

These data are potentially useful in a number of research areas, for example, in Taxonomy, pollination biology, apicultural improvement, natural resource management, and vegetation history.

A contribution to the ultrastructural knowledge of the pollen exine in tribe Inuleae Cass. (Asteraceae)

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The Inuleae *sensu stricto* comprise 38 genera and 480 species, mainly with a Eurasian and Mediterranean distribution (ANDERBERG, 1994). Using light microscopy, STIX (1960) and LEINS (1968, 1971) studied the pollen exine wall structure of five genera and 11 species included in the *Inula* pollen type, and 32 genera and 137 species representing 20 other pollen types. The only transmission electron microscope studies of pollen exine in the Inuleae were carried out by SKVARLA & TURNER (1966) (*Inula britannica*) and SKVARLA et al. (1977) (*Alagoppapus dichotomous*, *Blumea mollis*). These investigators pointed out the existence of a senecioid type wall structure in the pollen grains of the 3 taxa that they studied.

As a contribution to the knowledge of pollen wall structure in this tribe, we have investigated, with transmission electron microscopy, the pollen exine in the following taxa: *Asteriscus maritimus*, *Dittrichia viscosa*, *Pallenis spinosa*, *Limbaria crithmoides*, *Nauplius aquaticus*, and *Pulicaria paludosa*. In some cases, this study was complemented by scanning electron microscope observations of fractured exines. In the pollen of all the taxa examined, a well-defined senecioid pattern is noted. The spines are not solid, but have distinct structural modification for about 1/2-3/4 of their length. The only exception is the pollen of *A. maritimus* where only very reduced caecae were observed.

In general our results agree with those of LEINS (1968, 1971) and SKVARLA & col. (1966, 1977).

According to BOLICK (1978), EL-GHAZALY & ANDERBERG (1995), and ZAVADA & VILLIERS (2000), the reduction of the caecae and foramina indicates that the pollen of the Inuleae is primitive within the Asteroideae.

In our opinion, the unusual development of the caecae of *Asteriscus maritimus*, when compared with that of the other Inuleae, represents an adaptation to the especially harsh conditions of habitat for this species (seaside rocks exposed to the action of sun and salty wind). In fact, greater caecae constitute an efficient harmomegathic device, and are a good place for the accumulation of hydrophobic substances, for preventing the dessication of the male gametophyte.

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Orbicules: intriguing features of the anther

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In the anthers of flowering plants, gymnosperms, and seed ferns, tiny ($\pm 1 \mu\text{m}$) granules may occur on the radial and innermost tangential wall of secretory tapetum cells. These granules are called orbicules or Ubisch bodies. Orbicules develop simultaneously with the growing pollen exine and are composed of sporopollenin, similar to the pollen exine.

In higher plants, the ornamentation of the pollen exine and that of the orbicule wall often show striking analogies. These parallelisms are rooted in the homology of tapetum and sporogenous tissue. Since ornamentation of the pollen exine offers useful characters for systematics, orbicules might also have taxonomic value. We studied the ontogeny, diversity and systematic importance of orbicules in the order Gentianales with scanning and transmission electron microscopy. A typology based upon the morphological and ultrastructural diversity was worked out. The proposed typology offered a useful tool to evaluate the systematic importance of orbicule characteristics in the families of Gentianales. In Apocynaceae s.l. and Gentianaceae orbicule characters seemed to be strongly correlated with evolutionary trends in pollen and tapetum type features.

As mentioned above, orbicules are considered to be a general feature of species characterised by a secretory tapetum. An interesting fact is that hay-fever-causing angiosperms are characterised by the presence of secretory tapeta. We investigated the possible role orbicules may play as vector of allergens. The presence of orbicules was studied in 15 important European allergenic species. In all Betulaceae, Chenopodiaceae, Poaceae, and Urticaceae species, orbicules were attached to the pollen exine, indicating the possibility of dispersal of orbicules into the atmosphere during anthesis. The localisation of homologs of the new birch allergen *Bet v 7* was studied at the ultrastructural level in Hazel anthers using immunocytochemical staining methods. The results of this study showed that orbicules of Hazel might act as effective vectors for homologs of *Bet v 7* and that the debris of Hazel anthers represent another vector of allergens after the pollen season.

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EVOLUTION OF ANGIOSPERM POLLEN CHARACTERS

Pollen evolution in cordaites and early conifers

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Modern conifers, according to S.V. Meyen (1984), represent the "summit" of evolution among gymnosperms possessing the most advanced seeds. It can be confirmed by the realizing the fact that they possess the most advanced pollen too. The origin of two main features of modern conifer pollen grains – strict distal germination and the eusaccus in bisaccate condition – should be understood to reveal the trend of pollen evolution in the clade of pinopsids.

Since R. Florin (1938 – 1945) it is generally accepted that conifers has originated from Euramerian cordaites (the Cordaitanthaceae). Florin has derived *Potomiesporites*, monosaccate pollen of the earliest conifers from the cordaitalean pollen *Florinites*, but now it is highly doubtful, as *Florinites* possesses an eusaccus and a

single distal aperture, while *Potoniesporites* seems to be protosaccate prepollen. M. A. Millay and T. N. Taylor (1976) have shown that *Florinites* is rather a final stage of an independent evolutionary lineage within cordaites, which had began from the eumonosaccate prepollen of *Felixipollenites*.

Cordaitina prepollen produced by the both Angaran families of cordaites, i. e. the Vojnovskyaceae and Ruffloriaceae, seems to be more "primitive" possessing a single torus-like saccus with an ultrastructure which can be described as intermediate between protosaccate and eusaccate. But at the same time Angaran cordaites produced another type of pollen grains which could be designated as "tub-like" (*Acusporidatina*, *Cladaitina*, *Reticulatina* and other pollen genera). They were round or oval with a rectangular depression on the proximal side. The ectexine was rather thick especially at the distal side imitating a single protosaccus attached to the proximal side (subinfratorma Distalsaccini Döbner, 1970). Both ectexine surface and inner ultrastructure could be rather diverse in different genera, but the general absence of distal aperture in this type of palynomorphs indicates that it has to be assigned to prepollen. Surprisingly such prepollen occurs both in the Vojnovskyaceae and the Ruffloriaceae and even in some primitive Angaran conifers (*Kungurodendron*) suggesting a case of geographic parallelism.

Almost all other types of pollen grains produced by primitive conifers can be easily derived from *Potoniesporites*. The main phylogenetic "stem" of early conifers leads from the Emporiaceae, Utrechtiaceae and Thuydiaceae with *Potoniesporites*-prepollen to the Voltziaceae whose members have produced protobisaccate pollen grains assignable to *Triadispora* and *Alisporites*. Pollen grains of *Triadispora* seemed to possess double germination demonstrating proximal trilete mark together with distal aperture located between sacci, while *Alisporites* was more advanced pollen with a single distal aperture.

The Majonicaceae together with the close genus *Sashinia* represent most probably the "lateral branch" in the conifer phylogeny. Their pollen (*Laekisporites* of the Majonicaceae and *Scutasporites* of *Sashinia*) was protobisaccate and seemed to possess double germination too, although their proximal aperture looked differently than that of *Triadispora*.

Surprisingly *Nuskoisporites* (pollen grains of *Ortiseia*) looks more primitive than *Potoniesporites*, although *Ortiseia* seems to be the most advanced (and the youngest) member of the Utrechtiaceae. In having radial symmetry *Nuskoisporites* resembles *Cordaitina* and one can assume that all three genera have evolved from the common ancient predecessor.

The distribution of different types of pollen among early pinopsids suggests that the separation of conifers from cordaites (if it was a single event) has happened very early in the evolutionary history of cordaites, roughly at the same stage, when the cordaites themselves have been divided into three families (Cordaitanthaceae, Vojnovskyaceae, and Ruffloriaceae). The immediate ancestors of conifers had possessed apparently the *Cordaitina*-like pollen, which remained later only in the Angaran families of cordaites. Many features of coniferalean pollen grains (e. g. double germination, bisaccate condition, etc.) evolved most likely independently in several parallel lineages.

Origin of the angiosperms: Palynological evidence revisited

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Interpretation of fossil pollen distribution, and taxonomic position has played an important role in developing hypotheses on the origin, and early diversification of angiosperms. Benchmark investigations in the 1970's reporting the successive occurrence of significant fossil pollen types through the Lower Cretaceous, coupled with surveys of extant pollen morphology of primitive angiosperms, laid the foundation for proposing the Lower Cretaceous origin and diversification of angiosperms. Over the past 30 years, however, ultra-structural investigations (SEM and TEM) on extant pollen, and a wide range of fossil pollen types (including dispersed, and *in situ* pollen) from the Permian, Triassic and Jurassic, suggest that a majority of Triassic, and Jurassic pollen types are similar to one another and probably have gymnosperm affinities, however, pollen with angiosperm features does occur in low frequency through the Triassic, and Jurassic. This suggests an alternative interpretation of angiosperm origins. The data alternatively suggest a Triassic origin of angiosperms within the Atlantic rift zone. During the Triassic and Jurassic species origination rates may have equaled the extinction rates resulting in the sparse occurrence of angiosperm pollen types from different geographic areas in the Atlantic rift (i.e., angiosperms initially had an extended lag phase). It was not until the Lower Cretaceous that origination rates

exceeded extinction rates (expansion phase of a species), resulting in the diversification of angiosperms through the Cretaceous. The earlier origin of angiosperms permits the consideration of new sister groups to the angiosperms, is more consistent with the molecular data, and is supported by the mega fossil data. These data may also call into question the usefulness of pollen characters for tracking the earliest angiosperms.

Fossil and living zona-aperturate pollen

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A survey on recent and fossil zona-aperturate pollen grains (i. e., with ring-like apertures running either meridionally or equatorially) within the angiosperms was undertaken. New findings of recent and fossil pollen are presented, from which the fossils can be tentatively attributed to *Nymphaeaceae*, *Araceae* and *Iridaceae*. Zona-aperturate pollen has evolved several times independently among basal angiosperms (*Nymphaeaceae*, *Eupomatiaceae*, *Annonaceae*, *Monimiaceae*, *Atherospermataceae*) and monocots (*Araceae*, *Iridaceae*, *Lomandraceae*, *Arecaceae*, and *Rapateaceae*), but not in eudicots (except of *Limnanthes* with its peculiar 2+2-synsulcate ring, BUCHNER et al., 1990). A ring-like aperture may divide the pollen grains in symmetric halves, or if running asymmetrically, it divides the pollen grains in two unequal halves. The zona-aperturate pollen generally differ in ornamentation and stratification not only in the respective apertural areas, but sometimes also outside the aperture. The polarity question is settled not for all taxa because of the lack of tetrad observations, but known for *Nymphaeaceae*, *Eupomatiaceae*, *Atherospermataceae*, some *Arecaceae*, and *Rapateaceae*. However, by conclusion from analogy of asymmetric halves, in *Araceae* and *Iridaceae* the apertural rings will probably run equatorially.

Is it not amazing that a ring-like aperture occurs in at least ten extant angiosperm families, more precisely in basal dicots and monocots? This apparently naive statement posed several questions, for instance: Why in so many families? Why does it occur in isolation within a family where most pollen are sulcate or inaperturate, as in *Annonaceae*? Why not in the eudicots? The zona-aperturate pollen type (fully zonate) is restricted to (some) basal angiosperms and some monocots families/genera, and is lacking in eudicots (except of the somewhat differing case of *Limnanthes*). The type is found also in fossils from the Cretaceous/Tertiary boundary (ZETTER et al., 2001). It might be a relict of early angiosperms, before the arrival ("invention") of the 3-colporate-configuration. Before the advent of eudicots-tricolporates an aperture ring was perhaps the best way for a target-oriented harmomegathic movement, i. e., to expand or to close a large area adapted for possible pollen tube formation/pollen germination. The apertural intine, the endexine (if present) and the reduced ectexine (likewise if present) form together with an "oncus-like" layer a very elastic band around the pollen grain. This band is elastic like an accordion or concertina. In dry state, the band is closed, in hydrated condition expanded, the ring opens widely, and the pollen germination can take place everywhere. Hence, the harmomegathic movement of expanding and closing the ring-like aperture is possible by the observed high degree of expansion. In contrast to operculate conditions, which might be of benefit in dry climates (preventing from excessive dryness), the zona-aperturate condition might be a benefit for taxa in humid environments.

More questions arise: why does, for example, the spectacular zona-aperturate condition occur alongside other aperturate conditions within the same genus? And why is the aperturate conditions sometimes not stable within a species, or even within a single pollen sac? A fine example is the case of *Rhaphidophora africana* where most of the pollen grains are extended sulcate to bisulcate (in SEM), but fully pollen also occur (GRAYUM, 1992: "separating into halves"). The percentage of fully zonate/bisulcate pollen may vary between individual plants. In general, within large monocot families, the aperture conditions is often diverse, for instance in *Araceae* (GRAYUM, 1992) or in *Arecaceae* (HARLEY & BAKER, 2001): inaperturate, sulcate, trichotomosulcate, bi-aperturate and zona-aperturate pollen even within genera can be found side by side, and this diversity may have been "an experiment by the playing/ gambling/ toying of nature". This hypothesis might also explain the lack of genuine zona-aperturate pollen conditions in eudicots.

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**Pollen evolution must be considered in a phylogenetic context:
insights from Magnoliales and implications for Proteales**

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Magnoliales, consisting of six families and about 3000 species of tropical to warm-temperate woody angiosperms, were long considered the most archaic order of flowering plants. This assumption was based on a special concentration of characters believed to be primitive in angiosperms, including monosulcate pollen with granular exine structure. However, all recent higher-level phylogenetic analyses have shown that Magnoliales are not the earliest-diverging angiosperms, urging revision of the status of the putatively primitive features of this order.

Here we show the implications of a recent phylogenetic study of Magnoliales (SAUQUET *et al.* 2003) for pollen character evolution, with an emphasis on Myristicaceae (with nearly 500 species, the second largest family in this group after Annonaceae), which exhibit interesting variation in their pollen morphology (SAUQUET & LE THOMAS 2003). In several instances, this approach, using cladistic scoring and parsimony optimization of character changes on phylogenetic trees, turns completely upside down long-standing ideas on pollen evolution. In particular, columellar exine structure is unambiguously resolved as the ancestral state of Magnoliales whereas granular exine structure evolved later within the order, at least once in the ancestor of a clade of four families and once within Myristicaceae. Other characters, such as the shape of pollen grain in Myristicaceae, turn out to be too homoplastic to be given any phylogenetic significance. These results illustrate the crucial importance of studying pollen evolution in a broader phylogenetic context, using hypotheses derived from additional sources of data.

These considerations will be crucial in a current project to reconstruct the biogeographic history of another order of angiosperms, Proteales, a curious assemblage of three very different families (Nelumbonaceae, 1 species, Platanaceae, 10 species, and Proteaceae, 1700 species) branching at the base of the eudicot clade of tricolpate angiosperms in all recent higher-level molecular analyses. In particular, the assignment of the extensive pollen fossil record of Proteaceae to modern taxa of the family needs complete revision to use the benefits of the cladistic approach and new data on phylogenetic relationships within Proteaceae (e.g. HOOT & DOUGLAS 1998).

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**The angiosperm genus *Gunnera* in the Mesozoic: comparison between
Tricolpites reticulatus and pollen of extant *Gunnera***

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With its very complete Antarctic distribution in Africa, South America, Southeast Asia, Tasmania and New Zealand, the angiosperm genus *Gunnera* of 30-40 species, is a model example of a Gondwanan group, for biogeographical studies aiming to reconstruct Southern Hemisphere history. In 1948, the botanist O. Selling noticed a remarkable similarity between the pollen of the Hawaiian *Gunnera petaloidea* and the fossil pollen species *Tricolpites reticulatus*. Based on this similarity, and subsequently confirmed by the studies of COOKSON & PIKE (1954) and JARZEN (1980), we know that *Gunnera* already occupied its present range of distribution by the Late Cretaceous (c. 80 my). Several reports of *T. reticulatus* also support the previous presence of *Gunnera* in other localities such as North America and India where *Gunnera* is almost, or entirely, absent today. A recently conducted morphological study based on scanning electron microscopy (SEM), identified three main types of pollen in extant species of *Gunnera*. Pollen type 1, characterised by an imperfect reticulum with sinuous undulating-crested muri, was identified only in the South American *G. herteri*. Pollen type 2, distinguished by a reticulum with equi-dimensional polygonal lumina, was found in the African species, *G. perpensa* of subgenus *Gunnera*, in the South American species of subgenus *Misandra*, as well as in the South American/Hawaiian subgenus Panke. Lastly, species from subgenera *Milligania* and *Pseudogunnera*, from New Zealand/Australia and South East Asia, respectively, have another type of pollen (type 3) which has a reticulum with lumina of variable shape and size. Within this type, two subtypes, a and b, were defined. Subtype 3a occurs in subgenus *Pseudogunnera*, here the lumina of the apocolpia are of a different shape and size from the lumina of the mesocolpia, while subtype 3b, represented by species of subgenus *Milligania*, has a reticulum with lumina identical in both the mesocolpia and the apocolpia.

The results of a comparative study between species of *Gunnera* showing these pollen types, and fossil specimens of *T. reticulatus* from the Antarctic Peninsula, the Kerguelen Plateau and southeastern Australia are described and discussed. According to the results of the study, there is a clear similarity between the Antarctic specimen from Vega Island (dated late Campanian- Maastrichtian) and pollen type 3, found in the Malayan species *G. macrophylla*, and in the species from New Zealand and Tasmania. The Australian fossil specimens from Beach McNamara and Beach Princes (both dated early to late Maastrichtian or early Danian) are different in morphology and could support a complex history for *Gunnera* in Australia. While the Beach McNamara specimen shows affinities to pollen type 3a of subgenus *Milligania*, the specimens of Beach Princes are more similar to pollen type 2, found in subgenera *Gunnera*, Panke and *Misandra*. Specimens from the Miocene of the Kerguelen Plateau (southern Indian Ocean) are very similar to pollen type 2 and this is in contradiction to previous results which had claimed a closer similarity between these specimens and the pollen of *G. macrophylla*. The comparison between pollen of extant species and fossil pollen can help our understanding of the biogeographical patterns of organisms. This study aims to enhance our comprehension of the biogeography of *Gunnera* and opens the door to new studies aimed at dating the phylogeny of the group.

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**Pollen evolution in *Amaranthaceae* - insights from electron microscopy
and phylogenetic analyses**

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Amaranthaceae s.s. (1000 species in approx. 70 genera, mainly tropical distribution) and Chenopodiaceae (1400 spp. in approx. 100 genera, chiefly temperate) together represent the most species-rich monophyletic clade (recently classified as Amaranthaceae s.l.) in the angiosperm order Caryophyllales. Pollen morphology in Amaranthaceae is most diverse and some complex features such as stellate pore ornamentation are found nowhere else among angiosperms.

With the aim to provide a robust phylogenetic framework for analyzing the evolution of pollen, the complete chloroplast *trnK* intron (2700 characters, including *matK*) was sequenced for all major groups in Amaranthaceae s.s., for representatives from Chenopodiaceae, and for allied Caryophyllid families. Maximum parsimony, maximum likelihood, and Bayesian analyses revealed a basal grade of *Bosea* (Macaronesian islands, Cyprus, Himalaya), followed by *Charpentiera* (endemic to Hawaii and the Australian Ridge). While Celosieae are supported as natural, most other currently recognized infrafamilial taxa were revealed to be para- or polyphyletic. Within former Aervinae, an Achyranthoid and an Aervoid clade were recovered. The gomphrenoid genus *Pseudoplatago*, sharing some pollen- and other morphological features with Amaranthoideae, is found within Gomphrenoideae rather than being in a position linking both subfamilies.

A SEM survey of pollen in Amaranthaceae s.s. (covering about 25% of the species) and selected Chenopodiaceae, accompanied by TEM studies, led to numerous pollen characters, the ancestral states of which were reconstructed on the molecular phylogeny. According to this analysis, spheroidal, pantoporate pollen with flat mesoporia and subglobose ectexinous bodies that are evenly spread on the aperture membrane is plesiomorphic in Amaranthaceae. The recently described metareticulate pollen architecture (with meshes of the reticulum being homologous to mesoporia) is reconstructed to have evolved once in the ancestor of a highly supported core Gomphrenoid clade that comprises the traditional Gomphrenoideae but excludes *Iresine*. Hook-shaped and stellately arranged ectexinous bodies (stellate pore ornamentation) appear to have evolved at least twice in parallel. Unlike other morphological characters used in previous classification systems, pollen characters in general represent morphological synapomorphies circumscribing many clades recovered with molecular data.

Olacaceae and other Santalales: contributions from palaeopollinology and phylogeny

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Olacaceae are the most primitive members of the order Santalales. An order characterised by the inclusion of most of the woody hemiparasitic angiosperms. In fact the Olacaceae is the only family in the Santalales containing autotrophic taxa. In the pollen fossil record the genus *Anacolosidites*, remarkable for its tri-diploporate morphology, is attributed to the Olacaceae, in particular to the genera *Anacolosia*, *Cathedra*, and *Ptychopetalum* which share very similar pollen morphology with the fossil genus. The earliest records of *Anacolosidites* are Maastrichtian. Other fossil pollen genera associated with the Santalales: *Loranthacidites*, *Gothanopollis*, and *Florschuetzia*, are also abundant from the Late Cretaceous onwards.

We have revised *Anacolosidites* using morphometrical tools in order to identify fossil pollen records that may be linked to pollen of current day genera of Olacaceae, based on our own observations plus three previous palynological surveys of the family. At the same time studies of the phylogeny of the Santalales using molecular and morphological data have also been carried out.

Only a few records of *Anacolosidites* appear to be erroneously attributed to Olacaceae, while the majority may well be ancestral to the extant genera *Anacolosia*, *Cathedra*, and *Phanerodiscus* from tribe Anacolosae; but not *Ptychopetalum* from tribe Olaceae. The particular distribution of *Anacolosidites* records through space and time allow us to postulate a biogeographic history compatible with an earlier proposed hypothesis of Olacaceae paleoecology. Since the Maastrichtian *Anacolosidites* records are associated with tropical or paratropical climates. The biogeographical history proposed is compatible with Wolfe's boreotropical hypothesis, especially when considering, not only a southward migration of tropical vegetation but also, more precisely, the reduction of the Eocene paratropical flora in the Northern Hemisphere, leaving only the current disjunct tropical areas.

An estimation of clade ages for our molecular phylogenetic hypothesis was realised using the Non Parametric Rate Smoothing method, calibrated with the oldest record of *Anacolosidites*. As an independent test of

age calibration, an earlier method of age estimation for the origin of the Santalales was also used. Combining age estimation of cladogenetic events with the current distribution of Olacaceae, results in a hypothesis of 'vicariance' events which also agrees with Wolfe's modified boreotropical hypothesis for most of tribes of Olacaceae. For a few genera with disjunct distributions some 'long distance' dispersal events have been identified during the Miocene.

These studies, applied to other families within the Santalales, offer opportunities to combine phylogenetic, palaeobotanical, palaeoclimatic and palaeogeographical data, thus providing a new hypothesis on relationships between earth history and cladogenetic events. In particular suggesting that taxa with Gondwanan distributions or, more precisely, with endemic sub-taxa in tropical Africa, Southern Africa and Asia, are not only the result of tectonic events, but also follow a modified boreotropical hypothesis.

Single pollen grains to tetrads and pollinia in Apocynaceae

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The two families Apocynaceae and Asclepiadaceae were grouped together into one family, the Apocynaceae s.l. (Endress & Bruyns 2000). Within the family Apocynaceae s.l. five subfamilies are recognized: Rauvolfioideae Kostel. (rather than Plumerioideae K. Schum.), Apocynoideae Burnett, Periplocoideae R. Br. ex Endl., Secamonoideae Endl. and Asclepiadoideae R. Br. ex Burnett. The Asclepiadoideae comprise four tribes: Fockeae H. Kunze, Meve & Liede, Marsdenieae Benth., Ceropogieae Orb., and Asclepiadeae (R. Br.) Duby (Endress & Stevens 2001).

The Rauvolfioideae typically have the corolla lobes sinistrorsely contorted in bud, the anthers are free from the style head, and 3- or 4-colporate pollen grains. The Apocynoideae are characterized by having the corolla lobes dextrorsely contorted in bud, anthers adnate to the style head, and 3-porate grains. The Periplocoideae are characterized by having tetrads (in most genera) or free pollinia (four per anther) which are shed onto translators. The pollinia consist of tetrads and are not covered by a pollinium wall. The translator consists of an adhesive disc, a stalk and an adhesive lined receptacle (onto which the tetrads or pollinia are shed at anthesis). The Secamonoideae are characterized by having five pollinaria each of which consists of four pollinia (consisting of tetrads and not covered by a pollinium wall). The pollinia are attached to a corpusculum by one or more caudicles. The manner in which the pollinia are attached to the translator is variable in this subfamily. The Asclepiadoideae are characterized by having five pollinaria, each of which consists of two pollinia, each attached to the corpusculum by a caudicle. The pollinium consists of single pollen grains and is surrounded by a pollinium wall. The genus *Fockea* Endl. (tribe Fockeae) differs from the other tribes in that the pollinium consists of tetrads, and is not surrounded by a pollinium wall.

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Phylogenetic analyses and the early evolution of pollen characters in angiosperms

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Phylogenetic analyses based on molecular and morphological data suggest resolutions of many long-standing controversies on the early evolution of angiosperm pollen morphology and ultrastructure.

Despite continued uncertainty on relationships between angiosperms and other seed plants, the increasingly robust rooting of the angiosperm phylogenetic tree among the "ANITA" groups (*Amborella*, *Nymphaeales*, *Illiciales*, *Trimenia*, *Austrobaileya*) clarifies the original pollen morphology of angiosperms. Contrary to hypotheses that the first angiosperms had granular exine structure, the ANITA rooting implies that

globose monosulcate pollen with irregular columellar structure and a continuous tectum was ancestral, and regular columellar structure and a foveolate-reticulate tectum arose soon after. The oldest recognized Cretaceous angiosperm pollen may represent this grade of evolution. Granular structure was independently derived from columellar within Nymphaeales, Magnoliales, and Laurales, as well as in "higher" eudicots such as Fagales (Amentiferae). This agrees with developmental evidence that granular structure in Nymphaeales is a modification of columellar structure. These results refute one of the arguments for a relationship between angiosperms and Gnetales, Bennettitales, and *Pentoxylon* (the anthophyte hypothesis), namely the granular exine structure of these groups. Conversely, they remove obstacles to a relationship with "seed ferns" with alveolar exine structure (e.g., *Caytonia*, glossopterids) and/or Triassic Crinopollen (with reticulate-columellar structure and thick endexine), which seemed less likely when granular structure was assumed to be primitive.

Magnoliales have been traditionally considered primitive but now appear to be a more derived early line. In Myristicaceae, members with granular exine structure are nested among columellar taxa and thus appear to be derived. Myristicaceae and Magnoliaceae (which are also columellar) are basal to *Degeneria*, *Galbulimima*, *Eupomatia*, and Annonaceae, which are united by a shift to granular structure. Within Annonaceae, granular monosulcate pollen (as in *Anaxagorea*) is ancestral, but it gave rise to columellar monosulcate and disulcate single grains and inaperturate tetrads, some of which underwent reversals to single inaperturate grains. The reticulate monoporate tetrads of Winteraceae were derived from foveolate-reticulate monosulcate pollen of the type seen in their sister group, Canellaceae. In Laurales, after an early shift to inaperturate pollen, the exine underwent stepwise reduction and granularization, culminating in the fragile exines of Lauraceae.

Session c1

BASIC AEROBIOLOGY/ MONITORING/ NEW TECHNIQUES: POLLEN

The use of a "solid phase cytometer" for a monitoring application to pollens and molds counts

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The solid phase cytometer (Chemscan system) is an ultrasensitive microbial analyser, designed to date and count microbial cells on a solid surface. The sensibility is sufficient to detect one cell. The solid surface may be a filter membrane or a glass slide.

Cells are labelled with fluorescent markers and analysed with a laser scanning system using a sophisticated discrimination process that can discriminate labelled cells from background environment such as autofluorescence particles. The process of cell labelling and laser scanning can be completed within one hour of sample collection.

This method is already validated for monitoring of process water in pharmaceutical industry and more recently for the detection of the airborne microorganism.

For monitoring pollen and molds, we work in three steps :

- Examination of the autofluorescence of pollens. That permit to count the total number of pollen of the sample in a few minutes. The sensibility and the reproducibility are very good.
- Production of specific rabbit antibody for the main allergenic or not particles that we want to study: betula, dactylis, parietaria, urtica, alternaria. These antibodies are controlled and conjugated with FITC (fluorescein isothiocyanate conjugated)
- Specific analysis of each kind of particles to study their own spectrometry (red and green).

Then mixing two and three kind of particles we can discriminate each specific particles and as they are fixed on a membrane it is possible to count them with a very high precision (one grain).

The last trials must help us to discriminate urtica and parietaria.

May the definition of Pollen Season influence aerobiological results?

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Pollen Season, Period of Maximum Pollen Production, Pollination Season, Pollination Period, Main Pollination Period, Main Pollen Season, Principal Pollination Period, Effective Pollen Season, Atmospheric Pollen Season, are amongst the terms most frequently used in the aerobiological literature to define the period of time in which most of the annual total pollen concentration is recorded. Similarly, different criteria are used to delimit this period, depending on the author, the pollen type and geographical area involved. The period is most commonly defined as the time of the year in which a certain percentage of the annual total pollen is recorded, the most frequent values used being 90%, 95% or 98% (Nilsson and Persson 1981, Torben 1991, Galán et al. 1995). In other cases, the period is defined in terms of the time elapsing between two days on which specific values or conditions are registered (Mullenders et al. 1972, Lejoly Gabriel & Leuschner 1983, Pathirane, 1975, etc.).