranes occur just below the microsporocyte plasma membrane (Fig. 1B). No starch, ektexine, or appreciable cellulose was detected at the pre-meiotic stage;

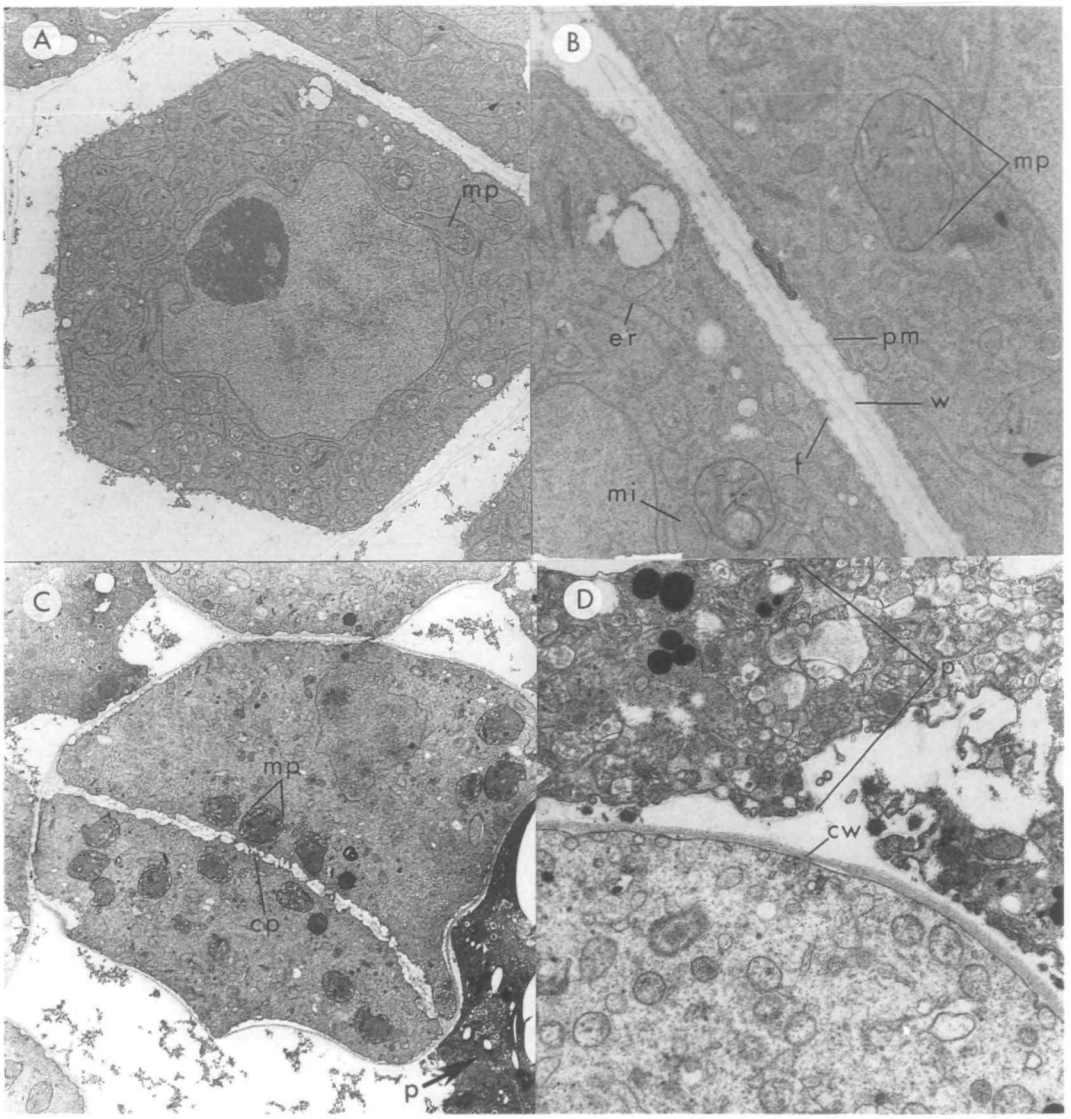


FIG. 1. A, Microsporocyte prior to meiosis. Note nucleus, nucleolus, dense concentration of mitochondria and endoplasmic reticulum, and microspore plastids (mp). × 11250. B, Close-up of region between two microsporocytes in A, showing endoplasmic reticulum (er), mitochondrion (mi), plasma membrane (pm), and remnant wall layer (w). Fibrillar material (f), presumably callosic, is appressed to the outer plasma membrane. Note densely staining lamellar body (between plasma membrane and callose wall) and young microspore plastid (mp). × 10700. C, Dyad stage, after the first meiotic division. Note the several microspore plastids (mp) positioned adjacent to the cell plate (cp). Processes of the periplasmodium (p) have invaded the dyad intercellular spaces at this stage. × 3750. D, Edge of outer dyad wall, showing thin, callosic cell wall (cw) and highly convoluted membrane of periplasmodium (p). × 10700.

however, the early microsporocyte cell walls are pectic-rich. At the dyad (post meiosis 1) stage, prominent intercellular spaces are evident between the dividing microsporocytes (Fig. 1C). A thin callosic wall surrounds the dyad (Fig. 1C, D). Numerous microspore plastids were observed to be positioned mostly adjacent to the cell plate, with microspore nuclei positioned distal to the plane of divison (Fig. 1C).

The walls of the tapetal cells begin to break down during the microspore dyad stage (Fig. 2A). Globular deposits are prevalent in the tapetum,

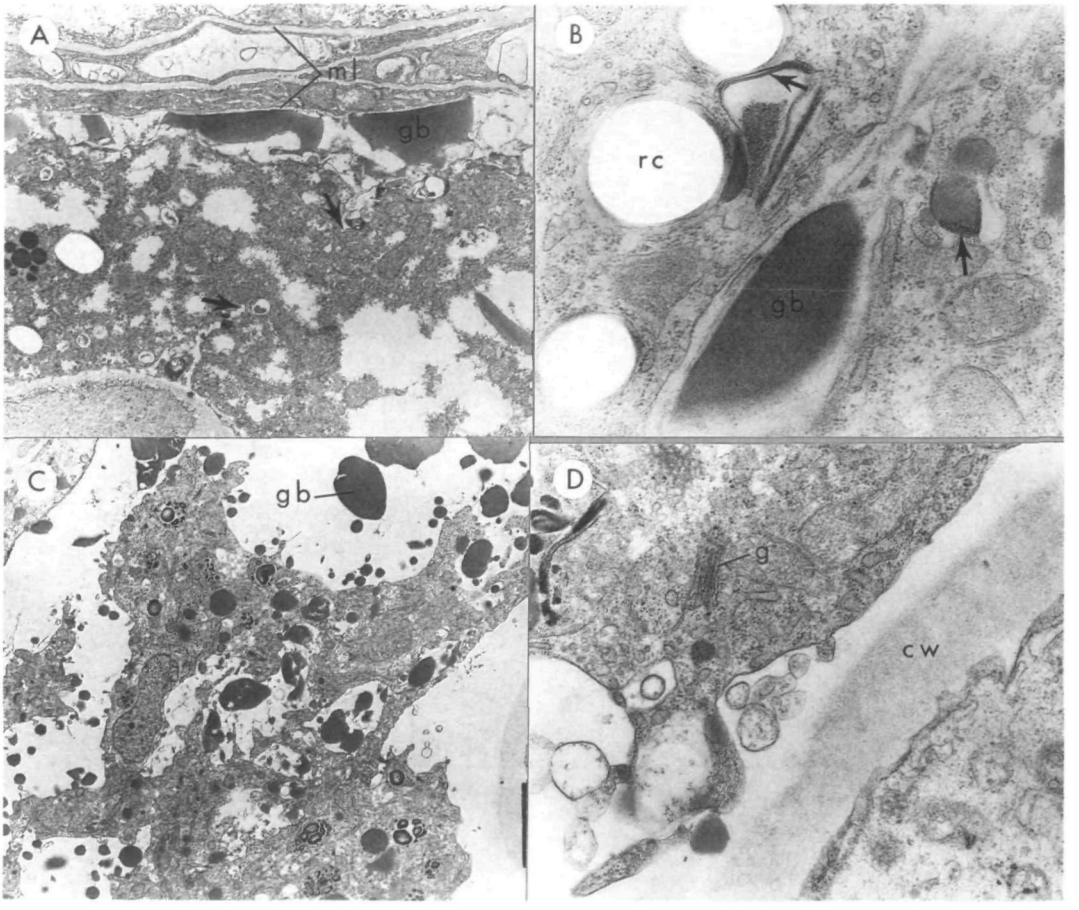


FIG. 2. A, Early dissolution of radial walls of tapetum (arrows). Note middle layer (ml) and globular deposits (gb). ×8070. B, Close-up of globular deposits (gb) between adjacent tapetal cells, showing membrane lamellae (arrows). Note open hole at location of raphide crystal (rc). ×23000. C, Periplasmodium following complete dissolution of tapetal walls. Note numerous, irregular globular bodies (gb). ×4270. D, close-up of junction of periplasmodium (upper left) and tetrad callose wall (cw). Note numerous golgi (g), mitochondria, and ribosomes of periplasmodium. ×20000.

especially on the outer tangential and radial walls (Fig. 2A). The globular deposits are associated with apparent membrane lamellae (Fig. 2B) during their formation. With the breakdown of the tapetal wall, periplasmodial processes invade the intercellular spaces of the microsporocytes (Figs 1D, 2C). The periplasmodium contains a system of numerous vesicles and highly convoluted sacs at its junction with the dyad wall (Fig. 1D). Raphide crystals (Fig. 2B) occur scattered throughout. Numerous golgi bodies, mitochondria and ribosomes are evident in the periplasmodium at its interface with the developing microspores (Fig. 2D).

Microsporogenesis is successive, resulting in tetragonal tetrads of microspores (i.e. with the axes of pairs of microspores oriented approximately perpendicularly; Fig. 3A). At this tetrad (post meiosis II) stage of development, the tapetal plasmodium has completely invaded the locular cavity (Fig. 3A). The variably sized globular deposits, having the staining properties of sporopollenin (i.e., osmiophilic and homogeneous) but no ektexine staining reaction (i.e. negative auramine O fluorescence), occur throughout the periplasmodium, particularly adjacent to the inner anther wall (Fig. 3A). The early tetrads have a rather thick outer wall (Fig. 3A, B), which is composed of both callose (strongly aniline blue-fluorescent) and some cellulose (weakly cellufluor-

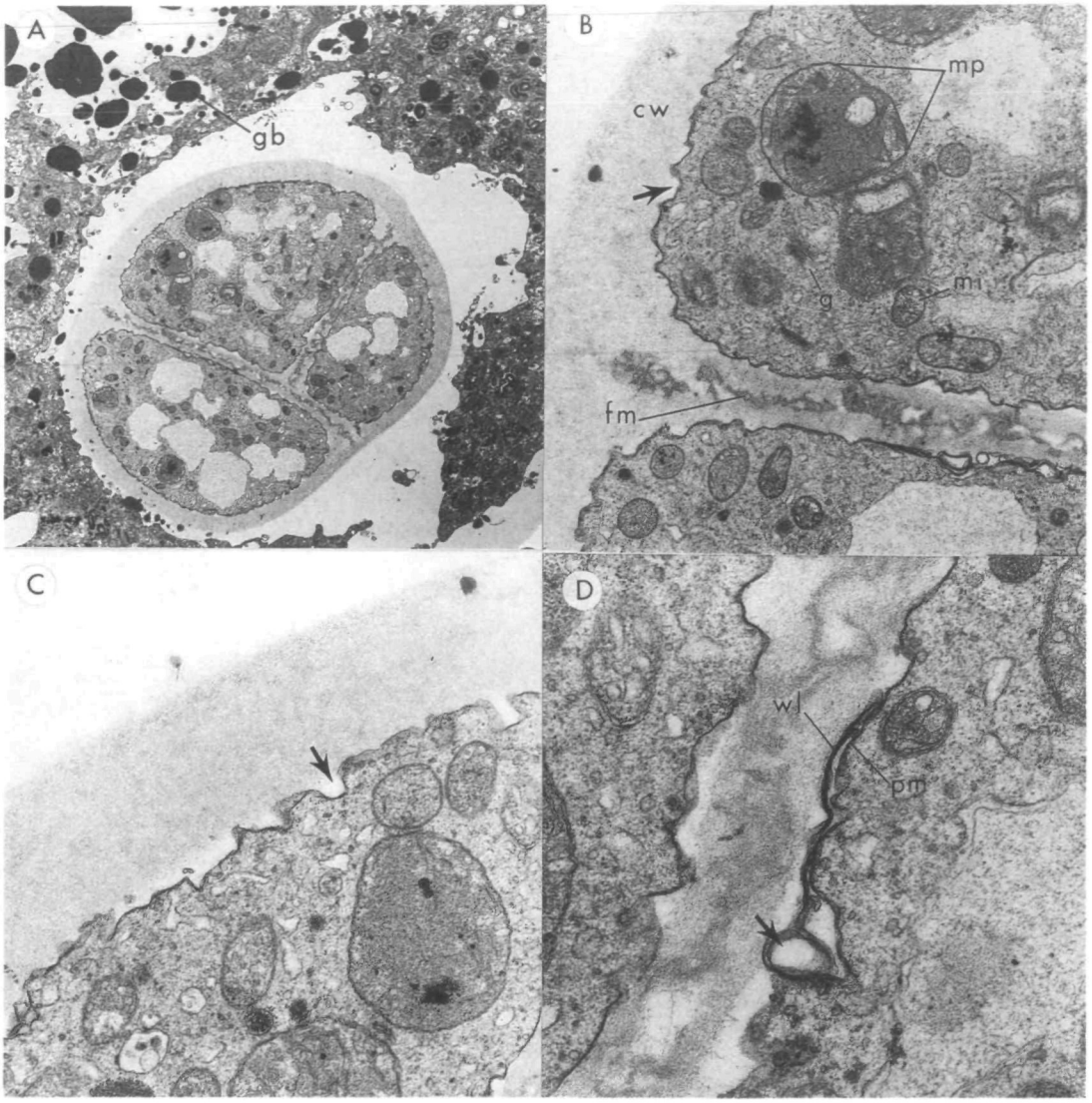


FIG. 3. A, Tetrad of microspores (showing tetragonal orientation of microspores) surrounded by the periplasmodium. Note electron-dense globular deposits (gb) of periplasmodium. × 3040. B, Close-up of tetrad, showing thick callose wall (cw) and fibrillar material (fm) at the junction of callose walls between adjacent microspores. Golgi bodies (g) and mitochondria (mi) are plentiful. Microspore plastids (mp) contain peripheral lamellar bodies and electron-dense deposits. Note invaginations (arrow) of plasma membrane. × 10970. C, Distal callose wall of tetrad, showing invaginations (arrow) of plasma membrane. Note mitochondria, plastids, and numerous small vesicles inner and adjacent to plasma membrane. × 15100. D, Close-up of callose wall between adjacent microspores of tetrad. A 'white line' extracellular membrane (wl) has developed exterior to plasma membrane (pm). A large vesicle (arrow) is also apparent at invagination of the plasma membrane. × 3220.

fluorescent). Between adjacent microspores, the median region of the wall exhibits a vesicular appearance, containing electron-dense fibrillar material (Fig. 3 B). The microspore cytoplasm at this stage contains numerous mitochondria and golgi bodies, several microspore plastids, and a number of coalescing vesicles (Fig. 3 B, C). Microspore plastids have peripheral lamellar bodies, vesicles, and inner electron-dense deposits (Fig. 3 B). The plasma membrane of each microspore

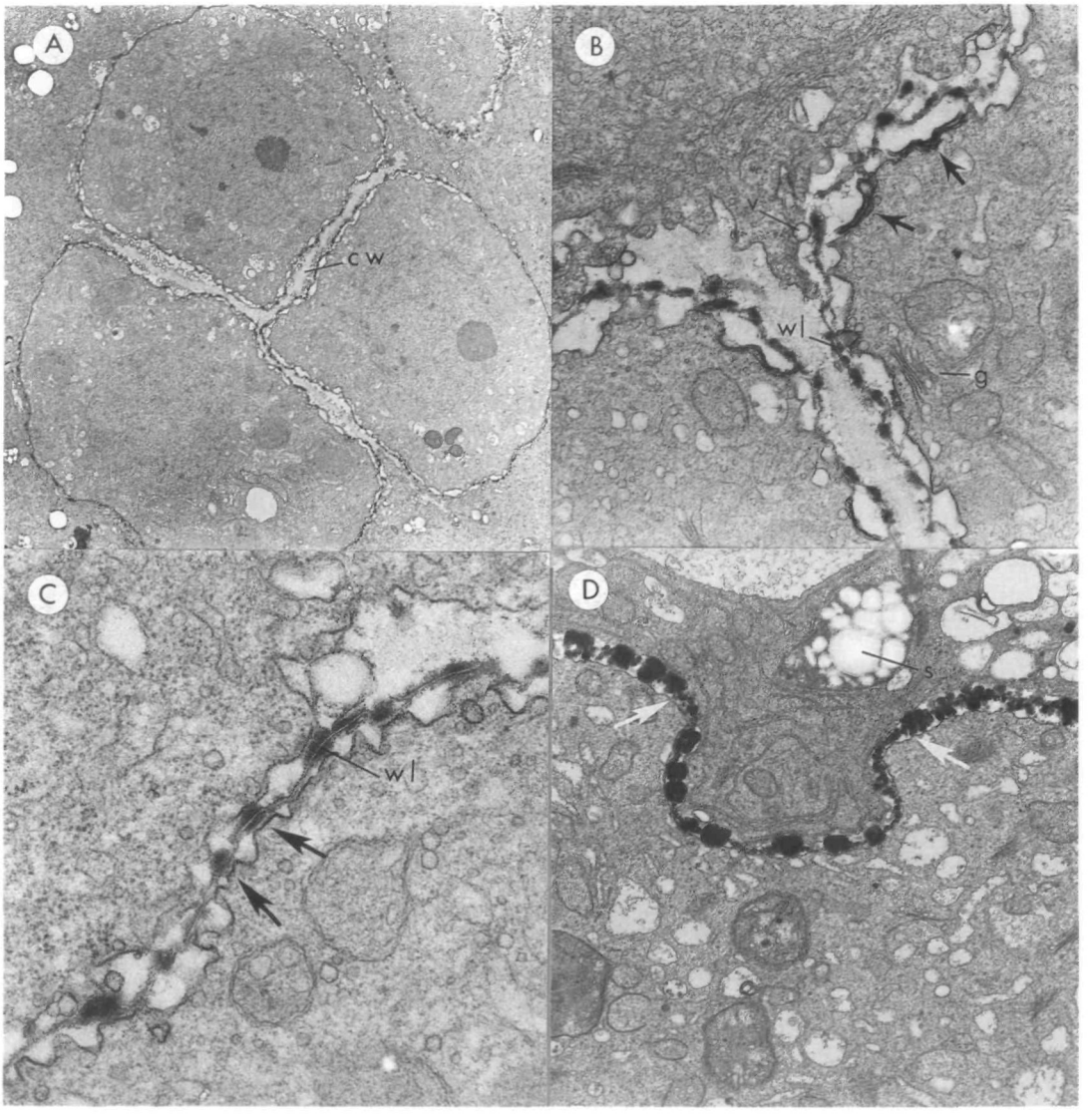


FIG. 4. A, Late tetrad stage (three microspores visible). Note that only the callose wall (cw) at the proximal side of the microspores remains. × 2820. B, Late tetrad stage at junction of two microspores; periplasmodium at upper left. Callose wall is deliquescing, particularly at distal regions of microspores. Note 'white line' (wl) extracellular membranes (at either side of callose wall) with bead-like deposition of exine material. Vesicles (v) and golgi bodies (g) are common at cytoplasm periphery, the latter appearing to have fused with plasma membrane (arrows). × 19270. C, Distal wall of microspore (lower right) at junction with periplasmodium (upper left), showing 'white line' (wl) extracellular membrane. Note bead-like depositions of exine at point of plasma membrane projections (arrows) flanking invaginations. × 39900. D, Close-up of recently released microspore (below) showing profusion of periplasmodial endoplasmic reticulum outside of inwardly collapsed apertural region (arrows). Note starch plastid (s) of tapetum. × 5990.

exhibits a characteristic 'rippling' pattern, resulting from the development of rather uniformly distributed invaginations (Fig. 3B, C, D). These invaginations typically have an inner electron-dense layer (Fig. 3C). Vesicles, of approximately the same dimension as the invaginations, are often observed at the membrane (Fig. 3B, D). Later in this tetrad stage, an extracellular membrane, having the appearance of a 'white line' (see Discussion) and consisting of an electron-trans-

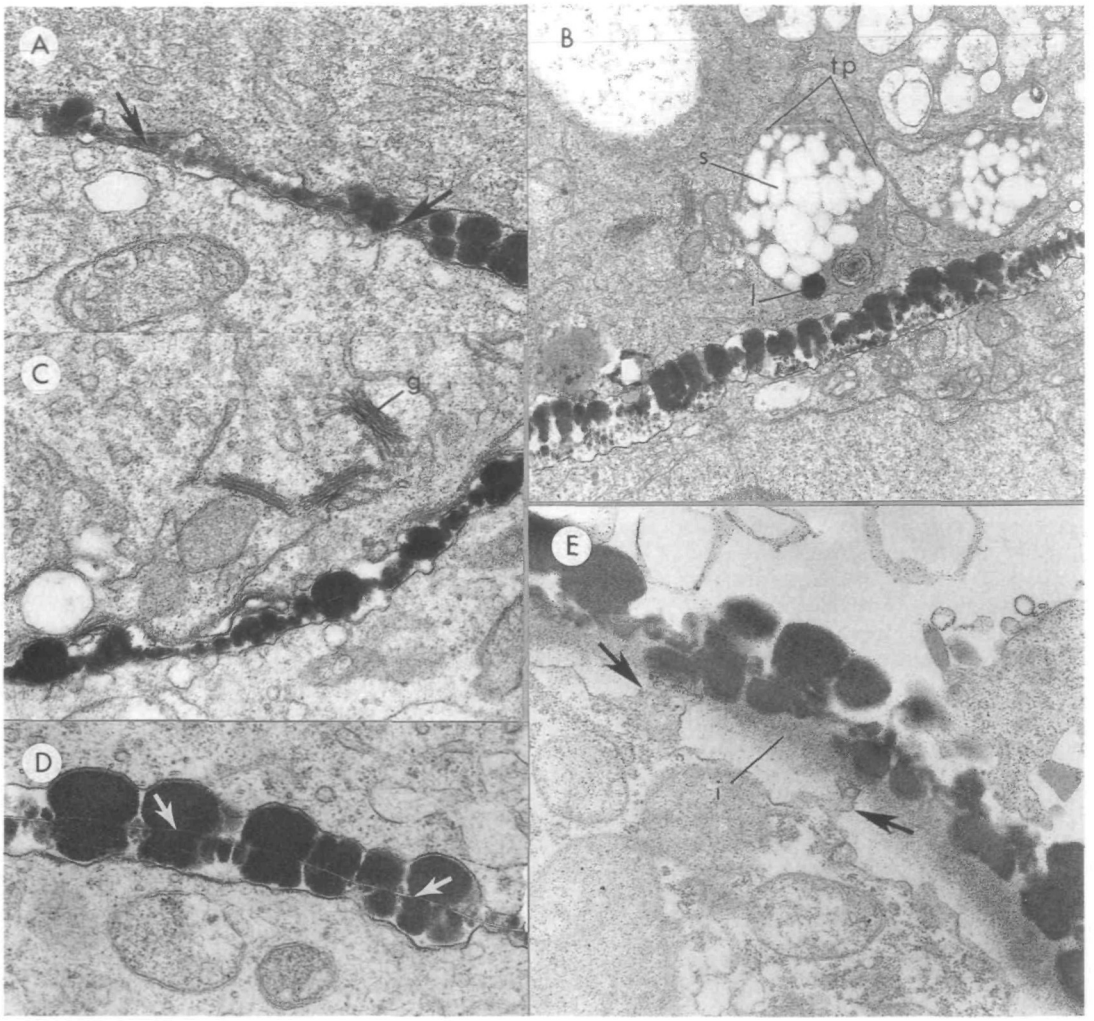


FIG. 5. A, Close-up of microspore wall after release of tetrad. Note numerous ribosomes in periplasmodium (above). Exine deposits are variable in size and are traversed by tangential 'white line' (arrows). × 32070. B, Periplasmodium adjacent to microspore wall. Note tapetal plastid (tp), full of starch (s) and apparent lipid body (l). × 11340. C, Golgi bodies (g) of periplasmodium, adjacent to wall of microspore (below). × 23940. D, Close-up of mature exine, showing vestigial 'white line' (arrows) between inner and outer layers of rod-shaped exine deposits. × 27070. E, Early deposition of intine (i). Note radially oriented extensions (arrows) of the plasma membrane. × 17280.

parent layer sandwiched between two electron-dense layers, develops between the plasma membrane and callose wall (Fig. 3D).

Subsequent to the formation of the plasma-membrane invaginations, the callose wall of the tetrad begins to break down (Fig. 4A). The proximal callose walls remain intact for a period of time after the distal walls have dissolved (Fig. 4A, B). Exine is deposited on to the extracellular membrane ('white line') simultaneously at both the distal and proximal regions (Fig. 4B). Exine is also deposited simultaneously (and to a comparable degree) on either side of the extracellular membrane (Fig. 4C). Numerous golgi bodies are present in the microspore cytoplasm and are often seen as recent excretion products at the plasma membrane. Outward projections of the plasma membrane (flanking the invaginations) generally serve as the sites of initial exine deposition (Fig. 4C). Exine develops initially as bead-like deposits (Fig. 4C), these increasing in size to the large rod-shaped structures found in more mature grains

(see Fig. 5D). Vesicles of variable size are common on either side of the developing exine wall (Fig. 4C).

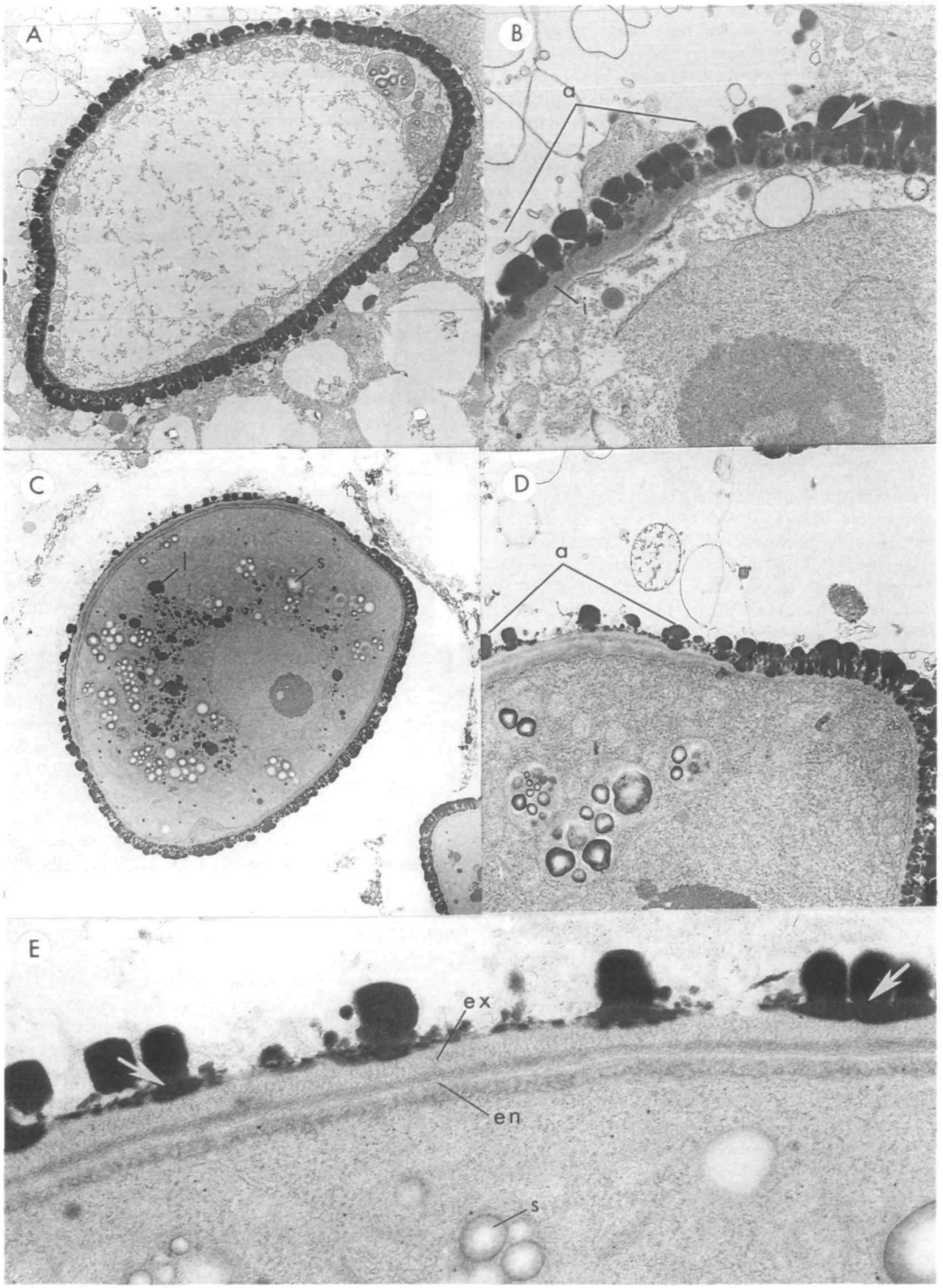
Soon after the release of the microspores from the tetrad, the future apertural region has collapsed inward (Fig. 4D). Prominent parallel stacks of endoplasmic reticulum occur in the periplasmodium within the pocket formed by the collapse of the microspore aperture (Fig. 4D). The exine wall becomes considerably thicker after microspore release (Figs 4D, 5A, B), and exhibits auramine O fluorescence (ektexine positive). The extracellular membrane ('white line') is clearly visible and extends tangentially through the rod-shaped exine elements (Fig. 5A, D). The periplasmodium contains numerous mitochondria, ribosomes, endoplasmic reticulum, golgi bodies, and tapetal plastids (Fig. 5B) at this stage. The tapetal plastids consist of double membrane-bound organelles with spherical electron-dense, lipid-like deposits and electron-transparent starch deposits (Fig. 5B). Starch was distributed throughout the periplasmodium, as evidenced by a positive IKI staining reaction. Tapetal plastids are occasionally in close proximity to the developing microspore wall (Fig. 5B). Golgi bodies of the periplasmodium are commonly seen adjacent to the periplasmodial membrane at the outer pollen exine wall (Fig. 5C). The mature exine consists of oblong rod-shaped elements with granular deposits present in the inner exine layer (Fig. 5D). At a slightly later stage, deposition of the fibrillar intine is initiated (Fig. 5E). Occasional radially oriented, finger-like projections were seen appressed to the intine during its deposition (Fig. 5E).

After being released from the tetrad, the microspores enter the vacuolate stage (Fig. 6A). This stage is characterized by the presence of a large central vacuole which displaces the remaining cytoplasm to the periphery of the microspore. The exine is fully developed at this point (Fig. 6A, B). A vestige of the tangentially oriented extracellular membrane ('white line') is clearly visible in the rod-shaped exine elements (Fig. 6B). Fibrillar intine deposits occur inner to the exine wall (Fig. 6B). This fibrillar material is cellulosic, as confirmed by positive cellufluor fluorescence. Vesicles are occasionally visible in the peripheral cytoplasm, apparently fusing with the plasma membrane (Fig. 6B). The intine of the apertural region is significantly thicker than that of the non-apertural region at this stage (Fig. 6B). After the vacuolate stage, microspores are fully expanded (Fig. 6C). The exine wall in the non-apertural region is relatively thick, consisting of laterally appressed rod-shaped elements with inner and outer layers of approximately equal thickness (Fig. 6C, D); minor granular deposits occur in the exine, particularly in the inner exine layer (Fig. 6B, D). In the apertural region the exine elements are further apart, and the inner exine layer is considerably reduced (Fig. 6D). The intine of the non-apertural region is faintly two-layered, the inner layer staining slightly more densely (Fig. 6D). The apertural intine is two-layered, having a relatively thick outer layer (exintine) traversed by channel-like structures and a thin, more homogeneous inner layer (endintine) (Fig. 6E). This apertural intine is pectic-rich (as determined by positive staining with alcian blue), but no differentiation between the two layers was observed. A gradation in thickness is evident in the intine at the junction of non-apertural and apertural regions (Fig. 6D). A thin, amorphous, and somewhat vesicular region may be visible proximal to the endintine (Fig. 6E). Starch grains and apparent lipid deposits are plentiful in the pollen-grain cytoplasm (Fig. 6C, D, E).

DISCUSSION

The development of both the tapetum and pollen grains in *Xiphidium coeruleum* shows some significant features in comparison with other monocotyledons. During tapetal development, large globular deposits are secreted within the binucleate cells prior to microsporogenesis. These deposits are similar to the so-called 'lipid globules' or 'lipid bodies' in *Tradescantia* (Mepham and Lane, 1969; Tiwari and Gunning, 1986*b*) and to the 'lens-shaped bodies' in *Heliconia* (Stone, Sellers, and Kress, 1979), but differ from the latter in being considerably more variable in shape and size. As in *Heliconia*, the tapetal deposits in *Xiphidium* show evidence of an internal lamellar structure, at least during their formation (see Fig. 2B). The exact chemical composition of these tapetal 'globules' is not known at present. Because of their similarity to the exinous wall in composition and staining

FIG. 6. A, Microspore at vacuolate stage. Note large, central vacuole and peripheral cytoplasm. ×3400. B, Close-up of stage at A, showing early deposition of fibrillar intine (i) in region of aperture (a). Note numerous vesicles inner to plasma membrane. ×8670. C, Mature, fully expanded pollen grain. Note starch (s) and lipid (l) deposits. ×2450. D, Junction of apertural region (a) and non-apertural region (right). Note two-layered nature of intine. ×4110. E, Close-up of apertural wall region, showing electron-dense exine with vestigial 'white line' (arrows) and two-layered intine comprised of outer exintine (ex) and inner endintine (en). Note starch plastid (s). ×8590.



properties, it was thought possible that they contain sporopollenin and may be homologous to the orbicules or Ubisch bodies occurring in taxa with a secretory tapetum (Echlin and Godwin, 1968; Heslop-Harrison and Dickinson, 1969) or to the sporopollenin deposits observed in colchicine-treated anthers of *Tradescantia virginiana* (Tiwari and Gunning, 1986*c*). However, the globules show no ektexinous staining reaction at any stage of development, as they do not fluoresce with auramine O and do not survive the acetolysis treatment of Dickinson and Bell (1973).

Just prior to meiosis, the cell walls of the tapetum begin to break down. Invasion processes of the periplasmodium soon occupy the intercellular spaces of late microsporocytes and eventually surround microspore dyad and tetrad stages. Raphide crystals, double membrane-bound plastids, and the aforementioned globular deposits are present in large numbers in the periplasmodium. The plastids present here have no direct role in pollen wall formation and probably function simply as carbohydrate reservoirs. The tapetal periplasmodium eventually forms a syncytium, as no evidence of original cell boundaries can be seen in any later stages. By the tetrad stage the periplasmodial membrane has become closely appressed to the microsporocyte callosic wall. This periplasmodial membrane ('perispore membrane' or 'PSM' of Tiwari and Gunning, 1986*a*) forms, at an early stage, highly proliferative convolutions, which define intricate cavities (see Fig. 1D). Numerous mitochondria, ribosomes, endoplasmic reticulum, golgi bodies, and plastids occur in the periplasmodium periphery at this stage, indicating a high level of metabolic activity; this may support a function for the periplasmodium in secreting degradative enzymes or sporopollenin precursors. No microtubule arrays (as reported for *Tradescantia* by Tiwari and Gunning, 1986*a*) were definitively observed in the tapetum; however, specific staining procedures for microtubules were not utilized in this study.

Microsporocyte development initiates with the secretion of a thin fibrillar cell wall layer (pulled away from the plasma membrane); this layer is presumably the degraded remains of the original cellulosic/pectic cell wall of the sporogenous tissue. The cell walls of the tapetum have mostly or completely dissolved by this stage, suggesting that enzyme secretions which function in the breakdown of the tapetal wall also effect cell wall dissolution of the sporogenous tissue, described, for example, in *Tradescantia* by Mepham and Lane (1969). The pectic-rich nature of the microsporocytes in *Xiphidium* support the hypothesis that pectinases are responsible for the dissolution of tapetal and microsporocyte cell walls. The function of the distinctive microspore plastids, consisting of double-membrane-bound organelles enclosing peripheral electron-transparent vesicular structures and electron-dense (presumably lipid) bodies, is probably as carbohydrate reservoirs. By the time of microspore maturity the plastids have accumulated considerable starch (see Fig. 6C, D, E) and presumably function in providing energy stores for the growing male gametophyte. Lipid-like bodies in mature pollen grains may be carotenoids, perhaps responsible for the yellow pollen coloration (personal observation).

Microsporogenesis in *Xiphidium*, as in all other Haemodoraceae (Stenar, 1927; Dellert, 1933; de Vos, 1956; Simpson, 1988), is confirmed (see Stenar, 1938) to be successive (i.e. in which meiosis II follows cytokinesis of meiosis I). The callose wall which develops around the microspore tetrads is distally homogeneous but exhibits a vesicular appearance (with more electron-dense fibrillar material; see Fig. 3B) between adjacent microspores. This fibrillar material becomes less prominent at later stages and may simply be the boundary region between microspore cell walls at the completion of cytokinesis. At the early tetrad stage the plasma membrane contains characteristic invaginations (see Fig. 3B, C, D), identical to those found in other monocotyledons (Dickinson, 1970; Stone *et al.*, 1979). The direct cause of these invaginations is not apparent. Minute vesicles are prevalent just inner to the plasma membrane at this stage (Fig. 3C), as are occasional larger vesicles, of about the size of an invagination (Fig. 3D). Microtubules, often implicated in membrane conformational changes, were not observed. The ultimate ultrastructural origin of these plasma membrane invaginations warrant further attention, as they may serve as the template for the subsequent development of exine pattern (see below).

Exine deposition in *Xiphidium* is associated with a single, tangentially oriented extracellular membrane (resembling the 'white line' of Godwin, Echlin and Chapman, 1967, and of Stone *et al.*, 1979, or the 'lamella' of Heslop-Harrison, 1971), which develops in the early tetrad stage. No primexine layer was observed. This extracellular membrane, the 'white line', consists of a unit-membrane-sized electron-transparent layer bordered on either side by slightly thicker electron-dense layers. It is apparently derived directly via a secretion of the plasma membrane, as suggested by the observed fusion of vesicles and/or golgi bodies (see Fig. 3B, D). Soon after the initiation of the

'white line', the deposition of exine begins and the surrounding callose wall begins to break down. The fact that the distal wall of each microspore breaks down first, while the proximal callose wall region remains intact for a longer period of time (Fig. 4A) supports the hypothesis that tapetal (not microspore) enzymes are responsible for callose breakdown (Vasil, 1973). Exine deposition initiates upon the relatively flat 'white line' at the site of near contact with the finger-like projections of the microspore plasma membrane (flanking the invaginations; see Fig. 4C). Bead-like deposits of electron-dense material develop simultaneously on the inner and outer surface of the 'white line'. Whether these deposits are sporopollenin in composition or, alternatively, constitute a primexine precursor is not known at present. No ektexinous staining reaction was observed at this early tetrad stage, perhaps indicating that the deposits are primexinous (i.e. primarily cellulosic templates); however, the absence of an observed positive ektexine staining reaction at this stage may be explained by the scanty volume of deposits present. The deposits do exhibit TEM staining properties similar to those of the mature exine of the pollen grain wall, which does test positive for ektexine.

The apparent occurrence in *Xiphidium* of recently excreted golgi bodies in the microspore cytoplasm during early exine deposition (Fig. 4A) may be cited as evidence that these golgi bodies are actively involved in secreting enzymes or structural precursors utilized in exine or primexine synthesis. Interestingly, this initial deposition of exine occurs simultaneously at both distal and proximal walls of each microspore. Even on the distal surface, which has a considerable thickness of callose wall, there are deposits of exine both inner and outer to the 'white line'. If indeed callose is relatively impermeable to material transport (Mackenzie and Heslop-Harrison, 1967), this observation would support the notion that the enzymes responsible for at least the initial exine deposition arise from the microspore (not periplasmodial) cytoplasm. However, when the callose wall does completely deliquesce, a corresponding finger-like projection of the periplasmodial membrane usually occurs opposite that of the microspore plasma membrane, suggesting that the periplasmodium functions in exine deposition, at least at this later stage. After the release of the microspores from the tetrad, numerous golgi bodies occur near to (and apparently fuse with) the periplasmodial membrane, adjacent to the outer exine wall. In contrast, little cellular activity is evident within the microspore cytoplasm at this stage. Thus, these observations support the idea that the periplasmodium functions directly in exine wall development, either in the direct deposition of sporopollenin or, as has been demonstrated for other taxa, via the infusion of an initial primexine matrix with sporopollenin.

The exine appears to be fully developed soon after release of the microspores from the tetrad. The mature exine of *Xiphidium* basically consists of closely appressed rod-shaped (baculate) structural elements. The exine may be described as two-layered, with the inner and outer layer delimited by a vestige of the 'white line' extracellular membrane, which is persistent even in fully mature pollen grains (Simpson, 1983). The outer and inner exine layers of *Xiphidium* exhibit identical staining properties, both with light and transmission electron microscopy. Both layers exhibit ektexinous staining properties (positive for auramine O fluorescence). Granular deposits are prevalent between the major columellate elements, particularly in the inner exine layer (see Simpson, 1983).

The distal surface of the baculate exine elements in *Xiphidium* determine the distinctive verrucate sculpturing occurring in this genus (and in most members of the tribe Haemodoreae; see Simpson, 1983). A central question to consider, then, is the developmental basis for the determination of the exine pattern in *Xiphidium*. Although this cannot be answered satisfactorily from the present study, it seems probable that the invaginations of the plasma membrane provide a template for the mature exine pattern. Each baculate structural element forms on the 'white line' extracellular membrane at the point of contact of the finger-like projections (flanking the invaginations) of the microspore plasma membrane. Curiously, a corresponding finger-like projection of the periplasmodial membrane is usually present opposite that of the microspore plasma membrane, resulting in the simultaneous bead-like deposition of primexine on either side of the 'white line' (see Fig. 4C). However, the projections of the periplasmodial membrane arise well *after* the development of those from the microspore plasma membrane, following degradation of the callose wall, and thus are not involved in the establishment of the initial pattern. The question now becomes: what brings about the invagination of the plasma membrane and why are the invaginations of such a consistent size and shape? Current studies by the author utilizing three-dimensional reconstruction of TEM serial cross-sections may help to elucidate this aspect of exine pattern formation.

Exine deposition in *Xiphidium* is quite different from that found in tectate-columellate monocotyledons. *Xiphidium* lacks the development of a

cellulosic fibrillar thickening (primexine) layer and the subsequent formation of radially oriented probacular vesicles, which occur, for example, in the tectate-columellate *Lilium* (Dickinson, 1970) and *Typha* (Takahashi and Sohma, 1984). In contrast, early exine deposition in *Xiphidium* is strikingly similar to that occurring in *Heliconia* of the Zingiberales (see Stone *et al.*, 1979), in which sporopollenin is deposited on one to several extruded 'white lines'. In fact, in the mature pollen grain of *Heliconia*, the entire non-apertural wall and the bacula of the apertural wall are typically divided into an inner and outer layer by virtue of one or more persistent 'white lines', identical to the 'commissure' in the exine of *Xiphidium*. Thus, the two-layered nature of the exine in most Haemodoraceae may be structurally homologous to that found in at least one member of the Zingiberiflorae. This is of systematic significance in assessing familial relationships. For example, the similar pollen wall development in the Haemodoraceae and Zingiberales, if homologous, would provide a synapomorphy supporting the close relationship of the two taxa proposed, for example, by Walker (1990). In contrast, Dahlgren and Rasmussen (1983) proposed a classification of the Bromeliiflorae in which the Haemodoraceae (and Pontederiaceae) are most closely related to the Typhales (consisting of the Typhaceae and Sparganiaceae). Because *Typha* has a *Lilium*-like exine wall development (Takahashi and Sohma, 1984), acceptance of this latter scheme would necessitate hypothesizing the independent and convergent evolution of the common developmental pattern seen in *Xiphidium* and the Zingiberales. Further work is needed to affirm this, as pollen wall developmental studies are particularly scanty in this complex.

It may be of significance that exine development in *Xiphidium* most closely resembles the initial deposition of nexine 1 in *Lilium*, in which tangentially oriented 'white line' extracellular membranes are secreted and serve as the site of sporopollenin secretion. Based on this similarity in development, it seems probable that exine deposition in investigated members of the Haemodoraceae (*Xiphidium*) and Heliconiaceae (*Heliconia*) is homologous to nexine 1 deposition in the Liliaceae (*Lilium*). Because the tectate–columellate exine structure is considered primitive among monocotyledons (e.g. Zavada, 1983) and because the Liliales (*sensu stricto*) is deemed by most phylogeneticists to hold a very basal position in monocot evolution, it seems likely (by outgroup comparison) that the non-tectate–columellate exine structure in the Haemodoraceae (Simpson, 1983), Pontederiaceae (Simpson, 1987), and Zingiberales (Kress, Stone and Sellers, 1978) is derived from a more ancestral tectate–columellate condition. Thus, the non-tectate–columellate condition in these taxa may have evolved by loss of the early developmental stage of exine development. The question then arises: do the observed similarities reflect convergence or common ancestry? Studies of pollen wall development in the taxa currently presumed most closely related to the Haemodoraceae are vital to the investigation of this intriguing possibility. It would be quite interesting to compare exine development in other members of the Bromeliiflorae (*sensu* Dahlgren and Rasmussen, 1983). A study of pollen wall development in the family Pontederiaceae, the presumed sister group of the Haemodoraceae (Simpson, 1990) is in progress by the author and will be most informative in assessing whether the similar non-tectate–columellate structure in these two taxa has a common (and presumably homologous) developmental basis. In addition, the superorder Bromeliiflorae contains three taxa (Typhales, Bromeliaceae, and Velloziaceae) all of which possess a tectate–columellate exine structure (Ayensu and Skvarla, 1974; Nilsson, Praglowski, and Nilsson, 1977; Brighigna, Fiordi and Palandri, 1981; Zavada, 1983; Simpson, unpublished). Knowledge of patterns of wall development may be very useful in assessing wall homologies and in clarifying the phylogenetic relationships among these taxa.

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