(SS10) Exine development and pattern formation, unifying ultrastructural and genetic approaches

Date: August 28
Place: Room 5336 (oral), Room 6302 (poster)
Organizers: Stephen Blackmore, Nina Gabarayeva & Michael Hesse
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Purpose: The science of palynology is founded entirely upon the extraordinary organisational diversity and resistance to decay of the exine. Not surprisingly therefore, there has always been great interest in understanding how the complex, elaborate and often taxon-specific patterns of exine organisation are developed and have evolved. Traditionally these questions have been addressed by microscopy: first optical microscopy and later electron microscopy. There has also been a strong interest in the theoretical basis of pollen and spore symmetry control, number and placement of germination sites, and surface pattern formation. However, in spores the control of perispore (or perine) sculpturing remains poorly understood with more information urgently needed. More recently, there have been dramatic advances in the molecular genetics of pollen development based on insights from the model plant, *Arabidopsis*. Much of this new research has been undertaken in Japan.
The symposium aims to bring together experts from the ultrastructural, theoretical and genetic research areas in order to develop a unified understanding of exine organisation. In doing so it hopes to overcome the tendency in modern science for disciplines to specialise and diverge, each developing its own audience and terminology. Whilst the symposium will be primarily of interest to those with an interest on the development of pollen grains and spores, the insights it generates will also assist in interpreting forms encountered in palaeopalynological or systematic investigations.

Oral Presentation

Aug. 28 [PM2] Room: 5336

Chair: Nina Gabarayeva

14:30-14:50 **[Introduction to the Symposium SS10] Reviewing the past, present and future of exine development** SS10-O01 (37)

Stephen Blackmore

14:50-15:10 **Pollen structure and development in Nymphaeales: Insights into exine evolution in an ancient angiosperm lineage** SS10-O02 (514)

Mackenzie L. Taylor, Jeffrey M. Osborn

15:10-15:30 **Microsporogenesis and exine substructure in *Trochodendron aralioides* Siebold & Zuccarini. (Trochodendraceae)** SS10-O03 (200)

Yu-Chwen Hsu, Wann-Neng Jane, Su-Hwa Chen

Chair: Stephen Blackmore

15:30-15:50 **Primexine development in *Passiflora racemosa* Brot. The hidden side of development** SS10-O04 (141)

Nina Gabarayeva, Valentina Grigorjeva, Yana Kosenko

Aug. 28 [PM3] Room: 5336

Chair: Stephen Blackmore

16:20-16:40 **Exine development in *Passiflora racemosa* Brot.: post-tetrad period. The hidden side of development** SS10-O05 (140)

Nina Gabarayeva, Valentina Grigorjeva, Yana Kosenko

16:40-17:00 **Genetic pathways for pollen wall formation in Arabidopsis** SS10-O06 (590)

Zhong-Nan Yang, Yue Lou, Xiao-Feng Xu, Cheng Zhang, Hai-Shuang Chang, Jun Zhu

17:00-17:20 **How plants conquered the land: An EvoDevo analysis of the spore wall** SS10-O07 (552)

S. Wallace, D. J. Beerling, A. Fleming, C. H. Wellman

Chair: Nina Gabarayeva

17:20-17:40 **[Concluding remarks to Symposium SS10]** SS10-O08

Stephen Blackmore

Poster Presentation

Aug. 28 [PM1] Room: 6302

13:30-14:30 **Sporoderm ultrastructure in *Anthoceros agrestis* Paton. (hornworts)** SS10-P01 (409)

Svetlana Polevova

Acetolysis test of the developing exine in *Nicotiana tabacum* L. SS10-P02 (410)

Svetlana Polevova, Natalia Matveyeva, Anna Smirnova, Igor Yermakov

Pollen morphology and localization of Ni in some species of *Alyssum* Ni-hyperaccumulators SS10-P03 (400)

Dolja Pavlova, Vicenta de la Fuente, Daniel Sánchez-Mata

SS10-O01 (37)

Reviewing the past, present and future of exine development

Stephen Blackmore

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This presentation provides an introduction to the symposium on “exine development and pattern formation, unifying ultrastructural and genetic approaches”. It will review the historical background to exine development, recognising an early phase of research based initially on optical microscopy and, later, on electron microscopy and an exciting recent phase, integrating evidence from molecular genetics and insights from colloidal behaviour and self assembly. A detailed understanding of exine development was only possible after the advent of electron microscopy and, interestingly, much of the early electron microscope research aimed at discovering universal phenomena or “common features of exine deposition”. More recently, as a greater number of taxa have been investigated the emphasis has switched to trying to understand the origins of the often taxon-specific characteristics of the exine. A similar situation applied following the advent of molecular genetic studies, with most initial work having focussed on the model plant *Arabidopsis thaliana* but with an ever wider range of plants being investigated. Slightly looser parallels can be drawn between some early mathematical

analyses of pollen symmetry, by Wodehouse and others, which sought to find underlying laws of pollen and spore organisation, and the impact of concepts from colloidal behaviour and self-assembly. We are now very much closer to being able to relate new findings on patterns of gene expression to the particulars of exine development and to place this in a functional and evolutionary context. Exine development remains of profound importance in plant reproductive biology and the field has an exciting future as the prospect of an experimental approach integrating ideas from models of self assembly, with a developmental genetic approach visualised through electron microscopy.

Keywords: pollen and spore ontogeny, sporopollenin, pattern formation, self assembly.

SS10-O02 (514)

Pollen structure and development in Nymphaeales: Insights into exine evolution in an ancient angiosperm lineage

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Nymphaeales, or water lilies, comprise a lineage that diverges from the basal-most, or second-most basal node of the angiosperm phylogenetic tree. Nymphaeales consist of three families (Cabombaceae, Hydatellaceae, Nymphaeaceae) with eight-to-nine genera and <100 species, all of which are aquatic. Despite having few taxa, Nymphaeales exhibit great variation in ecological and morphological traits and because of this, are particularly well-suited for studies of the early evolution of reproductive traits in angiosperms, including pollen characters. In this presentation, we will describe mature pollen characters for all water lily genera and developmental characters for select taxa. We will discuss the adaptive and phylogenetic significance of traits such as pollen size, exine layer ultrastructure and thickness, aperture morphology, and tapetum type. Pollen grains are dispersed as monads in all genera except *Victoria,* in which grains are held in permanent calymmate tetrads. Permanent tetrads and dyads also occur occasionally in *Victoria's* sister genus *Euryale*. Pollen grains are monosulcate in Cabombaceae, Hydatellaceae, and in *Nuphar*, the earliest-diverging genus in Nymphaeaceae, whereas all other Nymphaeaceae have a ring-like aperture. The exine is tectate-columellate in all water lilies, but the relative ultrastructure of each layer varies. For example, *Barclaya*, *Brasenia, Cabomba,* and *Trithuria* exhibit a thick infratectum and robust columellae, whereas those in 'core' Nymphaeaceae exhibit a thin infratectum and narrower columellae. *Cabomba* exhibits prominent tectal/columellar microchannels, as do some Nymphaeaceae; however, microchannels in Nymphaeaceae are distinct from those of *Cabomba.* All microchannels are first apparent in the free microspore stage, concurrent with tapetum disassociation. Pollen grains of two genera exhibit major sculptural elements: supratectal rods in *Cabomba* and spines in *Nuphar*. The spines of *Nuphar* are ultrastructurally different and have an earlier ontogenetic origin than the sculptural rods of *Cabomba*, or the minor tectal ornamentation of other water lilies. Tapetum ontogeny and structure also varies within water lilies, with secretory, amoeboid, and transitional types all present. This indicates that tapetal development is more labile in basal angiosperms than previously thought and that this lability was likely present early in angiosperm history. Nymphaeales exhibit a wide range of pollination mechanisms, including beetle, bee, fly, and wind pollination, as well as cleistogamy, and also variation in carpel structure. Thus, water lilies are an excellent system within which to study the functional correlations among pollen characters, pollination mechanisms, and post-pollination development. Potential correlations among dispersal unit size, exine ultrastructure, and pollination or post-pollination biology will be discussed.

Keywords: exine ultrastructure, Hydatellaceae, pollination, post-pollination development, tapetum.

SS10-O03 (200)
Microsporogenesis and exine substructure in *Trochodendron aralioides* Siebold & Zuccarini. (Trochodendraceae)

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This study aims to elucidate the anther wall development, pollen wall development and exine substructure of T*rochodendron aralioides* Siebold & Zuccarini, a tree lacking vessel elements in its wood. The anther wall development is of the basic type, which is comprised of an epidermis, an endothecium layer, three middle layers and a tapetum. Anther-tapetum is of the glandular type and the cells are uniseriate. Pollen grains are tricolporate and 2-celled at the time of shedding. Microspore mother cells undertake meiosis with simultaneous cytokinesis to produce tetrahedral tetrads enclosed within callose wall. Before protectum development begins, a glycocalyx layer is inserted against the callose, and the plasma membrane is invaginated, exclusive of the future apertures. Subsequently, the probacula are elongated under the protectum and arise basally from the plasma membrane. The foot layer and endexine formation are concomitant with the callose wall dissolution. The foot layer is thick, and the endexine is thin. The foot layer and the endexine both are continuous. The intine is initially formed in the vacuolated stage. The hollow Ubisch bodies are observed on the inner surface of tapetum, in anther locule and on pollen surface after the vacuolated stage. The numerous anthers of a single flower are at some different development stage both in protandrous and protogynous individuals.

Keywords: Ubisch body, anther wall development, callose.

SS10-O04 (141)
Primexine development in *Passiflora racemosa* Brot. The hidden side of development

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The aim of this study was to trace in detail pre-pattern stages and the primexine development during the tetrad period by TEM. At the most early tetrad stage the microspore surface has an intricate form: portions with numerous outgrowths undergo subsequent deep invaginations, forming long pockets – a typical picture of active endocytosis. The whole plasmalemma is covered by globular osmiophilic droplets with a cog wheel outline, which seem to be of tapetal origin. Having occurred inside invaginations of the plasmalemma, some of these lipoid droplets are engulfed in the process of endocytosis, others remain in the periplasmic space. At the next step a system of microfilament bundles and RER cisternae, disposed perpendicularly to the plasmalemma, were observed in the cytoplasm, ready to be involved in primexine patterning. In places, numerous Golgi vesicles promote the appearance of the initial glycocalyx in the periplasmic space. At the middle tetrad stage, due to

contraction of microfilaments, the plasma membrane becomes regularly invaginated. The glycocalyx fills up these invaginations, forming first cup-like clusters, then one-sided convex clusters, resembling a lens in their form: these are sites of future lumina of the exine reticulate pattern. At the next step the glycocalyx consists of two layers: a new, more dense inner layer appears all around the plasma membrane surface. The two glycocalyx layers consist of distinct radially oriented worm-like units. At last, young columellae appear in the inner glycocalyx layer: free columellae, predestined to be located on the bottom of the future lumina, are formed under lens-like clusters, whereas larger columellae, covered with tectum – the constituents of the future muri - are layed down between these clusters. Some portions of the microspore surface – the future ring-like aperture sites - lack glycocalyx and undergo invagination, outlining the three future opercula. All the young columellae grow gradually in height during the late tetrad period. The data obtained give support for the idea that all the events observed in the periplasmic space correspond to the first three self-assembling micellar mesophase: from spherical micelles through cylindrical micelles to "middle" mesophase – a layer of hexagonally packed cylindrical micelles. These processes seems to be possible due to increasing concentration of surface-active glycoproteins with addition of lipoid substances, located in the periplasmic space.

Keywords: substructure template, self-assembly.

SS10-O05 (140)

Exine development in *Passiflora racemosa* Brot.: post-tetrad period. The hidden side of development

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The literature often suggests that in *Passiflora* ectexine and endexine are intermixed. A detailed ontogenetic study was necessary to check this opinion. Our results have shown that after disintegration of the callose special cell wall and free access of sporopollenin from the tapetum, the columellae grow in 4-5 times in hight and in width. In the narrow periplasmic space, at the basis of columellae, a dark-contrasted lamella appears, discernible as a typical "white line". Then, two or three such lamellae are added to the initial one, showing a distinct border between ectexine and endexine. This separating layer is also seen in our SEM fractures of mature pollen grains. After the establishment of this layer the periplasmic space becomes wider and is filled with a new generation of the glycocalyx, whereas the layer of glycocalyx above this, surrounding the columellae, gradually disintegrates. Then, the lower portions of the columellae (regarded earlier as columellar "roots") appear first as semi-transparent, lace-like structures, with radially oriented units revealed in their substructure. These lace-like columellar roots gradually accumulate sporopollenin. In aperture sites, where columellae are absent, the endexine lamellae are especially pronounced, but loosely disposed, and the glycocalyx is highly ordered, showing radial substructure and many small spherical granules. At the next developmental step large, loosely disposed sporopollenin granules appear at the lower part of aperture sites. Thus, the aperture sites are defined by loosely arranged endexine lamellae from outside and by separate granules from inside. The final stage is the appearance of stacks of cord-like structures or fenestrated lamellae, formed from spherical granules, at the basal part of the interapertural regions. A special test, involving potassium permanganate contrasting, used to differentiate endexine from ectexine, has shown more intensive contrast in white-lined lamellae and in all the structures, disposed under them. Hence, it would be reasonable to consider the exine as having distinctly delimited ectexine and endexine, not intermixed layers. The data obtained give support for the idea that white-lined endexine lamellae appear on the basis of so-called neat

(laminate) micelles, after this the whole micellar cycle, observed during the tetrad period, is repeated in the periplasmic space, giving the framework for all other endexine structures, described above.

Keywords: substructure template, self-assembly.

SS10-O06 (590)
Genetic pathways for pollen wall formation in Arabidopsis

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Pollen is formed inside the locules of the anther. The pollen wall formation initiates at the tetrad stage with the primexine formed inside the tetrad. After microspore release from the tetrad, the mature pollen wall is formed. It comprises of exine (sexine and nexine) and intine. The exine is controlled by sporophytic tapetal cells and the intine is controlled by the haploid microspore. Several genes including *NPU*, *RPG1*, *NEF1* and *DEX1* have been reported to be involved in primexine formation in Arabidopsis. Although many genes involved in sexine development have been reported, nexine development remains poorly understood. Here, we report a nexine identity gene in Arabidopsis thaliana, *Nexine Layer Controller* (*NLC*), and present genetic pathways for pollen wall formation. *NLC* is exclusively expressed in the tapetal layer at tetrad stage. The knockout of *NLC* shows absence of nexine and intine layers. However, the expression of *ATUSP*, a gene essential for intine formation, is not affected in this mutant. This suggests that *NLC* controls nexine development, and the development of the intine layer is dependent on the presence of the nexine layer. In tapetal cells, there exists a genetic pathway "*Dyt1-TDF1-AMS-MYB103-MS1*" for tapetal development and function with *MYB103* regulating sexine formation. Double mutant analysis and in situ hybridization showed that *NLC* acts downstream of *AMS* to regulate nexine development. Therefore, in tapetal cell, the genetic pathway "*Dyt1-TDF1-AMS-MYB103*" regulates sexine development, and the genetic pathway "*Dyt1-TDF1-AMS-NLC*" regulates nexine development.

Keywords: AT-Hook, pollen wall, nexine, intine, tapetum.

SS10-O07 (552)
How plants conquered the land: An EvoDevo analysis of the spore wall

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Many theories have been advanced regarding the key innovations required to enable plants to successfully colonise terrestrial habitats. One such proposed innovation is the development of a durable spore wall structure, containing a sporopolleninous exine layer, to withstand attrition and a desiccating and UV rich environment. A significant amount of study has been conducted with regards to the molecular genetics of pollen wall (the derived homologue of the spore wall) development in the angiosperm, *Arabidopsis thaliana* (L.), particularly with respect to the exine layer and sporopollenin biosynthesis. However, research into the molecular genetics of spore wall development in basal plants has thus far been extremely limited. By examining the results of a fully replicated microarray analysis at early and mid stages of moss sporogenesis, up and down regulated

genes have been compared with those known to be involved in pollen wall development, therefore allowing the identification of candidate genes likely to be involved in the development of the spore exine, the spore wall as a whole, and consequently the ability of the sporophyte to survive in the terrestrial environment. The involvement in spore wall development of selected candidate gene(s) will be verified by conducting gene knock-out and gene swap experiments involving the basal plant and moss model species *Physcomitrella patens* and the higher plant, *A. thaliana*. Ultimately this experimental approach will test the hypothesis that the biochemical and developmental pathway required for pollen wall development in higher plants is ancient and has been conserved over ~400 million years of land plant evolution.

Keywords: exine, sporopollenin, angiosperm, moss, gene knock-out.

SS10-P01 (409)
Sporoderm ultrastructure in *Anthoceros agrestis* Paton. (hornworts)

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Spores of *Anthoceros agrestis* are large, rounded-pyramidal, and with a trilete scar. The outlines are circular to subcircular in the polar view and elliptic in the equatorial view. The distal hemisphere is coarsely reticulate with lumina of hexahedral or irregular outlines. There are high conical processes (=spines) with curved tips on the furcation of the muri. The spines are slightly distorted, often with several (two or three, rarely four) tips, which are irregularly curved and give spores a shaggy appearance. Small granules of different sizes are visible on the smooth floor of the lumina. The proximal hemisphere is flat to dihedral. The laesurae, which have straight and thickened margins, extend to the equatorial zone. The laesurae are of the same length. Their thickenings turn into an equatorial rim on their extremities. Similarly to the distal hemisphere, the proximal hemisphere is also coarsely reticulate but there are no protuberances on the muri. The lumina are slightly elongated along the radius of the spore. The rim diverges from thickened margins of bordered laesurae like tree branches. The lumen floor is scabrate as well as the muri, occasionally with granules of various sizes. The endosporium is very thin, electron-translucent, microgranulate, and thickened under the laesurae. The exosporium is massive, granular, of variable thickness; it forms spines and muri. There are two layers of the exosporium: outer exosporium-1 and inner exosporium-2. Exosporium-2 has three strata, which differ in their structure and gradually turn one into another. The inner stratum is made of small granules with very narrow and electron-translucent gaps between them. Inner margins of laesurae are uneven and look torn. The middle exosporium-2 stratum is made of rather large granules with wide gaps between them filled with electron-dense substance. This is the thickest layer in the region of spines. Exteriorly large granules of the outer stratum are fused in a solid tectum. The exosporium-1 is a thin and interrupted layer, composed of many small granules and rare large granules. The endosporium is lost in acetolyzed spores; some peculiarities of different exosporium parts are preserved, including electron-dense substance in the gaps between exosporium granules. The wall of mature spores in *Anthoceros agrestis* consists of granules of different size and shape and does not have any homogeneous or lamellar layers. The sporopollenin is electron-translucent in granules and electron-dense between them.

Keywords: *Anthoceros*, hornworts, spore, sporoderm ultrastructure, exosporium.

SS10-P02 (410)
Acetolysis test of the developing exine in *Nicotiana tabacum* L.

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Sporoderm development is divided into the tetrad and the post-tetrad periods. At the early tetrad stage, the forming sporoderm is completely dissolved by acetolysis. At the middle tetrad stage, tectum and bacula appear first, and the foot layer follows them in the nonapertural region. The microspore wall is thickened and acquires a lenticular shape in the area of the future aperture. The lenticular body is resistant to acetolysis and contains osmiophilic elements, which are more electron-dense than bacula in nonapertural regions. The tectum in some places consists of electron-dense granules, often fused into a single layer. The bacula were rooted in a thin discontinuous footlayer. Osmiophilic elements in the lenticular body resist acetolysis unlike tectum and, even to a greater extent, bacula, the latter after acetolysis become lace-like and have many rounded cavities. Acetolysis causes similar changes in the microspore wall at the late tetrad stage. Spherical cavities, uniform in size and shape, are still detectable in bacula of the acetolyzed exine, as well as in the foot layer. The tectum at the late tetrad stage becomes significantly thicker than at the middle tetrad stage. After acetolysis, it remains electron-dense, homogeneous, with a few narrow perforations. The electron dense elements of the endexine, that appear at this stage in the nonapertural sporoderm and in the aperture margins, are also resistant to acetolysis. The lenticular body at this stage is permeated by acetolysis-resistant, electron-dense thick lamellae. The analysis of microspore autofluorescence, caused by UV, has shown that there are no appreciable amounts of fluorescing sporopollenin components in early tetrads. Step by step, these components accumulate from the poles to the equator. The microspore was completely surrounded by a uniformly fluorescent wall at the middle tetrad stage. The aperture areas were most brightly fluorescent at the late tetrad and at the early stage of free microspores. These data suggest that sporopollenin components completely cover the surface of the microspore at the late tetrad stage, accumulating in the aperture regions in large amounts.

Keywords: *Nicotiana tabacum*, pollen grains, sporoderm development, sporopollenin.

SS10-P03 (400)
Pollen morphology and localization of Ni in some species of *Alyssum* Ni-hyperaccumulators

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Pollen morphology of five *Alyssum* species growing on serpentines in different places in the Mediterranean region were studied, described and compared. Cluster analysis was performed to show similarity between species and their populations. The shape of the pollen grains varies among the species and among the grains within the same anther. The pollen grains are 3-colpate, prolate, with long and tiny colpi reaching the poles. The ornamentation of the exine varies from micro-reticulate to reticulate between the species. Pollen sterility/fertility was also calculated. The highest percentage of sterile pollen (73.76%) was calculated for *Alyssum murale* and the lowest

(9.54%) for *A. bertolonii*. All species are representatives of sect. Odontarrena (C.A.Meyer) Koch well known as Ni-hyperaccumulators. Nickel and other representative elements present in pollen, anthers and filaments were studied by inductively coupled plasma-mass spectrometry (ICP-MS). The stamen parts of all species were micromorphologically analyzed by scanning electron microscopy (SEM) coupled to an Energy-Dispersive X-Ray Probe (EDX). Accumulation of Ni was detected in the filaments and anthers of all studied species and rarely in the pollen grains. The high concentration of Ca in pollen is noteworthy, and probably acts as a barrier against high concentrations of heavy metals like Ni.

Keywords: serpentine flora, nickel, stamen, sterile pollen, Mediterranean.