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Evolutionary significance of granular exine structure in the light of phylogenetic analyses

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ABSTRACT

In 1973 Van Campo and Lugardon recognized granular structure as a third major type of exine structure in seed plants, in addition to columellar (restricted to angiosperms) and alveolar (restricted to other seed plants, such as cycads and saccate conifers). Because they found granular structure both in other seed plants (non-saccate conifers, Gnetales) and in angiosperms (some Magnoliales, monocots, and "Amentiferae"), they suggested it might be ancestral in angiosperms. This suggestion was elaborated by other workers and supported by studies of Le Thomas and Lugardon on Annonaceae (Magnoliales), which appeared to show origin of columellae by various modifications of granules. Phylogenetic (cladistic) analyses of seed plants based on morphological and molecular data modify this scheme considerably but reaffirm the systematic interest of granular structure. In conifers and Gnetales (which probably form a clade), granular structure appears to be derived from alveolar (as in Pinaceae, Podocarpaceae and extinct outgroups of conifers). Molecular analyses root the phylogenetic tree of angiosperms among *Amborella*, Nymphaeales, and Austrobaileyales, which have columellar and related exine structures, implying that granular exines were derived within angiosperms. This contradicts earlier views that granular structure provides evidence for a relationship between angiosperms and outgroups such as Gnetales and Bennettitales. Phylogenetic analyses indicate that granular structure was derived from columellar within Magnoliales and Laurales, in each of which it is an important synapomorphy of a major subgroup; the same may also be true for Fagales. However, phylogenetic analyses of Annonaceae confirm that granular structure is ancestral in this group and columellar is derived, essentially as a reversal to the ancestral state in angiosperms. In Fagales granular structure is associated with wind pollination, but not in Magnoliales and Laurales; however, in all three cases it may be correlated with reduction in exine thickness.

1. Introduction

One of the most important scientific contributions of Bernard Lugardon was his article with Madeleine Van Campo recognizing granular exine structure (structure grenue) as a third major type of infratectal structure in seed plants (Van Campo and Lugardon, 1973). Two years earlier Van Campo (1971) had clarified the previously confused distinction between columellar structure, with a layer of radial columellae connecting the nexine and tectum, which is restricted today to angiosperms, and alveolar structure, with chambers surrounded by more or less complete partitions, which is found only in non-angiospermous seed plants (gymnosperms), such as saccate conifers (Pinaceae and Podocarpaceae), cycads, and *Ginkgo*. Van Campo and Lugardon (1973) recognized that several groups of both angiosperms and other seed plants (including Gnetales and non-saccate conifers such as Araucariaceae and Cupressaceae: Plate I, 1–4) fit into neither of these categories, having instead more or less densely aggregated subspherical granules below the tectum. Discovery of granular structure had to await the development of transmission and scanning electron microscopy (TEM, SEM), since the granules are too small (generally 30–80 nm) to be resolved by light microscopy (LM) and are visible only in TEM sections or SEM images of broken exines.

2. Pre-molecular views on the evolution of granular structure

Because granular structure differed from columellar and alveolar structure in occurring both in angiosperms and in other seed plants, Van Campo and Lugardon (1973) suggested it might be ancestral in angiosperms. Most of the taxa where they recognized granular structure from published reports and their own studies were "Amentiferae" such as Betulaceae and Juglandaceae (Fagales sensu APG, 1998, 2003; Plate I, 5). They listed several supposed "more or less gymnospermous" characters of these plants, but they acknowledged that most botanists had come to interpret them as highly advanced. Another group where they identified granular structure, Apocynaceae (Asteridae; Plate I, 6), was universally thought to be advanced. But in a survey of monocots and putatively more primitive "Magnoliidae" (in the original paraphyletic sense of Takhtajan, 1966; Cronquist,

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1968), they also found granular structure in Annonaceae (Plate I, 7), in the Magnoliales, then widely considered the most primitive of all angiosperm orders.

The hypothesis that granular structure was ancestral in angiosperms was reaffirmed by Lugardon and Annick Le Thomas (Le Thomas and Lugardon, 1974, 1976; Le Thomas, 1980/1981), who documented the presence of granular structure in additional Annonaceae. These exines appear glassy under LM; in his broad LM and SEM survey of pollen of Annonaceae, Walker (1971) had described them as "microtectate." As further evidence, Le Thomas and Lugardon (1976) and Le Thomas (1980/1981) described a series of transitional structures within Annonaceae, with mixed granules and columellae and/or aggregated or enlarged granules that approach columellae in form.

In a study on ultrastructure of Early Cretaceous angiosperm pollen, Van Campo, Lugardon, and I (Doyle et al., 1975; Fig. 1) reviewed exine structure concepts and elaborated the argument that some occurrences of granular structure are derived from columellar, notably those in Fagales. Triporate pollen with granular structure, known in the dispersed state as the Normapolles group, which intergrades with typical fagalean pollen in the Tertiary, appears only in the Late Cretaceous, after a long period during which angiosperms were represented first by monosulcate and then by tricolpate and tricolporate pollen (Doyle, 1969; Muller, 1970; Wolfe et al., 1975; Doyle, 1978). This picture was subsequently strengthened by discoveries of in situ Normapolles pollen in Late Cretaceous flowers from Europe and North America, which appear to represent extinct lines within Fagales (Friis, 1983, 1984; Friis et al., 2006b). Conversely, Early Cretaceous monosulcate and tricolpate pollen types have been associated with fossil flowers that can be related to living groups of "magnoliids" and "basal eudicots" (such as Platanaceae and Buxaceae), respectively (Crane et al., 1995; Friis et al., 2006a).

Soon after Van Campo and Lugardon (1973) recognized granular structure, Walker and Skvarla (1975) proposed a related scheme, based on their discovery that some Magnoliales, namely *Degeneria* (= Degeneriaceae) and *Eupomatia* (= Eupomatiaceae), have essentially homogeneous exines that they termed atectate. They argued that these were even more primitive than granular exines, which they regarded as the next step in a trend leading to columellar. They proposed a similar trend in other seed plants, beginning with atectate but leading through granular to alveolar structure. Walker elaborated these views in subsequent articles (Walker, 1976; Walker and Walker, 1984), in which he documented the diversity of exine structure in other "magnoliids."

These views were questioned by Doyle et al. (1975), who suggested that atectate structure was an extreme form of granular structure, derived from types in which the granules are so closely aggregated that they are almost indistinguishable, as in some Annonaceae (Van Campo and Lugardon, 1973; Le Thomas and Lugardon, 1974; Plate I, 7). They also cautioned that other "magnoliids" such as Winteraceae combine putatively primitive features such as vesselless wood with columellar structure, blurring the argument based on association of atectate structure with other primitive characters.

Doyle et al. (1975) and Doyle (1978) also questioned the hypothesis that atectate or granular exines were ancestral in non-angiospermous seed plants. They noted that presumed plesiomorphic fossil relatives of living conifers, namely Cordaitales and Late Paleozoic lebachian or "voltzialean" conifers, have alveolar structure, whereas granular structure occurs in modern families that do not appear until the Mesozoic. In putative Devonian relatives of seed plants (progymnosperms), Doyle et al. (1975) interpreted spores of *Archaeopteris* as granular, based on Pettitt (1966), but closer examination of Pettitt's figures led Doyle (1978) and Doyle and Donoghue (1986) to reinterpret them as alveolar, with many small alveolae forming a spongy mass, as in Carboniferous medullosan "seed ferns" (Taylor, 1978; Taylor and Rothwell, 1982; Osborn and Taylor, 1994). In Doyle (1978) I termed this structure "spongy alveolar" to distinguish it from types with better-defined radial alveolae extending most or all of the way from nexine to tectum, as in both non-saccate cycads and *Ginkgo* and living and fossil saccate taxa, which I called "honeycomb alveolar." Taylor and Rothwell (1982) showed that these two types differ in their development in medullosans and cycads. Unfortunately, this distinction is somewhat subjective and has caused much confusion and apparent conflict in the literature, as noted by Osborn and Taylor (1994).

The view that granular exine structure was ancestral in angiosperms appeared to be supported by the first attempts to apply cladistic methods to seed plant and angiosperm phylogeny, using morphological data. Phylogenetic analyses of seed plants by Crane (1985) and Doyle and Donoghue (1986), which represented angiosperms as a single taxon with generalized "magnoliid" features, placed angiosperms with living Gnetales and fossil Bennettitales and *Pentoxylon* in a clade called "anthophytes," which was in turn linked with "Mesozoic seed ferns," variously including glossopterids (actually Permian), *Caytonia*, and corystosperms. One of the characters linking angiosperms with other anthophytes was granular structure (lumped with columellar in Doyle and Donoghue, 1986), which was inferred to be derived from alveolar structure, the type found in the Mesozoic seed fern taxa (Taylor et al., 1984; Pedersen and Friis, 1986; Zavada, 1991; Osborn and Taylor, 1993, 1994). In a subsequent analysis of angiosperms that used other anthophytes and *Caytonia* to define ancestral states, Donoghue and Doyle (1989) found that the most basal angiosperms (the sister group of all the rest) were Magnoliales. The two features that supported this result were granular infratectal structure and a continuous tectum, with the remaining taxa united by a shift to columellar structure and a reticulate tectum.

This picture was muddied somewhat in later seed plant analyses that included several angiosperm taxa (Nixon et al., 1994; Doyle, 1996). In Nixon et al. (1994), the angiosperm tree was rooted between either *Chloranthus* (Chloranthaceae) or *Casuarina* (Fagales) and other angiosperms, whereas the analysis of Doyle (1996) inserted *Caytonia* (which has alveolar structure) above granular anthophytes as the sister group of angiosperms. However, both analyses implied that the ancestors of angiosperms had granular structure: in Nixon et al. (1994) angiosperms were nested within the granular Gnetales, and in Doyle (1996) the most basal line was Nymphaeales, which at the time were thought to be granular, and this state was inferred to be homologous with that in *Pentoxylon*, Bennettitales, and Gnetales.

A morphological cladistic analysis of Annonaceae by Doyle and Le Thomas (1996) supported the views of Le Thomas and Lugardon (Le Thomas and Lugardon, 1974, 1976; Le Thomas, 1980/1981) by placing *Anaxagorea* and other taxa with granular monosulcate pollen in a series of lines at the base of the family, and nesting groups with columellar monosulcate and tetrad pollen within the family.

Plate I. TEM sections of exines showing granular infratectal structure, from Van Campo and Lugardon (1973). (see on page 200)

1, 2. *Ephedra distachya* (Gnetales).
3. *Agathis alba* (Araucariaceae).
4. *Cupressus arizonica* (Cupressaceae).
5. *Juglans regia* (Juglandaceae).
6. *Nerium oleander* (Apocynaceae).
7. *Piptostigma calophyllum* (Annonaceae).
8. *Cyrtosperma hastatum* (Araceae). Scale bar = 1 µm. Cy = cytoplasm, En = endexine, F = foot layer, G = infratectal granules, In = intine, Or = orbicule, T = tectum.