Pollen Wall Development

The succession of events in the growth of intricately patterned pollen walls is described and discussed.

J. Heslop-Harrison

When the angiosperm pollen grain is shed from the anther, it consists of a haploid plant, the male gametophyte, which is enclosed in the wall of the parent spore. The role of the male gametophyte is to deliver two gametes to an ovule of another flower, where they will perform two fertilizations, one to give the zygote, and the other to produce the first nucleus of the endosperm. The wall of the pollen grain fulfills an important protective function during the hazardous journey between the anther and the stigma, a function for which it is singularly well adapted, for it reveals remarkable strength and chemical inertness. The principal wall material has been named sporopollenin by Zetzsche (*1*). Although sporopollenin does undergo slow autoxidation, it must certainly rank as the most resistant wall polymer produced by plants.

Because of the immunity of the walls of spores and pollen grains to most physicochemical degrading agents, they tend to be preserved in fossil and subfossil plant deposits when most other traces of biological structure have disappeared, and even to be concentrated as other components are lost. Thus it has been recorded that pollen grains and spores may be present to the extent of 4,000,000 per gram in naturally oxidized North Dakota lignite (*2*). Persistence in fossil deposits depends also on resistance to biological attack. Goldstein has reviewed the effects of fungal attack on modern spore and pollen walls, and Elsik has shown that similar predation took place in Cretaceous and early Tertiary times (*2*), but it is evident that even the avid phycomycetes make little headway in dealing with sporopollenin.

The author is professor of botany at the Institute of Plant Development of the University of Wisconsin, Madison.

Another striking feature of the pollen-grain wall is that in very many species it is elaborately sculptured and patterned. This patterning is so precise, distinctive, and consistent in its major features that it has formed the basis for a pollen taxonomy (*3*). This in turn has led to the growth of palynology, a science concerned with the utilization of pollen-coat characteristics for the identification of the plant species that contribute to coals, lignites, peats, and even the spore load of honey.

From the viewpoint of a biologist concerned with development and differentiation, the intriguing question is how such a remarkable structure as the pollen wall is produced. Generated around a single cell, accurately reflecting the genome, copied so precisely in such staggering numbers in each anther, and with detail executed in so refractory a material as sporopollenin, the patterning of pollen represents the very apotheosis of biological morphogenesis. It is the purpose of this article to report on some of the progress made in recent years in studying the development of pollen walls and to discuss its relevance to broader problems of pattern development in plants.

Pollen Wall Stratigraphy

The pollen wall has attracted to itself an intimidating terminology, as is perhaps inevitable in any biological field where the precise diagnosis of complex form is a desideratum. Erdtman, one of the leaders in the study of pollen taxonomy, has pointed out that terminologies may vary according to the purposes for which they are designed; thus morphological and morphogenetical classifications of spore wall components may lay stress on different aspects, each with entire justification. Fortunately Erdtman himself (*3*) has provided a basic terminology satisfactory for the purposes of this paper, and we will adhere to it, pleading the justification of space restriction for ignoring synonymy.

The exine of lily pollen seen in surface view in the Stereoscan electron micrograph on the cover provides in itself a conspectus of all the principal features of exine stratigraphy. The transmission electron micrograph of Fig. 1 of the sectioned pollen wall shows the strata. The electron-opaque outer layers comprise the exine; the inner fibrillar region is the intine. The exine consists of an outer, sculptured portion, the sexine, and an inner layer, the nexine. The sculpturing of the sexine is in the form of radially directed rods, the bacula, with enlarged heads; the heads of the bacula are linked above the level of the exine to form walls or muri, which produce the conspicuous reticulate pattern of the micrograph on the cover from which the remaining architectural principles may be readily deduced. The nexine is subdivided into an outer nexine 1 and an inner nexine 2, separated by a discontinuity seen as an electron-transparent line. The enclosures between the muri of the exine carry an amorphous or vaguely fibrillar material, seen in section in Fig. 1. This has been removed by the acetolysis treatment used in the preparation of the spore shown on the cover photo, but remains in the surface view seen in Fig. 11. In the fresh pollen this material is impregnated with oily, pigmented substances that contribute the characteristic color, stickiness, and odor of the lily pollen grain.

On the face opposite to that seen in the micrograph on the cover, the lily pollen grain is furrowed. The furrow or colpus is covered only by the nexine, and is the site of emergence of the pollen tube on germination.

The architecture of the lily exine provides a basis for understanding the structure of most other pollen walls. The heads of the bacula, instead of being linked, may be free, giving the so-called pilate exine. Alternatively, the heads may be connected in two dimensions, producing a completely or partially roofed condition, the tegillate exine. In this there is the inner nexine, the zone of voids traversed by the bacula, and the roof or tegillum; above this again may rise rods or spines in various more or less elaborate configurations.

Instead of the single colpus of the lily grain, there may be several; or the surface of the spore may be pocked with one or more circular or ellipsoidal apertures, covered only with a single exine layer corresponding in derivation to the nexine.

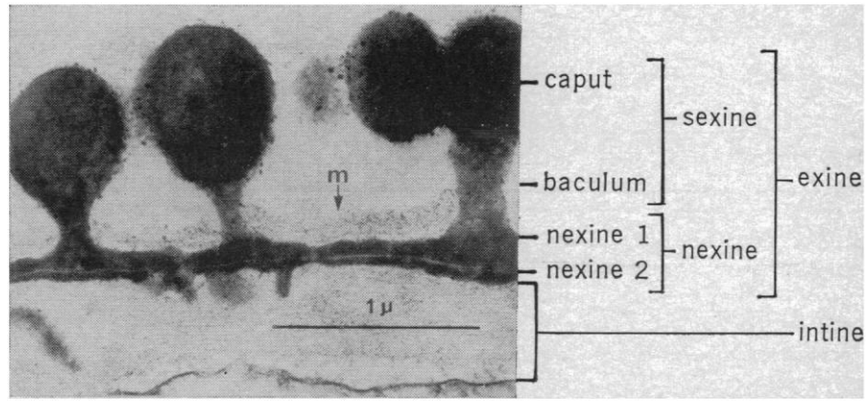


Fig. 1. Section of the walls of an untreated *Lilium* pollen grain showing the stratification, named according to Erdtman's terminology. The fibrillar matrix material, *m*, is removed by acetolysis, and does not appear in the scanning electron micrograph of the cover.

The Wall Materials

Three principal compounds are concerned during different periods of microspore wall formation: the polysaccharides—cellulose and callose—and the material of the exine already mentioned—sporopollenin. Cellulose, a β-1,4-linked glucan, is of course one of the major cell-wall constituents of green plants. Characteristically it occurs in plant cell walls in microfibrillar form, the microfibrils being 50 to 300 angstroms in width and of indefinite length (*4*). The finely fibrillar structure is often readily observed in electron micrographs of sectioned cell walls, particularly when the microfibrils are disposed in oriented arrays. Callose was first described by Mangin from the sporocyte wall (*5*); it is a β-1,3-linked glucan, but unlike cellulose it shows no microfibrillar organization (*6*). One of the special functional characteristics of callose as a wall material is the rapidity with which it can be both synthesized and destroyed. Accordingly, it commonly finds a role as a sealing or plugging agent, appearing, for example, as a sealant for wall pits after the severance of plasmodesmata due to injury (*7*).

Sporopollenin, with its extraordinary resistance to degradation, has presented a difficult subject for chemical investigation. The early work of Zetzsche and his collaborators revealed a reasonable community in the composition of sporopollenins from a variety of sources. Among flowering plants, for instance, the empirical formulas ranged from $C_{90}H_{134}O_{31}$ for the pollen of rye to $C_{90}H_{150}O_{33}$ for that of the date palm. Much of Zetzsche's analytical work was upon the spore coats of *Lycopodium clavatum*, a club moss the spores of which form the "lycopodium powder" of pharmacognosy. His conclusion was that sporopollenin was likely to be polyterpenoid in nature, but this has been shown to be improbable by the recent work of Shaw and Yeadon (*8*). These authors find that the spore walls of lycopodium and pine consist of 10 to 15 percent cellulose, an ill-defined xylan or hemicellulose fraction accounting for some 10 percent, a lignin-like fraction of 10 to 15 percent, and a lipid fraction of 55 to 65 percent. The polysaccharide component represents the inner spore wall, the intine. The "sporopollenin" of Zetzsche is resolved into a lipid and a lignin-like fraction. The lipid fraction produces as its most characteristic breakdown products simple mono- and dicarboxylic acids with an apparent maximum of 16 carbon atoms. Since spore walls do not respond to many of the usual microchemical tests for lignins, it is presumed that the lipid fraction acts in some way to protect the lignin moiety from chemical attack. It is noteworthy in this connection that the exine may respond more positively to lignin tests in early developmental stages, which suggests that as it matures lignin reactivity is masked by the accretion of other components (*9*).

Spore Development

The male gametophyte, transported in the pollen grain, is produced by division of the spore (strictly, microspore) protoplast, but this division occurs within the original spore wall, and is not accompanied by any change in the outer coats. The spores are produced in the anther, a compound sporangium, following upon the meiotic divisions in the mother cells or sporocytes. In the anther loculi, the sporocytes are surrounded by a tissue of secretory character, the tapetum. During meiotic prophase, the primary sporocyte walls are replaced by thick investments of callose, but massive protoplasmic strands develop between the cells so that the whole tissue in each anther loculus forms a syncytium. The sporocyte nuclei thus come to share a common cytoplasmic matrix, and this has important consequences, since it imposes the synchroneity of nuclear behavior that is so much a feature of meiosis in the angiosperm anther. At the conclusion of the meiotic divisions the haploid spores round up within the tetrad, each wholly enclosed by the callose wall and without plasmodesmatal community with sibs or parent (Fig. 2). The earlier period of nuclear interdependence in the sporogenous tissue is thus followed by one of nuclear independence, and this in turn has its significance (*10*). In consequence of the gene segregation and recombination occurring during meiosis, each daughter haploid nucleus will usually be genetically different, and some of the genetical diversity is shortly to be expressed in the haploid gametophyte generation—in some species, for example, in the synthesis of incompatibility substances. It is obviously essential for the assertion of this genetical autonomy that each spore nucleus should control its own volume of cytoplasm, and this is assured by the partitioning of the mother cell cytoplasm between the four spores and the isolation of each moiety by the callose wall. We will see later that the cytoplasm bequeathed by the mother cell undergoes a form of "clean up" before this partitioning process, related presumably to the elimination of long-lived messengers synthesized by the diploid parent nucleus or its precursors. The callose tetrad wall, at least in conjunction with the plasmalemma of the spore, is undoubtedly capable of ex-

cluding polymers, and appears also to be effective in restraining the entry of certain species of micromolecules. It acts therefore as a molecular filter in much the same manner as the membranes of the mammalian placenta (*11*).

Very rapidly after the cleavage of the mother cell cytoplasm, the formation of new walls begins in the individual spores. Progressively the patterning to be seen in the mature exine is developed in one of the most remarkable sequences of transformations known in plant morphogenesis. The events are summarized diagrammatically in Fig. 4. We will follow them now in further detail, and then examine the problem of their control.

The Tetrad Period

The process begins by the deposition of a new wall around each spore protoplast. This wall, the primexine, consists of a microfibrillar matrix penetrated by radially directed rods of material of different electron density (Fig. 5). The matrix material appears to be cellulose (*9*); it forms a continuous sheath around the spore except over those areas destined to become pores or furrows in the mature spore. In this way, right from the beginning of primexine growth, one distinctive feature of the exine pattern is foreshadowed. The question of what determines this conspicuous aspect of primexine pattern, the distribution of apertures, is obviously a very pertinent one. A clue comes from the observation of the cytoplasm immediately below the aperture region. In this zone it is often possible to discern a plate of endoplasmic reticulum applied closely to the plasmalemma, giving the striking triple-membrane configuration seen in Fig. 5. Since we see here a cytoplasmic component apparently concerned in the control of an aspect of wall morphogenesis, it is tempting to speculate how it may exert an effect. I have suggested that the apposition of the plate of endoplasmic reticulum to the plasmalemma prevents the local deposition of cellulose (*12*), and obviously this could be, simply by blocking the access of dictyosome vesicles carrying cellulose precursors and excluding microtubules concerned with microfibril orientation. However, the association of endoplasmic reticulum and plasmalemma, although invariably observed in such species as *Silene pendula*, is not necessarily so consistent in other plants, so it is premature to generalize.

Even should the disposition of the endoplasmic reticulum be a determinant of aperture location, the question of what in turn establishes the position of the endoplasmic reticulum obtrudes. The evidence concerning genetic control is discussed below. Here we may note that some part is played also by the spore environment, as shown by Wodehouse (*3*). In the lily tetrad, the colpus or furrow of the spores develops always toward the outside in tetrads where the spores are arranged in the common two-by-two position (Fig. 3). Clearly, orientation within the tetrad is the determinant here. However, where the spores are arranged in linear sequence, as occurs occasionally, the colpus is disposed randomly on one side or the other. What is innately determined is the production of a colpus. If the spore environment gives a guide, it is located appropriately. If there is no external guidance, random factors presumably determine position —a not unfamiliar situation in biological morphogenesis. It should be noted that it remains to be established positively for lily that the endoplasmic reticulum is consistently concerned in defining the location of the colpus.

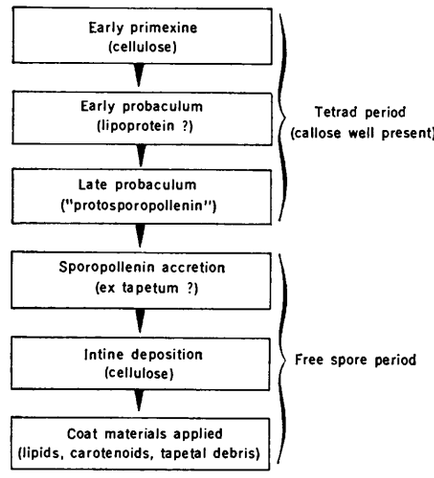
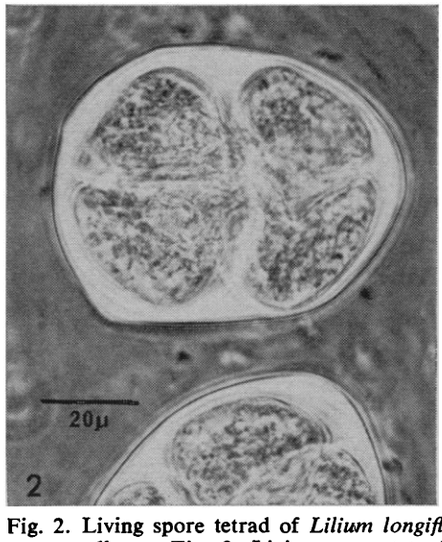


Fig. 4. Summary of events in pollen wall formation.

The other characteristic feature of the primexine is the array of radially directed rods traversing it from the earliest period of growth. These rods, the probacula, are the second major manifestation of pattern-determining processes, since their distribution over the surface of the spore and the interconnections which they form above and below the matrix material of the primexine are determinants of the principal structural features of the mature exine (*12*). Again there is a challenge to establish what forces operative within the spore protoplast locate the probacula and so translate genetic information into structural pattern. With regard to the role of external determinants, the same can be said of probacular disposition as can be said of the apertures: there are cases where orientation within the tetrad clearly plays some part, as it must in the lily spores of Fig. 3, but the intimate details of distribution that act to map out the final exine pattern (see cover) reflect the working out of a genetically determined program.

Just as the location of the apertures



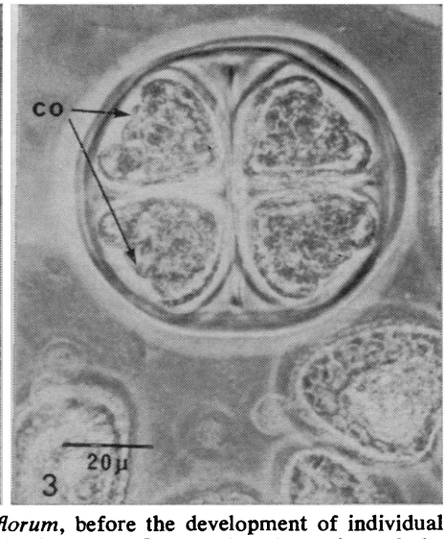


Fig. 2. Living spore tetrad of *Lilium longiflorum*, before the development of individual spore walls. Fig. 3. Living spore tetrad of *L. longiflorum* after formation of the primexines. The outwardly directed colpus regions (*co*) can be distinguished. A newly released spore lies toward the bottom right, revealing wall sculpturing.

may be related to prior disposition of endoplasmic reticulum, so a correlation has been observed between the position of the probacula and the distribution of structures within the peripheral region of the spore protoplast. In the earliest observations (*12, 13*) evidence was obtained of an association with elements of the endoplasmic reticulum, and Skvarla and Larson have published striking illustrations of membranous tubules running upward toward the bases of the probacula in the young spores of corn. Later, this relation is lost in corn, but in various caryophyllaceous species it may persist until the spores are released from the tetrads.

Not all investigations of primexine growth have revealed an association between the probacula and cytoplasmic components, and indeed such a relationship has been denied (*14*). It is perhaps significant that most of the clearest demonstrations of membrane profiles in the vicinity of the probacula have been with material fixed for electron microscopy with permanganate, and it is entirely possible that the permanganate image is of a derived structure. Nevertheless, this explanation merely raises the question of what it is derived from. Spores fixed with aldehydes before osmication commonly show vesicular components rather than tubules at the feet of the probacula: these could originate from dictyosomes, and be concerned with the delivery of material to the growing rods. If so, they might be expected to be in active movement in life, and the problem then concerns the nature of the forces that guide them to particular loci.

It has become clear (*15*) that pattern determination in the primexine takes place at a very early period indeed, since the critical events occur almost as soon as deposition of the fibrillar matrix begins. At this time, what appear to be ribosomes accumulate in aggregates beneath particular areas of plasmalemma, sometimes in polysome-like configurations (Fig. 6). From the plasmalemma above these areas lamellae arise, forming the cylindrical body of the probaculum. From the appearance in section, various interpretations of the probaculum structure at this period are possible. It could be formed of several pleated plates, of concentric cylinders, or of a single spirally disposed or pleated ribbon. Electron opacity and response to stains suggests that the lamellae may be lipoprotein. The ribosome association is characteristic of very early pattern formation, and later it cannot be discerned.

Spores removed mechanically from the tetrad in the early patterned period show evidence of the primexine even by optical microscopy. When the walls of such released spores are exposed to cellulase digestion, the patterning disappears, suggesting that removal of the matrix material permits the dispersal also of the probacula. This would be expected had the latter little or no connection among themselves at this time, even were they themselves resistant to cellulase attack (*9*). Two changes quickly ensue. New materials are injected into the probacula, and connections develop between neighboring heads above the matrix material, leading to the aspect seen in Fig. 7. The chemical change accounts for the increase in electron-density following osmication apparent in this figure. In conjunction with the development of connections outside of the primexine, it also gives a new identity to the patterned component, which is now no longer dispersed by cellulase digestion. Indeed, during this period of consolidation, a material with some of the properties of sporopollenin becomes associated with the patterned element, and this makes it possible to follow the further development of this component as an independent structure, since it acquires resistance to acetolysis. The scanning electron micrograph of Fig. 8 is of the patterned part of the primexine isolated by acetolysis at the time when the heads of the probacula have become linked to form a reticulum. At this period no coherent acetolysis-resistant

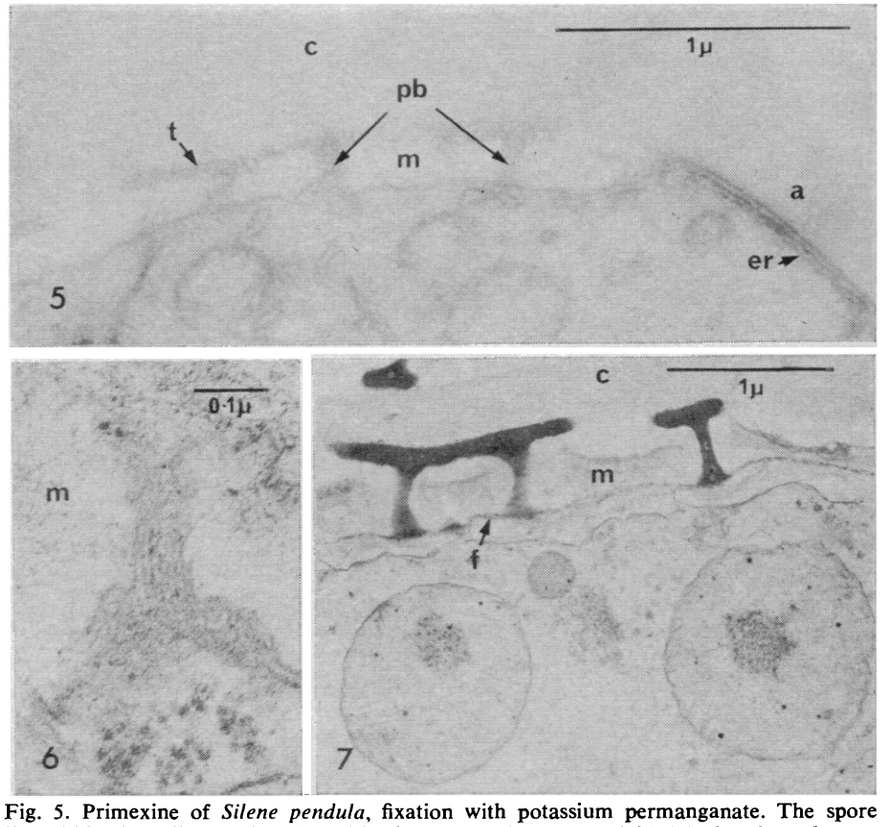
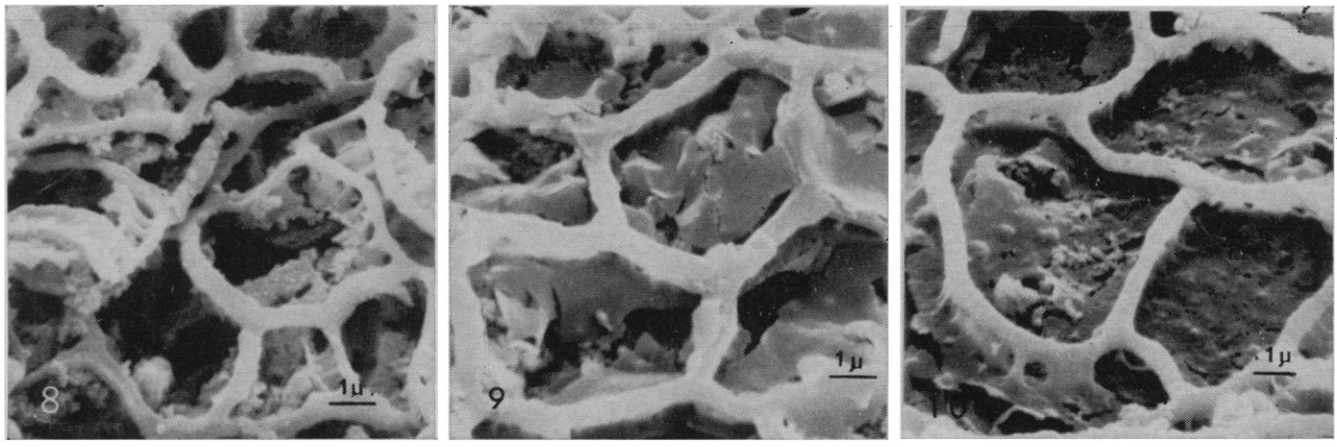


Fig. 5. Primexine of *Silene pendula*, fixation with potassium permanganate. The spore lies within the callose wall (*c*), and in the pore region at the right (*a*) the plasmalemma is in direct contact with the callose, with a plate of endoplasmic reticulum (*er*) below. The matrix material of the primexine (*m*) appears electron transparent with this fixation, and the probacula (*pb*) granular. The roof layer or tegillum is beginning to form (*t*). Fig. 6. Very early probaculum of *Lilium longiflorum*. Located below the probaculum are several ribosome-like bodies. The trunk of the probaculum rises from the plasmalemma, and is seen in this section to comprise 7–8 lamellae. The matrix material (*m*) reveals a vaguely fibrillar texture with this glutaraldehyde-OsO_4 fixation. Fig. 7. Older primexine of *L. longiflorum*, same fixation. The probacula are now consolidated and electron opaque, with linking muri above. In between, the matrix material (*m*) has withdrawn somewhat from the callose tetrad wall (*c*) at this stage. A foot layer —future nexine 1—is forming (*f*). The gap containing fibrillar or lamellar material between it and the plasmalemma may be an artifact, since fixation is difficult at this time, probably due to the limited permeability of the tetrad wall. The stage corresponds to that seen in the scanning electron micrograph of Fig. 8.



Figs. 8–10. Scanning electron micrographs of primexines of *Lilium longiflorum* isolated from tetrads by acetolysis. Fig. 8. Early stage of consolidation of the muri, corresponding to that seen in section in Fig. 7. Fig. 9. Intermediate stage, showing the development of a resistant foot layer between the probacula. Fig. 10. Still later stage showing continuous foot layer. The probacula can be seen at the bottom left. At this time the muri have the rounded appearance apparent in the mature exine (compare cover photo).

layer connects the feet of the probacula, but this soon develops between the plasmalemma and the matrix material, to give the tenuous foot layer seen in Fig. 9. This consolidates further, producing the nexine 1 of Fig. 1. The appearance is then as seen in Fig. 10, which is of a spore wall isolated by acetolysis from a tetrad like that of Fig. 3. The sequence, Figs. 8 to 11, shows how the muri gradually acquire the rounded form apparent in the mature exine by growth while still in the tetrad.

The Free Spore Period

Release from the tetrad follows through the rapid dissolution of the callose wall. This dissolution is mediated by a callase, which has been shown by Eschrich to be phase specific, appearing only for a limited period in the history of the anther. The early breakdown products of the callose wall are 1,3-linked oligosaccharides such as laminaribiose and laminaritriose, and these also have but a transient existence in the anther fluid before further breakdown to glucose (*16*).

The "protosporopollenin" of the late primexine is resistant to acetolysis, but it is nevertheless more reactive chemically than the sporopollenin of the mature exine. It is probably significant for understanding the biosynthesis of sporopollenin that after release from the tetrads the spore walls pass through yet other phases of reactivity. Transiently, the exine responds to some, but not all, of the common tests for lignin, and reveals the presence of free aldehyde groups. This may indicate the accretion of the lignin fraction detected by Shaw and Yeadon. Thereafter reactivity is progressively lost, and the strikingly inert condition characteristic of the mature exine is reached.

The early period following release of the spores from the tetrads is marked by rapid growth, and in lily the spores speedily expand to about 2.8 times their volume within the tetrad. This expansion is evidently osmotically driven, and it gives some indication of the enhanced suction pressure produced by the removal of the restraint of the callose wall. Nutrients present in the anther fluid are swept into the spores during this growth, showing that the spore walls themselves are not effective as barriers to small molecules at this time (*11*).

The growth in volume involves a substantial increase in surface area, and this must mean a considerable thinning out of the material present in the primexine. Yet this is not accompanied by any very conspicuous contraction of the bacula, foot layer, or muri, so it must be assumed that there is a compensating accretion of new material. Banerjee, Rowley, and Alessio have investigated this period of exine development in detail in *Sparganium*, and here also growth is not accompanied by a thinning out of the exine, suggesting rapid addition of sporopollenin (*17*).

During the early phase of expansion in lily, the matrix material of the primexine is shredded and thinned out, so that only a loosely fibrillar residue remains. This persists between the muri, visible both in scanning (Fig. 11) and transmission (Fig. 1) electron micrographs. It can be shown by the use of solvents that this residue is principally cellulose, and its continued presence may be significant during the final phase of pollen maturation, when special surface materials are added. In exines where a continuous roof layer or tegillum is formed, the remains of the primexine persist in the cavities.

The Role of Tapetum

Since the earliest observations by Goebel and other developmental anatomists of the 19th century, it has been accepted that the tissue surrounding the sporogenous cells, the tapetum, is concerned with spore nutrition. Goebel recognized two types of tapetum, one secretory, and the other amoeboid or invasive. The secretory tapetum maintains a parietal position throughout sporogenesis until it loses its integrity as a tissue, while the cells of the invasive type grow into the spore mass and achieve intimate contact, particularly during the last period of spore maturation. Although intermediate types do occur, we shall see that the distinction is possibly an important one for understanding some of the characteristics of pollen walls.

A distinctive feature of the tapetum is that during the tetrad period its cells often acquire particles of sporopollenin-like material on the inner, locular faces. These particles or plaques are formed at the surface of the protoplast, and project into the gelatinized inner wall of the tapetal cells. They have sometimes been referred to as "Ubisch" bodies, but the name is inappropriate,

since they were first described and illustrated many years before the work of Ubisch (*18*). The tapetal plaques show all the features of sporopollenin in respect to staining properties and resistance to acetolysis, but they frequently appear to precede the spore exines during development in the acquisition of various characteristic qualities. Their presence on the tapetum clearly indicates that this tissue has the capacity to synthesize sporopollenin, and the precocious acquisition of this capacity has led to the suggestion that the principal synthesis is in fact located in the tapetum (*19*). According to this view, precursors of the exine material itself are transferred to the young grains in tapetal secretions, polymerization occurring on the surface of the young spores. A transfer of this kind could only take place after release of the spores from the tetrads, if the callose wall is indeed as impermeable as it appears to be. Nevertheless, tapetal contributions could be important during the phase of exine growth following tetrad breakup, contributing to the conversion of protosporopollenin into sporopollenin proper. Rowley in particular has urged this very plausible interpretation of events, and has pointed out that at least in those species with the invasive type of tapetum cytoplasmic strands could provide a route for substrate migration (*20*). Rowley has also shown that the electron-microscopic appearance of sporopollenin changes progressively during exine development, the early granular-lamellar aspect giving place to a more homogeneous one as the growth progresses. This translation is no doubt correlated with the change in reactivity already mentioned.

If the function of the tapetum is merely to secrete sporopollenin precursors, the role of the sporopollenin plaques studding its surface remains obscure. They could be no more than by-products, since they are not resorbed, and remain as a lining to the anther cavities even after the pollen has been dispersed. Yet it is premature to dismiss them as functionless. Carniel has pointed out the very curious fact that in the gymnosperm *Gnetum gnemon*, a plant with many angiosperm-like characteristics, the tapetal plaques are formed inside the tapetal cells, on the wall away from the anther loculus (*21*). They are thus not on the presumed route of migration of sporopollenin precursors to the developing spores, and are not bathed with locular fluid which might be expected to be charged with precursors. After the dissolution of the tapetal cells they remain as a lining to the loculi, just as in angiosperms. If there is a function, it may possibly be discharged in this final period, perhaps in connection with pollen dispersal.

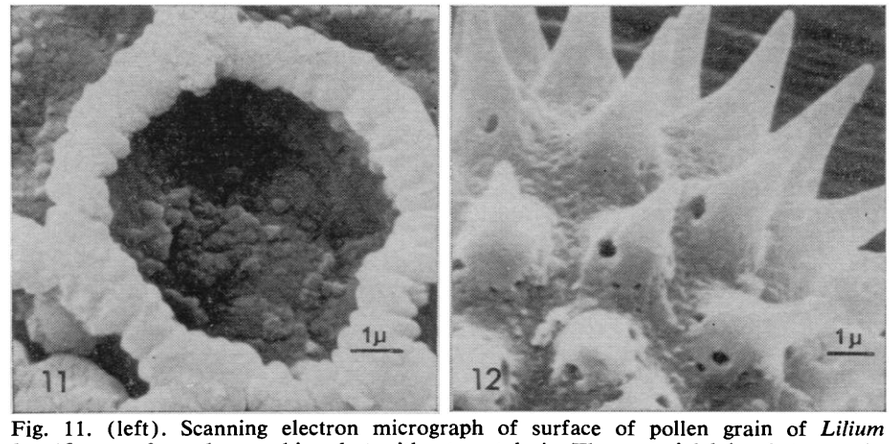


Fig. 11. (left). Scanning electron micrograph of surface of pollen grain of *Lilium longiflorum* after ether washing, but without acetolysis. The material lying between the muri is the matrix material seen in section in Fig. 1. Fig. 12. Scanning electron micrograph of the surface of a pollen grain of *Cosmos bipinnatus*. The spines rise from the tegillum or roof layer formed on the upper surface of the primexine. Their development mainly occurs after the release of the spores from the tetrad, and is associated with the activity of an invasive tapetum. Note that the spines have basal pores.

How Is the Exine Pattern Determined?

Since the events leading to exine pattern determination occur within the tetrad, and since the spores during the critical period are effectively isolated, the readiest conclusion is that the whole process is under the control of the haploid genome, in interaction only with the microenvironment within the tetrad, which presumably can be resolved into a system of constrictive wall forces and perhaps diffusion gradients of micromolecules. I expressed this view some years ago, when influenced by the revelation of some of the basic features of the mechanics of spore wall development within the closed tetrad system (*12*). Its implications are that the haploid spore nucleus is active very soon after meiotic segregation, and that it assumes total control of the cytoplasmic moiety bequeathed to the spore from the diploid mother cell.

The matter merits reconsideration now, in the light of increasing understanding of events accompanying sporogenesis. A striking feature of the meiotic prophase is that the ribosome population of the mother cell is radically modified, and this has been interpreted as indicating an elimination of long-lived messengers and other residual marks of the diploid sporophyte generation in preparation for the institution of the new gametophytic generation (*22*). New protein synthetic machinery is not set up until after the meiotic divisions, when, presumably, each haploid is provided with a new slate of ribosomes on which to inscribe whatever program may be called for in the early life of the gametophyte. Critical to our understanding of the control of wall patterning is the matter of timing. Which comes first, the initiation of the pattern-determining process, or the activation of the haploid spore nucleus, and the expression of this activation in the synthesis of new proteins?

Some of the evidence for lily has been reviewed above. Primexine formation begins very soon after the cleavage following meiosis II; a normal ribosome population is not yet fully restored in the spores at this time, but it is significant that what look like polyribosomes do become associated with the burgeoning probacula (Fig. 6). It is indeed as if the very earliest preoccupation of the spore cytoplasm were to establish a protein synthetic system concerned with this aspect of wall growth. Other preoccupations follow (*23*), and later it would be difficult indeed to say what part of the spore resources are in fact concerned with wall development. But the important point is that the critical pattern-determining event occurs in this very early period of independent life, and already the electron-microscopically visible appurtenances of protein synthesis are associated with it.

Genetic evidence concerning the control of pollen wall characteristics has been discussed by Godwin (*24*), who points out that segregation of different exine types in one and the same tetrad has never been recorded. Evidence of segregation of some kind would certainly be expected if exine features were exclusively under control of the spore nucleus. Furthermore, pollen wall differences associated with heterostyly behave as though they were determined exclusively by the diploid parental genome, since all spores in an anther share the same exine characters. Evidence of this kind points to control by the sporophyte rather than by the gametophyte of at least some spore wall parental tissues could determine such features. A means is known whereby properties as pollen color late in the course of development, and this is discussed further below. But in heterostyly, details of structural pattern in the exine behave as though they were programmed by the sporophyte (*25*), and presumably these features would be determined very early, within the tetrad.

How, then, might the sporophyte control spore wall pattern? Two possibilities present themselves. Either the pattern results from an interaction between the developing spores and neighboring parental tissues—specifically, the tapetum; or its development represents the working out of a program bequeathed from the diploid mother cell and carried in the cytoplasm through meiosis. The question of how far parental control is exerted directly during wall development was the center of an old controversy (*26*). The ultrastructural evidence is certainly good enough now to assure us that the living spore protoplast is intimately involved in the initial establishment of wall pattern, and that whatever may be the tapetal role in the later stages of pollen growth, it cannot be a factor intervening in any direct manner during the critical tetrad period. This leaves the possibility that parental control may depend upon the passage of determinants from the mother cell. The reorganization of the cytoplasmic ribosomes mentioned above occurs during the meiotic prophase, and it seems that, concurrently, far-reaching changes overtake the organelles and membranous elements of the cytoplasm. A class of structure not known in vegetative cells which we have named a multi-membraned body forms. This appears as a sphere bounded by one or more concentric paired membranes. The membranes enclose enclaves of cytoplasm, since characteristic cytoplasmic structures like mitochondria and spherosomes may appear within. An interesting feature is that from the external cytoplasm inward the concentric shells reveal progressively denser contents, presumably a reflection of increasing protein concentration. These bodies reach a maximum of numbers in the late meiotic prophase, when they are very abundant in the cytoplasm, and they are dispersed during the tetrad period. They could thus be the carriers of selected fractions of the maternal cell cytoplasm protected from the prophase reorganization, and their role could be to supply the spores with enzymes and perhaps structural proteins required during the early life of the gametophytes. A mechanism may thus exist for bequeathing short-term morphogenetic information from diploid sporocyte to daughter spore, and it could be in this manner that parental control over spore wall pattern is exerted.

The question of sporophytic or gametophytic control remains, therefore, unresolved. The balance of genetical evidence appears to favor sporophytic control, and it is possible to postulate how this might be managed. However, there is no evidence that firmly excludes the participation of the spore nucleus in pattern determination.

Nexine 2, Intine, and the Pollen Coat Materials

Two further walls are produced within the spore before its final maturation. One of these is the inner layer of the nexine, the nexine 2, which in some species begins growth before release of the spores from the tetrad. Like the remainder of the exine, it is composed of a material of the general class of sporopollenin, but its staining properties are often somewhat different from those of the sexine and nexine 1. There is no reasonable doubt that the material of the nexine 2 originates within the spore itself, and there is abundant evidence that it accumulates through the apposition of lamellae, not unlike those seen in the early probacular stage. Characteristically these lamellae seen in section reveal a central electron-transparent plate, variously estimated as 40 to 50 angstroms in thickness, on the surface of which the sporopollenin is deposited. The process is envisaged by Rowley as involving the participation of structures of unit-membrane dimensions, generated near the plasmalemma, which progressively accumulate a sporopollenin coat until they are incorporated in the nexine 2, or into its equivalent where the full stratification is absent. This process does not involve the development of any novel pattern, but it may be localized to particular areas of the existing pattern—for example, to reinforce the annular collar around the apertures of the grass pollen grain (*27*).

The intine, the last of the enveloping walls to appear within the spore, is cellulosic (*28*), and its formation does not appear to involve any principles not already known for primary wall formation in somatic cells. The microfibrillar organization is readily discernible in electron micrographs, and as is now well established in the growth of such walls precursor material is supplied by dictyosomes in the outer region of the cytoplasm. Vesicles of the "coated" type are apposed to the plasmalemma during intine growth, and microtubules also occur in periclinal orientation just within the plasmalemma. The microtubules are, however, rather thinly dispersed, and they show no preferred orientation.

In the final period of wall maturation, additional materials are applied to the outside of the exine. These coating substances probably discharge several functions, and their significance varies widely between species. In lily, the material is adhesive and serves to bind the pollen grains together in masses at the time of dispersal; it has therefore the characteristics of the *Pollenkitt* of Troll (*29*). The lily *Pollenkitt* contains a lipid component, and carotenoid pigments responsible for the characteristic yellow, orange or brown colors of the pollen in this genus. The coloration may be concerned either with the protection of the gametophyte from ultraviolet radiation during its period of exposure, or with attracting insects (*30*). Certainly the latter role is significant where the coating materials bear odorous essential oils.

The coat materials are synthesized in the tapetum, and are transferred, seemingly quite passively, at the time of the final dissolution of this tissue. They collect in the spaces between the muri of the exine, since the oleaginous component has a strong affinity for the residual material present in these spaces (Fig. 11) while being repelled by the exine proper.

Where the tapetum is invasive, the

coat materials may be applied directly by the tapetal plasmodium. Pankow, who studied their origin in *Galanthus nivalis,* concluded that they were injected into the exine cavities, and were not therefore truly superficial (*29*). Obviously tapetal products other than oils and pigments could be transferred in this manner, and indeed there are indications that tapetal cytoplasm may penetrate into the exine in later growth stages (*31*). In *Cosmos bipinnatus,* a composite with an invasive tapetum, an intimate association persists between the tapetal plasmodium and the exine while the spinules which decorate the tegillum are being formed. These spinules have basal perforations (Fig. 12) communicating with the internal cavities of the exine, and as Godwin and Chambers showed for lime pollen, tapetal cytoplasm may be entrapped. This offers yet another means whereby sporophytically determined properties could be imposed on pollen in the same manner as color in the lily's case. It is possible, for example, that the pollen-born substances concerned in sporophytic incompatibility systems could be carried this way (*23*).

Concluding Remarks

It is the aim of investigations of pollen wall growth to elucidate the devices through which the intricate detail of different components is molded in faithful conformity with genetical instruction. There is no doubt that any evidence gained must contribute, in equal measure, to our understanding of plant growth and morphogenesis in general, since so many manifestations of differentiation and development in plants do concern the cell wall. The modes of growth of the wall determine cell shape, the orientation being often related to the disposition of previous division planes. Secondary changes in wall structure and chemistry fit cells for manifold special functions in conduction, protection and support. In somatic cells, all of these processes occur in tissues where cells are in constant multidimensional interaction. The special attraction of the spore system is that it is a closed one. In the microcosm of a single cell cut off from all others, processes are worked out that are surely analogous to those taking place in other differentiating systems, but without the complication of these cellular interactions. The developing spore has therefore many of the advantages as experimental material possessed by microorganisms, while manifesting a kind of differentiation peculiarly associated with eukaryotes. We may expect much more from its exploitation in coming years as it becomes more and more the target for attack by the methods that have proved so productive with microbiological systems (*32*).

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