Osborn, J.M. and Taylor, T.N. (1994). Comparative ultrastructure of fossil gymnosperm pollen and its phylogenetic implications. In: M.H. Kurmann and J.A. Doyle (editors). Ultrastructure of fossil spores and pollen, pp. 99–121, Royal Botanic Gardens, Kew.

7. COMPARATIVE ULTRASTRUCTURE OF FOSSIL GYMNOSPERM POLLEN AND ITS PHYLOGENETIC IMPLICATIONS

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Abstract

Numerous studies have been carried out on fossil gymnosperm pollen detailing its overall appearance in transmitted light and, to some extent, its surface morphology as revealed by scanning electron microscopy. Fewer investigations, however, have addressed the fine structural nature of fossil pollen using transmission electron microscopy. Pollen wall ultrastructure from grains preserved within intact reproductive organs (*in situ*) has been documented from gymnospermous taxa of the following orders: Lyginopteridales, Medullosales, Callistophytales, Cordaitales, Glossopteridales, Voltziales, Coniferales, Caytoniales, Corystospermales, Cycadales, Bennettitales, and Pentoxylales. Several dispersed (*sporae dispersae*) pollen types are also known from a few of these groups as well as the Gnetales. Moreover, a number of other taxonomically enigmatic palynomorphs (both *in situ* and dispersed taxa) have been described at the ultrastructural level, and are currently classified as *incertae sedis*. Pollen morphology and ultrastructure of these groups have played an important role in recent phylogenetic studies, which have been based on a number of reproductive and vegetative characters. Although many palynological characters are considered conservative evolutionary features, they can also reflect several other biological and physical phenomena. For example, features associated with pollen ontogeny as well as pollination syndromes may be important influences, while the most prominent abiotic factors to consider are mode of preservation, degree of diagenesis, and a suite of preparation protocols. These features are discussed as they relate to phylogenetic interpretations and relevance of several salient palynological characters, including overall exine infrastructure, nexine organization, and saccus type and internal composition.

Introduction

The use of both extant and fossil pollen as a systematic tool in evaluating phylogenetic relationships among seed plants is well documented and extends back to the early days of palynology (e.g., Wodehouse, 1928). Since that time, palynological characters have become increasingly important for phylogenetic analyses, with investigations of pollen becoming broader in both number and type. The majority of studies on fossil pollen has involved the analysis of grains by light microscopy (LM). However, micromorphological and ultrastuctural investigations employing scanning and transmission electron (SEM and TEM) reveal structural features of phylogenetic significance otherwise not available by LM.

Many excellent studies have been carried out on both extant and fossil gymnosperm pollen detailing its overall appearance in transmitted light (e.g., Van Konijnenburg-van Cittert, 1971) and, to some extent, its surface morphology as revealed by SEM (e.g., Krassilov, 1977). Fewer investigations, however, have addressed the fine structure of gymnosperm pollen using TEM. For extant groups, those TEM studies that have been undertaken have principally focused on describing exine stratification in mature pollen, although several have examined the various stages of sporoderm ontogeny. Moreover, only a small number of investigations, as well as taxa, have been conducted on pollen ultrastructure of extant gymnosperms (for review see Kurmann, 1992). With regard to fossil taxa, although twenty orders of extinct gymnosperms are recognized (Taylor and Taylor, 1993), and fossil members of the four extant orders are also known, data on pollen fine structure for some groups are either entirely lacking or come from the evaluation of a single taxon.

The existing studies of fossil gymnosperm pollen can be categorized into two distinct groups, based on the types of grains that are investigated. These include palynomorphs that are isolated from sediments in a dispersed condition (*sporae dispersae*), and those grains that are recovered from within intact, megafossil reproductive organs (*in situ*). Numerous contributions have examined *sporae dispersae* grains, which have been recovered by standard acid maceration techniques, and addressed the nature of such palynomorphs as seen in transmitted light. Stratigraphic palynology has been useful in illustrating palynomorph distribution, diversity, and occurrence throughout the geologic column (i.e., biostratigraphy). However, one of the most significant constraints in studying dispersed grains is the fact that in most cases nothing is known about their parent plants. It is possible in some instances to compare *sporae dispersae* taxa with *in situ* grains (e.g., Thomas, 1987; Willard, 1989), and to evaluate overall grain morphology, surface sculpture, and the nature of the aperture or haptotypic mark in order to infer systematic relationships (e.g., Ward et al., 1989). Despite the fact that more than 50 dispersed taxa have been examined at the fine structural level (Table 1), for most, their taxonomic affinities remain equivocal.

Investigations that focus on *in situ* gymnosperm grains are typically afforded the opportunity to assess the systematic position of the plants that produced the pollen (Table 2). In a few cases, despite having complete morphological and/or anatomical data on the entire reproductive organ as well as information regarding pollen ultrastructure, the affinities continue to remain problematic (e.g., *Lasiostrobus*; Taylor, 1970; Table 2). Furthermore, pollen preserved *in situ* is of paramount importance in evaluating fossil gymnosperms from a biological perspective (Taylor et al., in press). For example, studies of *in situ* gymnosperm pollen have been instrumental in addressing several developmental and reproductive parameters such as microsporogenesis (Taylor, 1990), pollen germination and pollen tube growth (Rothwell, 1972), and the possible functional roles of the exine and sacci in pollination (Taylor and Zavada, 1986).

Tables 1 and 2 indicate the fossil gymnosperm taxa which are now known at the ultrastructural level. Although several new taxa have been investigated and others have been re-examined (Tables 1, 2) since the last review of fossil gymnosperm pollen ultrastructure was published (Taylor and Taylor, 1987), we will not review this topic here. Rather, the intent of this paper is to focus on the relative phylogenetic significance of several ultrastructural characters present in fossil gymnosperm pollen.

Ultrastructural Characters

In recent years, phylogenetic systematics (cladistics) has become a prominent component in palynology. An area that has received a significant amount of attention is the proposed phylogenetic relationships among extinct and extant seed plants as well as the question of angiosperm origins as elucidated by cladistic investigations (e.g., Crane, 1985; Doyle and Donoghue, 1986). These studies have been based on a number of both vegetative and reproductive features including several palynological characters. In addition to the four pollen characters used by Crane (1985) and the six employed by

TABLE 1. Selected gymnosperm taxa for which *sporae dispersae* pollen has been studied with transmission electron microscopy [1]

Taxon	Age
PTERIDOSPERMOPHYTA	
MEDULLOSALES (*Schopfipollenites*-type)	
Schopfipollenites sp. [2]	Pn
Schopfipollenites sp. [3]	Pn
GLOSSOPTERIDALES	
Protohaploxypinus spp. [4]	Pm
CONIFEROPHYTA	
CORDAITALES	
Florinites sp. [2]	Pn
VOLTZIALES	
Cheirolepidiaceae (circumpolloid grains)	
Circulina sp. [5]	Tr
Classoidites glandis [5,6]	Tu
Classopollis classoides [7]	Ju
C. harrisii [5]	Li/Rh
C. minor [8]	Ju
CONIFERALES	
Podocarpaceae	
Rugubivesiculites rugosus [9]	Cr
GNETOPHYTA	
Equisetosporites spp. [10]	Cr
Ephedripites sp.1 [11]	Cr
INCERTAE SEDIS	
Teichertospora torquata [12]	Dv
Nanoxanthiopollenites mcmurrayii [13,14]	Pn
Cannanoropollis janakii [15]	Pm
Marsupipollenites triradiatus [16]	Pm
Platysaccus leschikii [15]	Pm
Plicatipollenites malabarensis [15]	Pm
Praecolpatites sinuosus [16]	Pm
Protohaploxypinus limpidus [15]	Pm
Striatopodocarpites phaleratus [15]	Pm
INCERTAE SEDIS (CONT.)	
Bharadwajipollenites wielandii [17]	Tr
Equisetosporites chinleana [17,18,19]	Tr
Granamonocolpites luisae [17]	Tr
cf. *G. asymmetricus* [9]	Cr
Triadispora bölchii [20]	Tr
Unnamed bisaccate sp. [18]	Tr
Lueckisporites virkkiae [21]	Li
Lunatisporites noviaulensis mollis [21]	Li
Ovalipollis notabilis [21]	Li
O. ovalis [21]	Li
Araucariacites sp. [22]	Ju
A. hungaricus [8]	Ju
A. v. *granulatisporites* [6]	Cr
A. australis [23]	Cr
Cycadopitys sp. [8]	Ju
Inaperturopollenites limbatus [22]	Ju
I. ex gr. *hiatus* [8]	Ju
Spheripollenites scabratus [6]	Ju
Balmeiopsis limbatus [23]	Cr
Clavabisvesiculites sp. [9]	Cr
Cyclusphaera psilata [24,25]	Cr
Eucommiidites sp. 1 [11]	Cr
E. sp. 2 [11]	Cr
E. sp. [18]	Ju
E. sp. [26]	Cr
Granabivesiculites inchoatus [18]	Cr
G. cf. *inchoatus* [9]	Cr
G. sp. [18]	Cr
Granamultivesiculites sp. [9]	Cr
Monosulcites sp. 1 [11]	Cr
M. sp. [9]	Cr
Oculopollis maximus [27]	Cr
Punctamultivesiculites inchoatus [18]	
Verrumonocolpites conspicuus [18]	Cr
Unnamed (vestigial saccate) sp. [18]	Cr

[1]Age; Dv=Devonian, Cr=Cretaceous, Ju=Jurassic, Li=Liassic, Pn=Pennsylvanian, Pm=Permian, Rh=Rhaetian, Tr=Triassic, and Tu=Turonian.

[2] Pettitt, 1966; [3] Abadie et al., 1978; [4] Osborn, 1991; [5] Médus, 1977a; [6] Kedves and Párdutz, 1973; [7] Rowley and Srivastava, 1986; [8] Kedves, 1985; [9] Zavada and Dilcher, 1988; [10] Osborn et al., 1993; [11] Trevisan, 1980; [12] Foster and Balme, 1994; [13] Taylor, 1980; [14] Taylor, 1982; [15] Foster, 1979; [16] Foster and Price, 1981; [17] Zavada, 1990; [18] Zavada, 1984; [19] Pocock and Vasanthy, 1988; [20] Scheuring, 1976; [21] Scheuring, 1974; [22] Kedves and Párdutz, 1974; [23] Zavada, 1992; [24] Taylor et al., 1987; [25] Zavada, 1987; [26] Doyle et al., 1975; [27] Médus, 1977b.

TABLE 2. Selected gymnosperm taxa for which *in situ* pollen has been studied with transmission electron microscopy.

PTERIDOSPERMOPHYTA
- LYGINOPTERIDALES
  - *Crossotheca* sp. [1]
  - *Phacelotheca pilosa* [2]
  - *Potoniea illinoiensis* [3,4]
  - *P. carpentieri* [4]
  - *Schopfiangium varijugatus* [5]
- MEDULLOSALES (*Schopfipollenites*-type)
  - *Aulacotheca iowensis* [6,7,8]
  - *Bernaultia formosa* [9]
  - *B. sclerotica* [9]
  - *Boulayatheca fertilis* [10]
  - *Codonotheca caduca* [6,7,8]
  - *Dolerotheca* sp. [8,11]
  - *Halletheca reticulata* [8,11,12]
  - *Rhetinotheca patens* [4]
  - *R. tetrasolenata* [8]
  - *Schopftheca boulayoides* [8]
  - *Sullitheca dactylifera* [4]
- MEDULLOSALES (*Parasporites*-type)
  - *Parasporotheca leismanii* [1,4]
- CALLISOPHYTALES
  - *Idanothekion callistophytoides* [13,14]
- GLOSSOPTERIDALES
  - *Arberiella* sp. (*Protohaploxypinus*-type & *Striatopodocarpites*-type) [15]
- CORYSTOSPERMALES
  - *Pteruchus africanus* [16]
  - *P. dubius* [16,17]
  - *P. papillatus* [16]
  - unnamed sp. [18]
- CAYTONIALES
  - *Caytonanthus arberi* [19,20,21]
  - *C. kockii* [20]

CONIFEROPHYTA
- CORDAITALES (*Florinites*-type)
  - *Cordaianthus* sp. [13,14]
- CORDAITALES (*Sullisaccites*-type)
  - *Cordaianthus* sp. [13,14]
- CORDAITALES (*Felixipollenites*-type)
  - *Gothania lesliana* [13,14,22]
- VOLTZIALES
  - Voltziaceae
    - *Darneya peltata* [23]
    - *Sertostrobus laxus* [23,24]
    - *Willsiostrobus denticulatus* [23]
    - *W. cordiformis* [23,25]
    - *W. rhomboides* [23,25]
  - Cheirolepidiaceae (*Classopollis*-type)
    - *Hirmeriella* (=*Cheirolepidium*) *muensteri* [26]
    - *Classostrobus comptonensis* [27]
    - *Pseudofrenelopsis* sp. [28]
- CONIFERALES
  - Pinaceae
    - *Pinus* sp. [29]
  - Podocarpaceae
    - *Millerostrobus pekinensis* [30]
    - *Moreno fertilis* [31]
    - *Squamostrobus tigrensis* [32]
    - *Trisacocladus tigrensis* (*Trisaccites*-type) [33]
  - Taxodiaceae
    - *Drumhellera kurmanniae* [34]
    - *Elatides williamsonii* [35]
    - *Metasequoia milleri* [36]

CYCADOPHYTA
- CYCADALES
  - *Androstrobus balmei* [37]
- BENNETTITALES (=CYCADEOIDALES)
  - *Cycadeoidea dacotensis* [19,38]
  - *Leguminanthus siliquosus* [39]
- PENTOXYLALES
  - *Sahnia laxiphora* [40]

INCERTAE SEDIS
- *Erdtmanispermum balticum* (*Eucommiidites*-type) [41]
- *Erdtmanitheca texensis* (*Eucommiidites*-type) [41]
- *Lasiostrobus polysacci* [42,43]
- *Melissiotheca longiana* [44]

[1] Millay et al., 1978; [2] Meyer-Berthaud and Galtier, 1986; [3] Stidd, 1978; [4] Taylor, 1982; [5] Stidd et al., 1985; [6] Taylor, 1976a; [7] Taylor, 1976b; [8] Taylor, 1978; [9] Taylor and Rothwell, 1982; [10] Kurmann and Taylor, 1984; [11] Millay and Taylor, 1976; [12] Taylor, 1971; [13] Millay and Taylor, 1974; [14] Millay and Taylor, 1976; [15] Zavada 1991a; [16] Zavada and Crepet, 1985; [17] Taylor et al., 1984; [18] Osborn and Taylor, 1993; [19] Osborn, 1991; [20] Pedersen and Friis, 1986; [21] Zavada and Crepet, 1986; [22] Taylor and Daghlian, 1980; [23] Taylor and Grauvogel-Stamm, in prep.; [24] Taylor and Taylor, 1987; [25] Taylor, 1988; [26] Pettitt and Chaloner, 1964; [27] Taylor and Alvin, 1984; [28] Taylor and Alvin, in prep.; [29] Osborn and Stockey, in prep.; [30] Taylor et al., 1987; [31] Del Fueyo et al., 1990; [32] Archangelsky and Del Fueyo, 1989; [33] Baldoni and Taylor, 1982; [34] Serbet and Stockey, 1991; [35] Kurmann, 1991; [36] Rothwell and Basinger, 1979; [37] Hill, 1990; [38] Taylor, 1973; [39] Ward et al. 1989; [40] Osborn et al., 1991; [41] Pederson et al., 1989; [42] Taylor, 1970; [43] Taylor and Millay, 1977; [44] Meyer-Berthaud, 1989.

Doyle and Donoghue (1986), some of which overlap, a number of other characters have also been incorporated into other phylogenetic analyses of both gymnosperms and angiosperms (Table 3). The list of characters in Table 3 is not totally inclusive, and the degree to which it can be exclusively used is dependent upon the taxonomic rank at which a phylogenetic analysis is conducted (i.e., within a genus, a family, a division, etc.).

Nevertheless, it is clear that an array of morphological and fine structural features are available for evaluation. With both general access to TEM and a growing number of taxa being studied, the focus of many investigations has been the desire to identify a large number of characters and, as a result, infer homologies. Although many palynological characters are considered conservative features in terms of evolutionary change and manifestations of phylogeny, they can also reflect several other biological and physical phenomena. For example, events during pollen ontogeny as well as many facets associated with the reproductive biology of a taxon (e.g., pollination) may be important influences on observed fine structure. The most prominent abiotic factors to consider are mode of preservation, degree of preservational alterations (e.g., diagenesis), preparation protocols for TEM, and nature of the ultrathin sectioning plane. In the present paper, we address these features as they relate to phylogenetic interpretations and relevance of three salient palynological characters, including overall exine infrastructure, nexine organization, and saccus type and internal composition.

A. Gross exine infrastructure

A vast terminology has been developed for pollen wall ultrastructure based principally on the structural aspects of the sporoderm, as opposed to ontogenetic or functional features. Of the many systems of pollen wall nomenclature proposed, those of Faegri and Iversen (1989) and Erdtman (1969) are most widely recognized (see Zavada, 1984). Comparisons and discussions of these two schemes have received a significant amount of attention (e.g., Zavada, 1984) and will not be elaborated here. Briefly, however, the sporoderm as interpreted by Faegri and Iversen (1989) consists of three major layers: an outer ektexine and middle endexine (exine collectively), both composed of resistant sporopollenin, and an inner intine primarily consisting of cellulose. The sporopolleninous ektexine and endexine exhibit differential stainability in both LM and TEM preparations (see below). Erdtman (1969) also recognizes a three-layered sporoderm, composed of an outer sporopolleninous sexine and nexine (exine collectively) and an inner pecto-cellulosic intine. In Erdtman's classification, the wall layers are recognized based on their topographic positions.

Exine stratification is generally categorized into several general types, including homogeneous (atectate), tectate-alveolate ("honeycomb-like" type and "spongy" type), tectate-granular, intectate-granular, tectate-columellate, semitectate-columellate, and intectate-columellate (e.g., Doyle et al., 1975; Kurmann, 1992). However, researchers have differing views concerning interpretations of such terms as "alveolate" and "spongy" exines and, in practice, use these differently. In other cases a new set of terms may be created. The range of terminology is cumbersome and indeed problematic when attempting to address homologous wall layers. For example, similar names applied to structurally different layers by multiple authors may incorrectly imply homology. Therefore, the phylogenetic implications of how exine stratification is interpreted and described are critical, and necessitate consideration of all variables that may influence the observed structural features of the pollen wall.

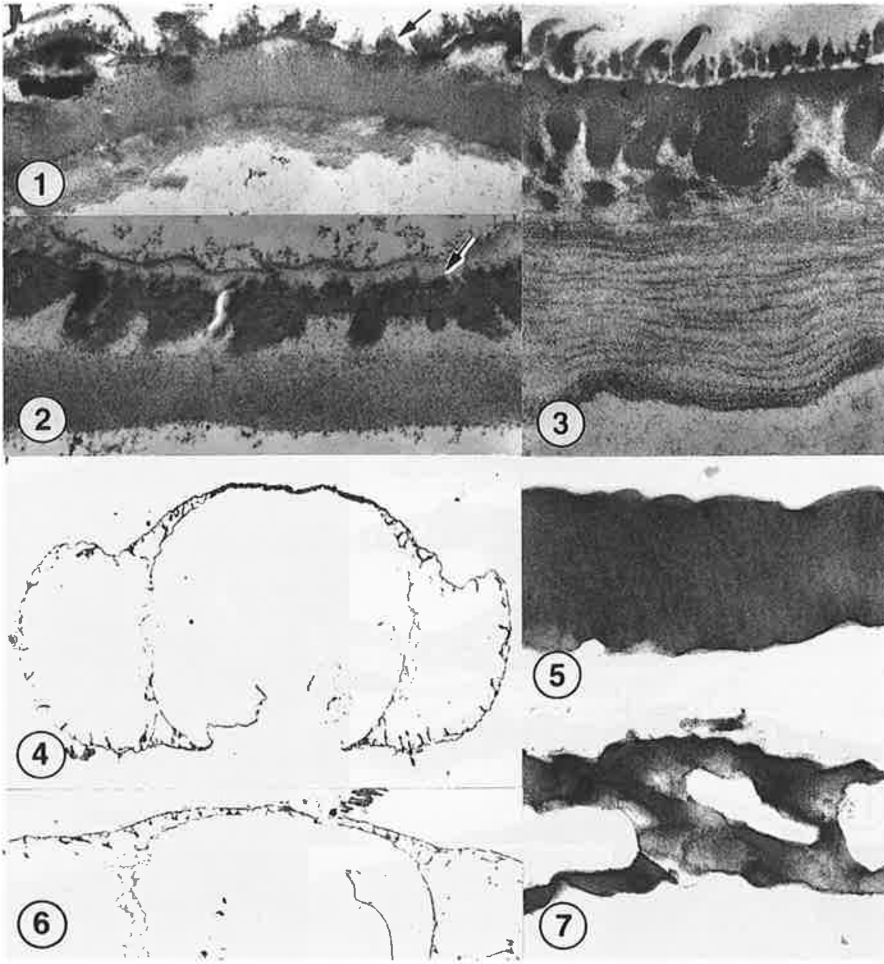
The exine clearly undergoes several major changes in ultrastructure during sporoderm ontogeny (Figs. 1–3). Relatively few studies have documented developmental sequences for fossil gymnosperm pollen (see Taylor, 1990). Those that have been published have resulted from examinations of multiple specimens of *in situ* grains. However, it is possible that the exine might be erroneously described if one were to only examine grains at a particular stage of development that were contained within a single pollen sac. For example, *Classopollis*-type grains isolated from the cones of the cheirolepidiaceous

TABLE 3. Selected palynological characters used in phylogenetic analyses

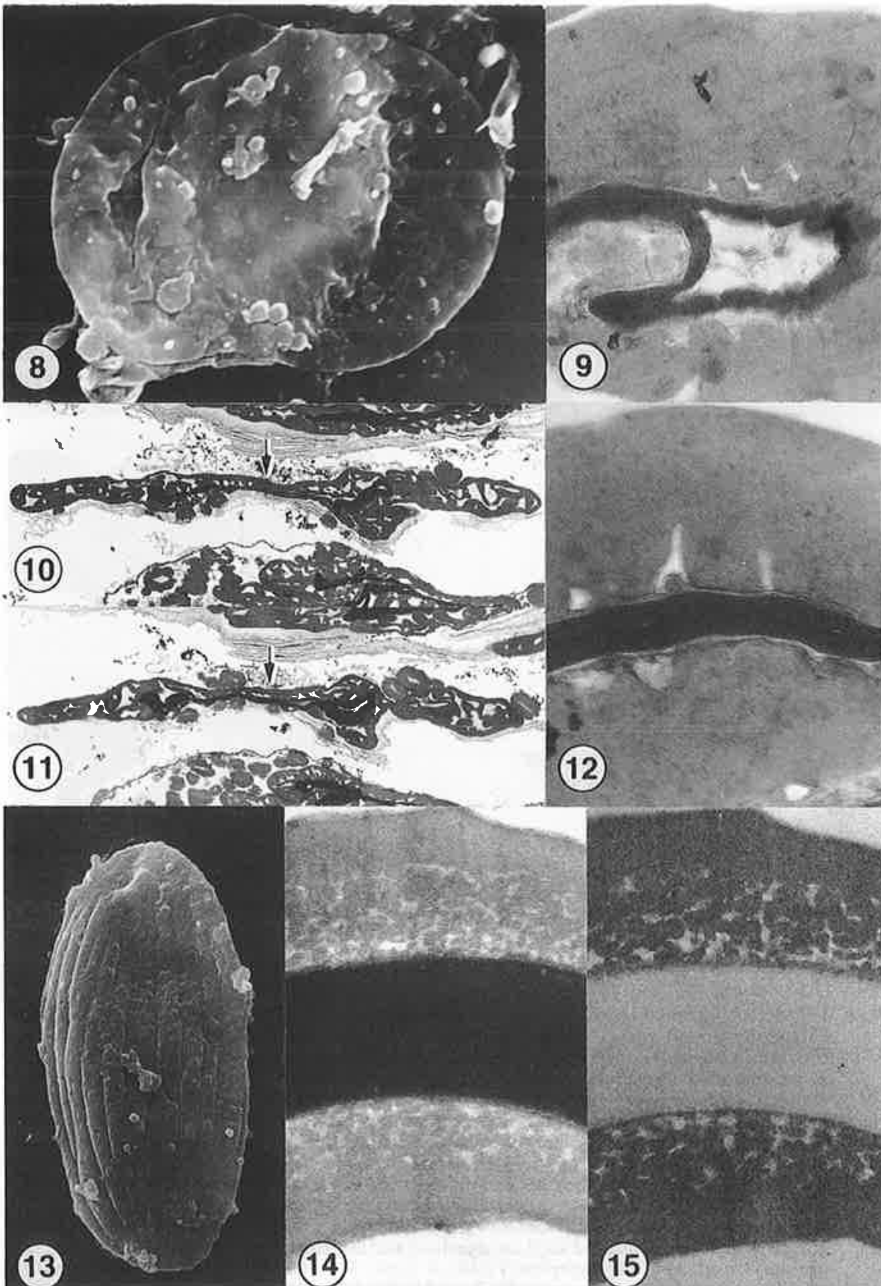
GROSS MORPHOLOGICAL FEATURE
Heterospory
Dispersal Unit
Tetrads acalymmate/calymmate
Shape
Size
Symmetry
Aperturate/inaperturate
Aperture type
Aperture position
Sulcus-pollen tube present/absent
Saccate/nonsaccate
MICROMORPHOLOGICAL FEATURE
Striate/nonstriate
Supratectal ornament present/absent
Surface sculpture
Sculpture attached/detached
Sculpture density
Sculpture detail
Aperture membrane sculpturing
ULTRASTRUCTURAL FEATURE
Gross exine ultrastructure (=stratification)
Exine partitioning
Sexine ultrastructure
Exine tectate
Tectum ultrastructure
Nexine ultrastructure
Nexine thickness over aperture
Endexine present/absent
Endexine present/absent in extra-apertural areas
Endexine thickness
Foot layer present/absent
Saccus internal composition

conifer *Classostrobus* (Taylor and Alvin, 1984) may have been characterized as either intectate-granular (Fig. 1), or as tectate-alveolate or tectate-columellate lacking a supratectal ornament (Fig. 2) if only immature grains had been sectioned. However, identification of additional ontogenetic stages demonstrated that the mature pollen of this taxon is in fact complex, consisting of a four-zoned, tectate-collumellate exine with supratectal spinules (Fig. 3).

It is also important to sample pollen sacs from different positions along the whole reproductive organ (e.g., distal vs proximal ends of a cone), as the organ may not exhibit synchronous pollen development. When possible, it is also advantageous to section entire pollen sacs, rather than mechanically or chemically macerating these structures in order to obtain pollen. The former approach permits identification of intra-locular structures such as resistant tapetal membranes and orbicules, which are important in documenting a complete ontogenetic series (Taylor et al., in press).



FIGS. 1-7. Exine infrastructure. **1**: Section through the cryptopore region of an immature *Classopollis* pollen grain. Note the homogeneous, non-lamellate nexine and dark staining, discontinuous sexine elements (arrow), × 10,000. **2**: Section through an unspecialized region of an immature *Classopollis* grain. This grain is older than the one illustrated in Fig. 1 and shows a better developed sexine and non-lamellate nexine. Note that the sexine consists of immature columellae and a superficially granular tectum (arrow) that results from poorly developed supratectal spinules, × 15,000. **3**: Section through an unspecialized region of a mature *Classopollis* grain. Note the well-defined lamellae within the nexine and the distinct columellae, homogeneous tectum, and supratectal spinules of the sexine, × 20,000. **4**: Median transverse section through a permineralized corystosperm pollen grain showing a homogeneous proximal wall. Note how the exine layers separate and gradually grade into an alveolate structure where the sacci are attached, and that the endoreticulations within the sacci are only attached to the outer sacci walls, × 1,060. **5**: Detail of the proximal wall from the permineralized corystosperm grain illustrated in Fig. 4 showing a homogeneous ultrastructure, × 31,500. **6**: Lateral transverse section through a permineralized corystosperm pollen grain showing an alveolate proximal wall, × 1,060. **7**: Detail of a laterally sectioned proximal wall from a permineralized corystosperm grain showing an alveolate fine structure, × 31,500.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Two related characters to note regarding developmental patterns are tectum thickness and exine partitioning (i.e., sexine thickness relative to nexine thickness). Zavada and Gabarayeva (1991) have recently shown in the tectate-granular exine of extant *Welwitschia* that the majority of the thick, homogeneous tectum observed in mature grains results from the developmental compaction and fusion of sporopolleninous granules. Consequently, the granular infratectal layer and the homogeneous tectum exhibit markedly different thicknesses during ontogeny. It is possible that this pattern could also occur in fossil groups; if immature grains are exclusively sampled, then exine descriptions and phylogenetic interpretations would be skewed.

The plane of section through a pollen grain and the nature of grain preservation are also key factors concerning interpretations of infrastructure. This is especially important with regard to studies of saccate grains. For example, bisaccate pollen of the Mesozoic seed fern group Corystospermales shows a distinctly different fine structure when sectioned medially as opposed to laterally. The proximal wall (cappa) in median sections is homogeneous (Figs. 4–5) and becomes tectate-alveolate in organization laterally (Figs. 6–7) where the sacci are attached (see below). The ability to detect these differences is the result of the permineralized nature of the fossils and the fact that multiple grains were individually serially sectioned (Osborn and Taylor, 1993). Until these structurally preserved specimens were examined, corystosperm pollen was known only from two studies based on compressed *Pteruchus* pollen organs. Principally due to preservation of the *Pteruchus* specimens, Taylor et al. (1984) were unable to detail the fine structure of the cappa, while Zavada and Crepet (1985) described the proximal wall to be ultrastructurally homogeneous with a few small lacunae. A similar type of organization has been detected in the bisaccate grains of another Mesozoic seed fern, *Caytonanthus* (Fig. 8; Caytoniales). Although pollen organs of *Caytonanthus* are also known only from compression specimens, serial sectioning (Osborn, 1991) indicates that the previously described alveolate exine (Pedersen and Friis, 1986; Zavada and Crepet, 1986) is prominent only in lateral wall regions (Fig. 11), and grades into a homogeneous infrastructure near the median area (Fig. 10), as in corystosperm pollen.

Preservation type may also affect interpretations of nonsaccate pollen. For instance, pollen ultrastructure of *Sahnia* (Pentoxylales) is known only from compression specimens, with the exine having a tectate-granular ultrastructure (Osborn et al, 1991). Grains exhibit a wide range of tectum and infratectum thicknesses resulting from differential degrees of granule packing (Figs. 9, 12). Although strata thickness in a tectate-granular exine may be a developmental phenomenon, as noted above for *Welwitschia,* it is clearly the result of preservational compression in *Sahnia* pollen (Figs. 9, 12). In fact, in some grain regions (Fig. 9), as well as in entire grains, the sexine shows a homogeneous ultrastructure.

In addition to mode of preservation, several other preservational influences (diagenesis) and the effects of temperature, chemical maceration, and electron microscopy preparation techniques are known to alter exine fine structure in both fossil and extant pollen (e.g., Sengupta, 1977; Niklas, 1980; Kedves, 1985; Kedves and Kincsek,

FIGS. **8–15**. Exine infrastructure. **8**: Proximal surface of a mature *Caytonanthus* pollen grain, × 3,000. **9**: Section of a compressed *Sahnia* pollen grain showing a dark-staining nexine and a light-staining sexine. Note that the sexine appears almost entirely homogeneous at left and tectate-granular at right, × 23,250. **10**: Near median transverse section through a compressed *Caytonanthus* pollen grain showing homogeneous sexine infrastructure (arrow), × 3,300. **11**: Lateral transverse section through the same compressed *Caytonanthus* pollen grain illustrated in Fig. 10 showing an alveolate sexine infrastructure (arrow), × 3,300. **12**: Section through a compressed *Sahnia* pollen grain showing a tectate-granular infrastructure. Note the thick, homogeneous tectum and weakly defined granular infratectum due to compression and compaction of granules, × 40,000. **13**: Polyplicate surface of a dispersed ephedroid palynomorph, × 2,700. **14**: Transverse section of a dispersed ephedroid palynomorph showing a darkly stained nexine and a lightly stained, tectate-granular sexine, × 31,500. **15**: Same exine region shown in Fig. 14, but from a different ultrathin section; note the lightly stained nexine and the darkly stained sexine, × 31,500.

1989; Rowley et al., 1990). For example, it is possible that the permineralized corystosperm grains noted above have been altered due to preservational phenomena, because the rocks in which they were preserved are known to have been thermally altered (see Osborn and Taylor, 1993). Nevertheless, the exine of these grains is still thought to be homogeneous in medial positions of the proximal wall (Figs. 4–5); this is based on comparisons with other Mesozoic bisaccates (*Caytonanthus*) that have a similar cappa fine structure but have not been subjected to the same thermal effects.

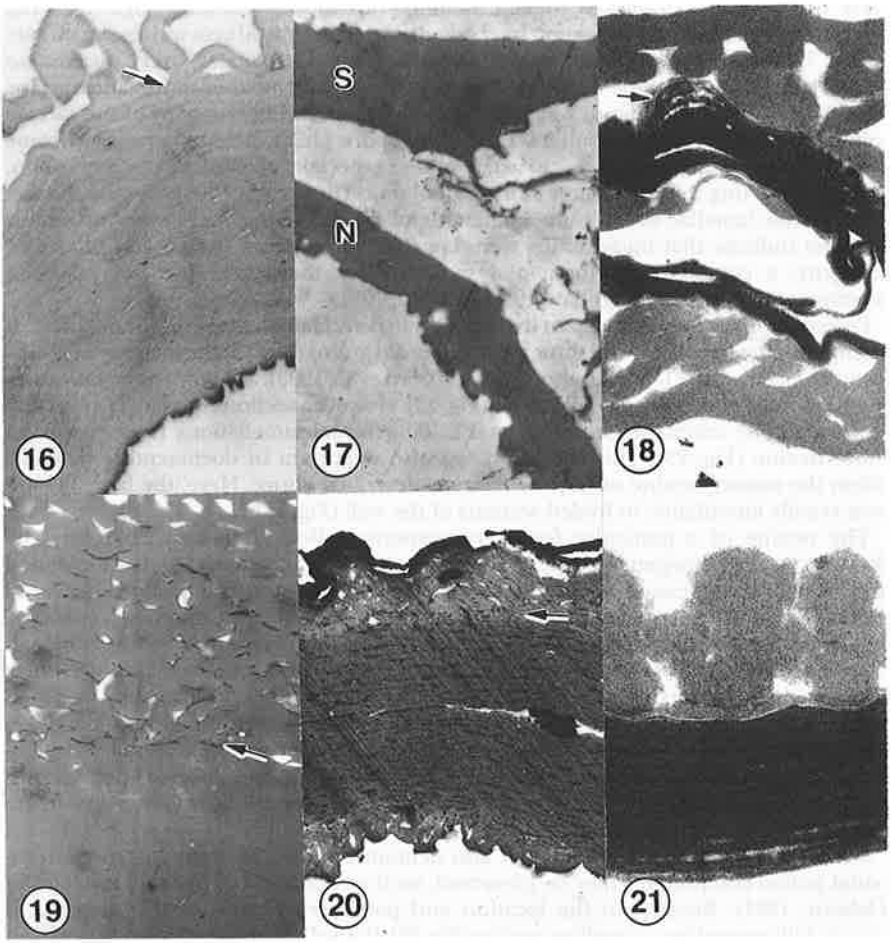
Regarding TEM preparation techniques, fossil exines have variable affinities for heavy metal stains. This can result in a number of anomalous conditions. In the majority of fossil gymnosperm grains examined to date, the sexine is light-staining and the nexine is dark-staining (e.g., Figs. 3, 9–12, 22, 24; also see discussion below). However, in some cases the exine may exhibit an opposite staining reaction (Figs. 14–15). The reasons for this are unclear, but the resultant sections afford the opportunity to detect exine strata at different levels of contrast and clarity. Such staining patterns have also been detected in fossil angiosperm pollen (Daghlian, pers. comm.).

B. Nexine organization

Most discussions of nexine organization have centered around proposed relationships between early and putatively primitive, extant angiosperms and the various gymnosperm groups to which they are suggested to be most closely related. Although the cladistic studies of Crane (1985) and Doyle and Donoghue (1986) differ with regard to several features, they both link angiosperms into a clade of highly derived seed plants, informally designated as "anthophyes", that also includes Bennettitales, Gnetales, Pentoxylales, and perhaps *Eucommiidites* pollen-producing plants (Pedersen et al., 1989). Many extant angiosperms regarded as primitive have monosulcate, tectate-granular pollen, as do all of the gymnospermous anthophyte groups. This has proven to be a problem concerning the investigation of many Early Cretaceous *sporae dispersae* grains and their identification as either angiospermous or gymnospermous (e.g., Zavada, 1984; Ward et al., 1989). Consequently, the distinction between pollen of these two groups has been primarily based upon both the appearance and structure of the nexine (=basal layer *sensu* Zavada, 1991b), specifically the nexine 2 layer or the endexine (Blackmore and Barnes, 1987).

The two layers of the nexine are divided into an the outer nexine 1 (=footlayer) and an inner nexine 2 (=endexine) based on their differential staining properties in light microscopy (e.g., with fuchsine B and auramine O; Faegri and Iversen, 1989). However, these two layers do not routinely show the same distinguishing affinities for stains used in TEM. As noted above, the nexine of most fossil gymnosperm pollen stains uniformly darker than the overlying sexine (Figs. 3, 9–12, 14, 22, 24), but opposite staining reactions may also take place (Fig. 15) and both the nexine and the sexine may appear homogeneous (Figs. 16–17). It is also possible that preparation artifacts, resulting from excessive staining for TEM, can lead to misidentification of nexine layers. In some cases, residual stains accummulate at the interface between the exine and the lumen of the pollen grain and superficially resemble a dark-staining inner layer (Fig. 16). This can be detected as an artifact if multiple sections from multiple grains are prepared using a range of stain regimes (Figs. 16, 19).

The endexine of extant gymnosperms is structurally lamellate at maturity, while the endexine of extant angiosperm pollen usually lacks lamellae except in apertural regions (e.g., Van Campo, 1971; Doyle et al., 1975). This character alone, however, may not be a good criterion for distinguishing between dispersed gymnospermous and angiospermous pollen for several reasons. For example, Blackmore and Barnes (1987) and Blackmore and Crane (1988) have suggested that differences in endexine structure of dispersed pollen may be indicative of ontogenetic stages rather than true structural dissimilarity. The timing of deposition of endexine materials differs between gymnosperms and angiosperms. The majority of endexine is deposited during the tetrad stage in gymnosperm pollen, whereas deposition of this wall layer is generally not initiated until



FIGS. 16-21. Nexine organisation. **16**: Section through the exine of a mature *Schopfipollenites* pollen grain showing a distinctly lamellate nexine (below arrow) and the lower portion of an alveolate sexine (above arrow), × 44,000. **17**: Section of the proximal wall near the aperture of a mature *Gothania* grain showing a non-lamellate nexine (N). Note also that the nexine and sexine (S) have the same affinity for TEM stains and appear homogeneous, × 22,500. **18**: Section through a mature *Caytonanthus* pollen grain showing a dark-staining nexine and light-staining sexine. Note that lamellae are only detectable in folded regions of the wall (arrow), × 20,100. **19**: Portion of an immature *Schopfipollenites* pollen grain showing a homogeneous, non-lamellate nexine (below arrow) and an alveolate sexine (above arrow), × 6,000. **20**: Section through an unspecialized exine region of an immature *Gothania* grain showing well-defined lamellae within the nexine (below arrow), × 18,000. **21**: Exine of an immature *Caytonanthus* grain showing a distinctly lamellate, dark-staining nexine, × 40,000.

the free-spore phase in angiosperms (e.g., Blackmore and Crane, 1988). It is clear from developmental studies of *in situ* fossil gymnosperm pollen that nexine lamellae also form at different stages. For instance, well-defined lamellations in the nexine of *Classostrobus* (Taylor and Alvin, 1984) and *Schopfipollenites* (Medullosales; e.g., Taylor, 1982) are only detectable in sections of mature pollen (Figs. 3, 16). However, when immature pollen of these two taxa are examined, nexine lamellae are notably absent (Figs. 1–2, 19). Interestingly, a contrasting situation has been observed in several taxa with *in situ* saccate pollen. Here, sections of immature pollen of *Gothania* (Cordaitales) and *Caytonanthus* exhibit well-defined nexine lamellae (Figs. 20–21) when compared with mature grains (Figs. 17–18). This is similar to what has been observed during ontogeny of the saccate pollen from several extant conifers. In the free-spore phase, nexine lamellae become stretched and tightly appressed to each other, especially during saccus expansion, thereby inhibiting their detection as individual units (Kurmann, 1990). Despite the fact that tripartite lamellae occur in the immature pollen of *Gothania* and *Caytonanthus*, this does not indicate that these grains were lamellate throughout the earliest phases of ontogeny; a complete developmental sequence for these taxa has not yet been determined (Taylor and Daghlian, 1980; Osborn, 1991).

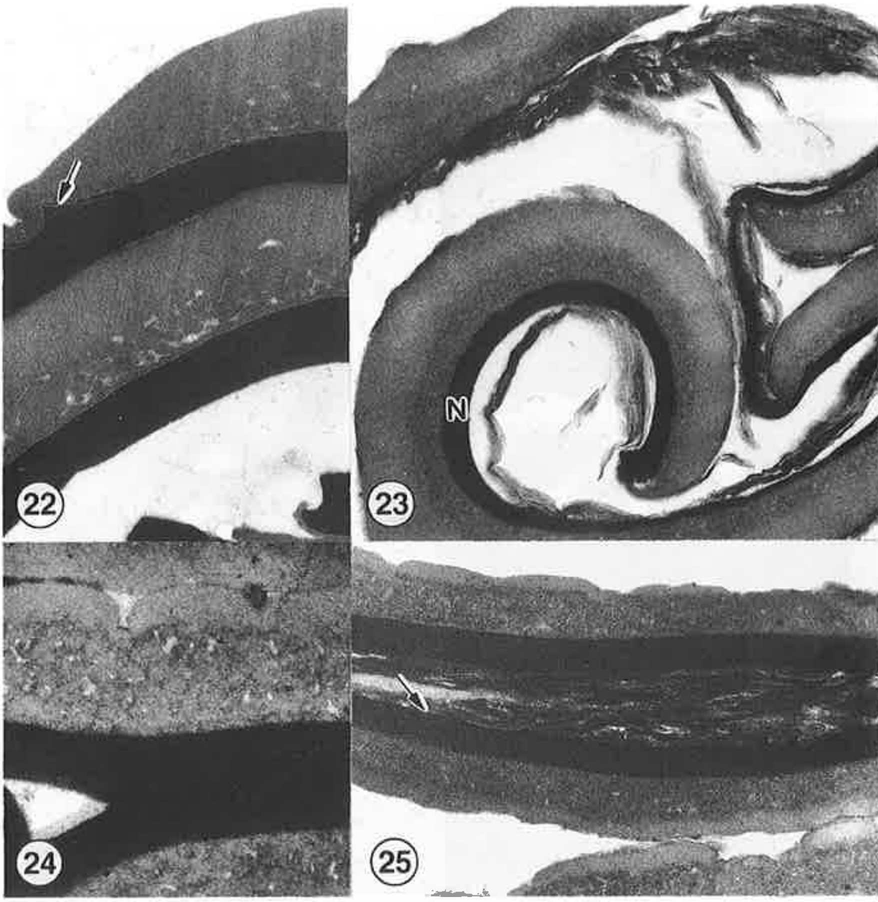
Preservation may also play a part in the ability to detect lamellae within the nexine. For example, in the case of several dispersed ephedroid grains (Fig. 13) the majority of grains sectioned lack nexine lamellae altogether (Osborn et al., 1993), although some may show an occasional lamella near a wall furrow (Fig. 22). However, sections of a single grain that has undergone extensive preservational folding reveal lamellations throughout the entire nexine (Fig. 23). This condition was also important in documenting lamellae within the mature nexine of *Caytonanthus,* as described above. Here, the lamellae are most readily identifiable in folded sections of the wall (Fig. 18).

The nexine of a particular fossil gymnosperm pollen grain may, therefore, be ultrastructurally homogeneous (i.e., lack tripartite lamellae) due to either developmental stretching and appression, or preservational compression and compaction. However, without detailed developmental data documenting a full range of intermediate stages, the degree to which ontogeny versus preservation affect the mature nexine is unclear. Consequently, the presence of lamellae provides useful information, although their absence within the nexine is clearly uninformative. For instance, one might be inclined to characterize the pollen of the bennettitalean genus *Cycadeoidea* as non-lamellate, because nexine lamellae have never been observed despite the preparation of multiple grains (Fig. 24). This assessment might inaccurately link the group closer to angiosperms, because the nexine of angiosperm pollen is characterized as non-lamellate except in the apertural regions of some grains.

Studies of *in situ Cycadeoidea* pollen also demonstrate that in some instances intra-exinal pollen components may be preserved, such as remnants of the cellulosic intine (Osborn, 1991). Because of the location and poor preservation of this material, it superficially resembles a lamellate nexine (Fig. 25). If a well-defined nexine had not been identified in these grains as a continuous, dark-staining layer consistent in thickness (Figs. 24–25), then the non-exinous layer (Fig. 25) could conceivably have been described as nexine. Intine preservation in other *in situ* and dispersed pollen grains from both gymnosperms and angiosperms is also possible, and it is advisable to employ appropriate preparation protocols (e.g., acid macerations, acetolysis) to chemically extract the oxidized wall layer (Zavada, pers. comm.) in order to preclude misidentification.

C. Saccus type and internal composition

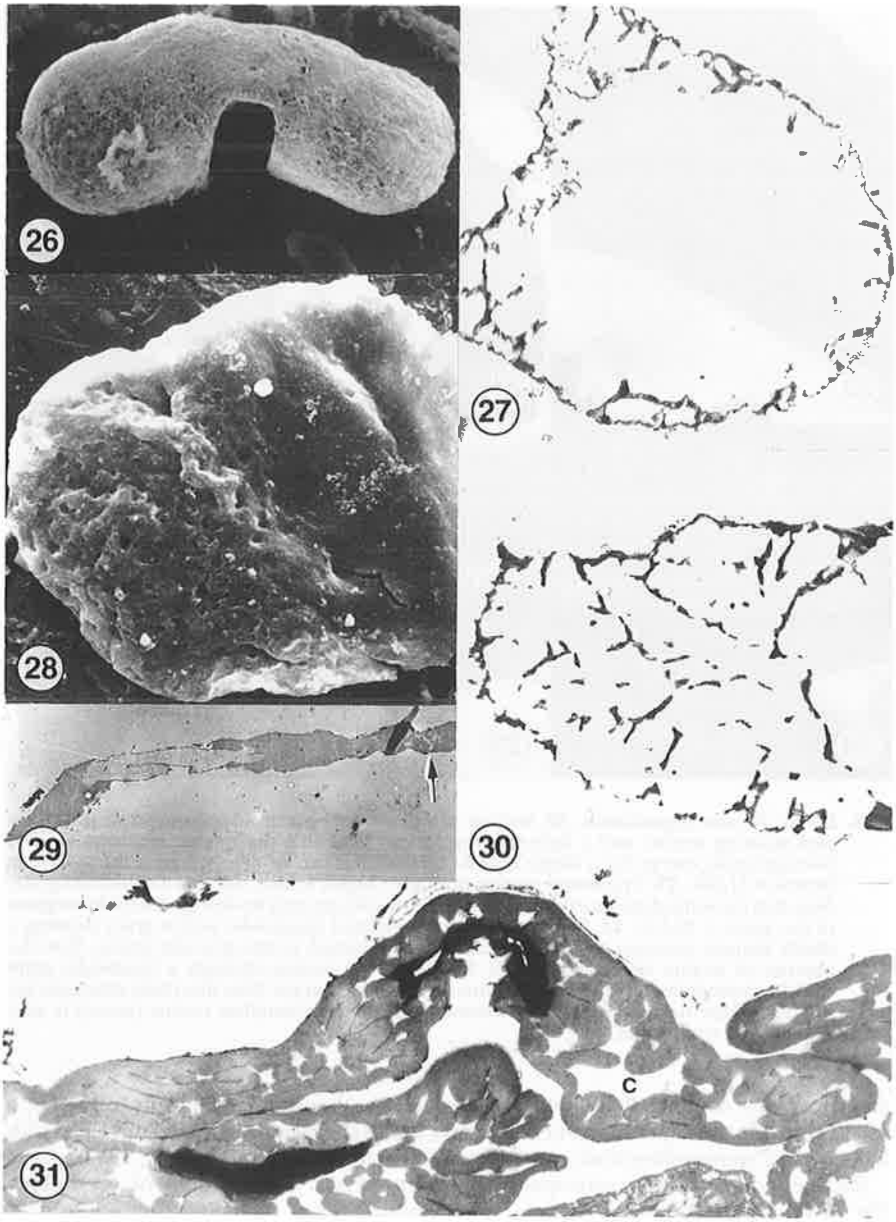
Extensions of the sporoderm, formed by separation of wall layers, are known from an array of pteridophyte and gymnosperm groups, as well as several angiosperm taxa. For the most part, these extensions are categorized by the degree of wall separation (i.e., the amount of space enclosed by the separated sexine and nexine layers). Spores and pollen grains with only slight separation of the exine are described as having a cavea (=cavum) or a camera, while those with a more extensively separated wall are said to have a pseudosaccus. The pseudosaccate condition is known in many lycopsid microspores (e.g., *Endosporites*; Brack and Taylor, 1972), while most angiosperms that have space in their exines are said to be caveate (e.g., Compositae; Blackmore et al., 1984). Both pseudosacci



FIGS. 22–25. Nexine organisation. **22**: Section of a dispersed ephedroid palynomorph showing a dark-staining nexine and a light-staining sexine. Note that the nexine is almost entirely homogeneous except for a single lamella (arrow) that can be detected near the sporderm furrow, × 31,300. **23**: Transverse section through a highly folded, dispersed ephedroid grain. Note that the individual lamellae within the nexine (N) can only be detected in folded regions of the grain, × 25,100. **24**: Section of a permineralized *Cycadeoidea* pollen grain showing a darkly stained, homogeneous nexine and a lightly stained, tectate-granular sexine. Note the absence of nexine lamellae, × 40,000. **25**: Transverse section through a *Cycadeoidea* grain showing 'pseudolamellae' within the lumen of the pollen grain. Note that these structures are not part of the nexine, as the lower boundary of the non-lamellate nexine (arrow) is well-defined and continuous, × 25,100.

and caveae are completely hollow. A saccus, on the other hand, is typically formed by a relatively extensive separation of exine layers, and is characterized as variably filled with a network of sporopolleninous plates, or endoreticulations (e.g., Traverse, 1988).

Saccate pollen occurs principally in gymnosperms. Four orders of seed ferns (Pteridospermophyta) are known to have saccate pollen, including Callistophytales, Glossopteridales, Corystospermales, and Caytoniales; the pollen of *Parasporotheca* (Medullosales) is also described as having vestigial sacci. Saccate pollen is also prominent in the Coniferophyta, and is known from Cordaitales, Voltziales (Voltziaceae), and Coniferales (Pinaceae and Podocarpaceae). Moreover, the pollen of one angiosperm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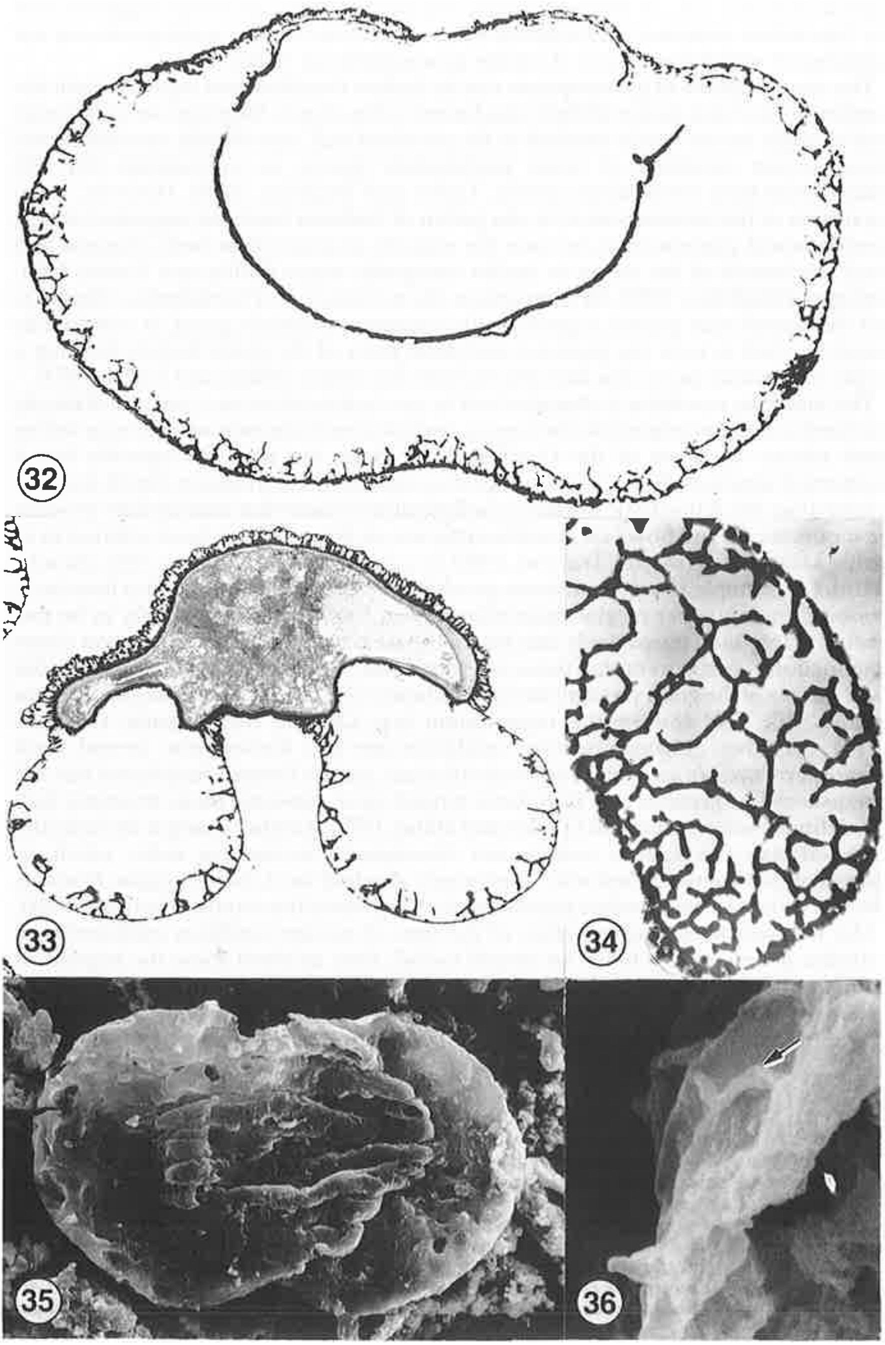
family (Lactoridaceae) has been characterized as saccate (Zavada and Taylor, 1986a; Zavada and Benson, 1987). However, the sacci in this family lack any type of conspicuous internal structure (i.e., an endoreticulum), and Crane (1990) has further suggested that the "saccus-like structures" of *Lactoris* as well as the caveae of other angiosperms are not homologous with the true sacci of various gymnospermous clades.

The saccate grains of gymnosperms can be further classified with regard to both the number of sacci and nature of their attachment to the corpus. Monosaccate pollen may have a single saccus that is attached to the proximal wall, superficially resembling the pseudosaccate condition of some pteridophyte spores, as in *Gothania* (Fig. 32, *Felixipollenites*-type cordaitalean grains; Taylor and Daghlian, 1980). However, distal separation of the monosaccus in *in situ* pollen of *Gothania* has been suggested to be a developmental phenomenon, because the majority of grains show both proximal and distal attachment of the saccus in earlier ontogentic stages (Millay and Taylor, 1974; Taylor and Daghlian, 1980). By comparison, the monosaccus of *Cordaianthus* (*Florinites*- and *Sullisaccites*-type grains), a geologically younger cordaitean genus, is consistently found attached to both the proximal and distal poles of the grain, thereby forming a single, continuous saccus that laterally encircles the corpus (Millay and Taylor, 1974).

The bisaccate condition is characterized by two independent sacci that are distinctly restricted in their attachment to the corpus, and is known from most seed ferns as well as other saccate members of the Coniferophyta. Here, the sacci are typically found transversely attached (Figs. 4, 26), although they may also occur in a more distally inclined fashion (Fig. 33). A third type of saccus configuration is somewhat intermediate between the monosaccate and bisaccate conditions; grains of this type have been referred to as having a transitional saccus (Traverse, 1988) or a girdling saccus (Osborn, 1991; Zavada, 1991a). For example, the *sporae dispersae* genus *Protohaploxypinus*, presumed to have been produced by a number of glossopteridalean seed ferns, has what appears to be two relatively large sacci transversely attached to the taeniate corpus. However, upon closer examination it is apparent that these two transverse bladders are continuous over the distal surface of the grain via slight lateral attachment (Fig. 35). Examinations of ultrathin sections with TEM confirm this organization (e.g., Osborn, 1991; Zavada, 1991a). A number of other enigmatic saccate conditions are also known from several fossil gymnosperm taxa. *In situ* pollen of the problematic species *Lasiostrobus polysacci* has 3–8 subequatorial projections that have been termed sacci; however, these structures lack well-defined endoreticulations (Taylor and Millay, 1977). Another example includes the dispersed taxa *Lueckisporites virkkiae* and *Lunatisporites noviaulensis mollis*, which, in addition to having two prominent, transversely attached sacci, have elongate bladders attached to the proximal surface paralleling the long axis of the corpus (Scheuring, 1974).

The ultrastructural determination of the type of saccate condition exhibited by a particular pollen grain is based on several factors. First, as noted above the majority of *Gothania* grains resemble the pollen of *Cordaianthus* in that the monosaccus is both proximally and distally attached to the corpus. Identification of a limited number of ontogenetically more mature grains (Fig. 32) indicates that the saccus is in fact distally

FIGS. 26–31. Saccus structure. **26**: Equatorial view of a permineralized, bisaccate corystosperm, × 800. **27**: Median transverse section through the saccus of a permineralized corystosperm pollen grain showing eusaccate condition. Note that the endoreticulations are discontinuous between the outer saccus wall (right) and the saccus floor, × 2,600. **28**: Proximal surface of a compressed *Pteruchus* pollen grain, × 7,000. **29**: Oblique section through a compressed *Pteruchus* grain showing sacci that superficially appear to be completely infilled with endoreticulations. Note that some space (arrow) within the right saccus can be detected, × 2,000. **30**: Lateral transverse section through the saccus of a permineralized corystosperm grain showing continuous endoreticulations extending between the outer saccus wall (left) and the saccus floor, thereby superficially appearing 'protosaccate', × 2,100. **31**: Oblique section through a compressed *Caytonanthus* pollen grain. Note that the left saccus appears extensively infilled with endoreticulations, but that a distinct cavity (C) can be detected in the right saccus. The darkly stained layer represents the nexine in the corpus region, × 9,284.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separated from the corpus. Secondly, sacci are believed to function in several ways, principally during pollination and pollination-related events. It is clear that sacci efficiently increase overall grain sizes without significantly increasing grain weight, and thereby provide for greater dispersal by wind. Others have suggested that sacci have a number of additional functions, which include: to harmomegathically maintain volumetric continuity; to physically ensure that the distal aperture is oriented against the nucellus to maximize siphonogamy; and to reduce competition with other pollen grains by maximally occupying the micropylar space, thereby physically preventing conspecific grains from entering that space (e.g., Chaloner, 1976; Zavada and Taylor, 1986b; Zavada, 1991a).

Perhaps a more salient aspect of saccus ultrastructure to address in a phylogenetic context is the nature of the internal sporopolleninous units, or endoreticulations. The degree to which gymnospermous sacci are filled with endoreticulations has become an important topic of discussion and phylogenetic speculation. Grains in which the endoreticulations are continuous between the corpus and the saccus walls (i.e., completely infill the saccus cavity) have been referred to as "protosaccate" (Scheuring, 1974). By comparison, when the saccus cavity is almost entirely hollow and the endoreticulations are restricted in attachment to the outer saccus wall (i.e., discontinuous), the grain is said to be "eusaccate" (Scheuring, 1974). The phylogenetic implication of the term 'protosaccate' as pleisiomorphic was the impetus for Meyen (1987) to refer to grains of this type as "quasisaccate", because eusaccate grains geologically precede protosaccate forms in the fossil record. However, if Foster and Balme's (1994) report of protosaccate grains from the Late Devonian is accurate, then Meyen's stratigraphic reservations would be less relevant. Nevertheless, descriptions of protosaccate and eusaccate grains from different major groups of gymnosperms have prompted speculation as to whether or not these two types of sacci are homologous (Crane, 1990). Doyle (1987–1988) has suggested that the protosaccate condition is indeed derived from the eusaccate type, specifically with reference to Caytoniales and Callistophytales.

In our opinion, the phylogenetic utility of this character is poor, and the degree to which the internal composition of the saccus can be homologized in seed plants is equivocal at best. First, the majority of *in situ* grains for which the protosaccate condition has been described has come from compression specimens. It is clear that this type of preservation has significant effects on saccus structure. For example, until recently ultrastructural data on corystosperm pollen have come exclusively from three compressed *Pteruchus* species (Taylor et al., 1984; Zavada and Crepet, 1985). These compressed grains have been important in providing information on a variety of features, especially surface morphology (Fig. 28). When ultrathin sectioned, the sacci appear to be extensively filled with endoreticulations (Fig. 29). However, discovery of permineralized *in situ* corystosperm pollen, as noted above, has provided the opportunity to make comparisons of grains preserved differently (Osborn and Taylor, 1993). The three-dimensionally preserved grains unequivocally demonstrate that corystosperm pollen is eusaccate (Figs. 4, 26). Therefore, although the sacci of compressed *Pteruchus* grains superficially appear to be protosaccate, the extensive infillings in fact represent the discontinuous endoreticulations that have been preservationally compressed.

The ultrathin section plane has also been shown to be important with regard to

FIGS. 32–36. Saccus structure. **32:** Equatorial section of a permineralized, mature *Gothania* pollen grain showing the monosaccus attached only to the proximal wall, × 333. **33:** Median section through an extant, bisaccate *Pinus* pollen grain showing the eusaccate condition, × 1,000. **34:** Lateral section through the saccus of an extant *Pinus* grain showing an extensive endoreticulum extending throughout the saccus, × 5,000. **35:** Proximal view of a dispersed *Protohaploxypinus* grain showing the taeniate cappa and girdling saccus, × 1,000. **36:** Saccus floor of a permineralized *Pinus* pollen grain showing endoreticulation attachment scars (arrow); the saccus cavity is to left, × 2,000.

interpretations of saccus structure. For example, serial sections through the same permineralized corystosperm grains indicate that although the grains are definitively eusaccate, as observed in medial sections (Figs. 4, 27), if lateral sections are examined the grains morphologically appear protosaccate (Fig. 30). This structure results from the sacci tapering off laterally where they approach attachment to the corpus. This sectioning phenomenon is also convincingly illustrated in comparable medial and lateral sections through the sacci of extant *Pinus* pollen (Figs. 33–34) as well as compressed grains of *Caytonanthus* (Fig. 31).

It is also possible that the ontogenetic age of pollen grains may contribute to their characterization as protosaccate. For example, in the dispersed protosaccate grains of *Triadispora*, the sacci are relatively small (10–12 μm wide) and grains are often found in tetrads, a condition suggesting dispersal at this stage (Scheuring, 1976). The possibility exists that these grains were superficially protosaccate only, because either the sacci had not yet fully expanded or they were developmentally destined to remain small in size. In extant *Pinus* pollen (e.g., Dickinson and Bell, 1970; Willemse, 1971), endoreticulations separate from the corpus wall while grains are in the late tetrad stage; however, *Pinus* sacci clearly expand during subsequent developmental stages to attain relatively large sizes. This is also illustrated in the mature pollen of Middle Eocene *Pinus*, where the corpus wall lining the saccus floor shows separation scars indicating the former attachment sites of endoreticulations (Fig. 36). Another indication that saccus size may be important in whether or not the endoreticulations tear away from the nexine is the fact that the only extant genus with protosaccate pollen, *Dacrydium* (Podocarpaceae), is also characterized by small sacci (Pocknall, 1981; Médus et al., 1989).

Finally, endoreticulation size may also play a role in the morphological determination of a particular grain as 'eusaccate' or 'protosaccate'. For example, corystosperm grains may be 'eusaccate' because of the delicate nature of their endoreticulations (Figs. 27, 30) which would conceivably be easily ruptured, or separated, from the underlying nexine during saccus expansion. Although serial sections through *Caytonanthus* grains indicate that they are eusaccate, the sacci are small and still densely filled with thick endoreticulations (Figs. 10–11, 31). Despite the fact that the sacci of *Caytonanthus* are smaller and more extensively filled than those of corystosperm grains, the relative robustness of *Caytonanthus* endoreticulations may preclude their separation from the corpus wall to the degree observed in other grains.

Conclusions

The present paper underscores a conservative approach in assessing the relative emphasis that should be placed on the morphology and ultrastructure of fossil pollen. Based on the above discussion, we encourage less 'homologizing' of particular exine features in fossil pollen, especially when those data are taken from published micrographs in the literature. For example, an important consideration concerns interpretations of the nexine, specifically the endexine (nexine 2) and the ectexinous footlayer (nexine 1). Nexine layers of fossil gymnosperm grains are highly variable in their affinities for TEM stains, and clearly in the presence or absence of lamellations. In fact, it is distinctly possible that the endexine *per se* is absent from many fossil grains. In an analogous system, in many extant pollen types the underlying intine frequently acts as support for the endexine and, consequently, both are lost following acetolysis (Blackmore and Crane, 1988). It is not unlikely that this loss could also occur during lithification in the case of fossil grains, and thereby calls into question the utility of such characters as "endexine present or absent" (e.g., Doyle and Hotton, 1991).

Although the primary emphasis here has been on developmental and functional effects on pollen form, as well as geological and methodological influences, the phylogenetic element may also be important. One of the most challenging aspects in the study of fossil pollen ultrastructure is gauging the relative importance of potentially phylogenetically significant data while taking into account, and either appropriately weighing-in or filtering-out, the other influences that may magnify or mask characters that are valuable in phylogenetic analyses. Therefore, when only a few, random sections from a single grain or a small number of grains have been studied, it is objectionable to make phylogenetic inferences about various ultrastructural characters observed in fossil pollen.

Acknowledgements

This investigation represents a portion of a dissertation submitted by the first author in partial fulfillment of the requirements for the Ph.D. degree from The Ohio State University (OSU). This study was supported in part by a Doctoral Dissertation Improvement Grant from the National Science Foundation (NSF; BSR–9016397), a student scholarship from the American Association of Stratigraphic Palynologists, and a Presidential Fellowship and a Graduate Student Alumni Research Award both from the Graduate School at OSU to JMO, as well as NSF grant DPP-9118314 to TNT. We thank Ruth A. Stockey (Botany Department, University of Alberta, Canada) for providing fossil pine material.

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