Session a1

POLLEN ONTOGENY AND DEVELOPMENT

Sugar physiology in lily anther compartments

Castro, A. J. & Clément, C.

Laboratoire de Stress, Défenses et Reproduction des Plantes, Université de Reims Champagne-Ardenne, UFR Sciences, Moulin de la Housse, BP 1039, 51687 Reims Cédex 2, France.

In order to better understand the various pathways regarding sugar physiology in the anther of lily (*Lilium* hybrid), both soluble and wall-bound invertase as well as the alpha and beta amylase enzyme activities were measured separately in the different compartments (envelopes, fluid and pollen), and correlated with the carbohydrate content during pollen ontogenesis.

Our results showed that glucose and fructose were widely present in the envelopes during the initial anther growth phase, whereas sucrose concentration was significantly higher in the locular fluid and the developing microspore at that period. Subsequently, both hexoses accumulated in all the three compartments during the pollen maturation stages, whereas the levels of sucrose decreased drastically to almost completely disappeared. A good correlation was noticed between the sucrose content and the sucrolytic activity, which was mostly related to the wall-bound insoluble enzyme fraction. A striking result was the existence of a decreasing gradient of glucose and fructose content towards the pollen grain, suggesting that the male gametophyte may act as a sink for the photosynthetic assimilates that reach the anther during its development. In addition, sucrose concentration was found to be meaningfully higher in the fluid when comparing with the envelope and pollen fractions, likely revealing a putative site for disaccharide storage.

On the other hand, two consecutive peaks of starch accumulation were observed in the anther envelopes at the meiosis and mitosis stages, respectively, whereas amylogenesis only occurred in the developing pollen during the maturation phase. Both alpha and beta amylase activities also varied remarkably between the two compartments. In this way, the alpha amylase activity diminished dramatically in the envelopes during microspore vacuolation, and then a new peak appeared during the maturation period. In the pollen fraction, instead of starch was absent from the gametophytic cells, noticeable alpha amylase activity was detected at the meiosis and during microspore vacuolation, and further stayed quite significant in the mature pollen grain. In addition, the beta amylase activity increased in the anther envelopes at the vacuolated microspore stage and during the late pollen maturation steps, whereas it remained low in the developing pollen as late as mitosis, and then it gradually increased until the end of the maturation period. All these findings indicate that the regulation of starch metabolism follows different pathways in each compartment of the lily anther.

On the basis of the results achieved in the present study, a synthetic scheme of sugar partitioning in the anther during pollen ontogeny is proposed.

Comparative sporoderm ontogeny of some selected Gymnosperms and Angiosperms

Gabarayeva, N. I.

Komarov Botanical Institute of Russian Academy of Sciences, St.-Petersburg 197376, Russia.

It has been found that in *Encephalartos altensteinii* (Cycadaceae) all the main characters of the exine appear within the tetrad period. The framework of the exine - the plasma membrane glycocalyx - appears first and consists of close packed cylinder-like units 120-160 nm in diameter, oriented perpendicularly to the microspore plasma membrane – of tufts, by Rowley and his coauthors (Rowley et al., 1981. One tuft corresponds to one future alveola. At middle tetrad stage, before sporopollenin accumulation has been initiated, it is already possible to observe the future pattern of the long-alveolate ectexine. At this time the microspore surface coating - glycocalyx -

is well developed and in radial sections shows a pattern of cylinder-like units. This pattern is very similar to that of *Ceratozamia mexicana* and *Stangeria eriopus*.

The main character of the glycocalyx framework is its dynamism: the units are capable of building up throughout the period of exine development. The invaginated character of the plasma membrane at the young stages of the *Encephalartos altensteinii* tetrad microspore development is typical actually for all species under ontogenetic study. This universal feature is probably connected with the mode of the glycocalyx growth. The glycocalyx units are built up continuously in their proximal part, from the side of the plasma membrane, including the late tetrad stage, when sporopollenin has already started to accumulate. The involvment of the plasma membrane movements (micromovements) into the process of building-up the glycocalyx framework is evident. In *Stangeria eriopus*, during mid-tetrad stage some portions of the plasmalemma are considerably invaginated, which results in widening of the periplasmic space and stretching out of the glycocalyx units. Oscillatory movements of the plasmalemma were confirmed by our stereometric study in *Stangeria eriopus* (Gabarayeva & Grigorjeva, 2002). Short stretching and detachment of the glycocalyx from the plasma membrane could be needed for radial growth of the glycocalyx units by self-asembly.

Sporopollenin acceptor particles (SAPs – Rowley & Claugher, 1991; Rowley & Skvarla, 1993) occur distributed alongside the walls of the cylinder-like units, bringing about a corresponding sporopollenin accumulation and the appearance of the long-alveolate exine pattern with cylindrical alveolae in *Encephalartos*. The initial accumulation of sporopollenin apparently occurs in the form of microglobules around SAPs with diameter 30-50 nm. Subsequent sporopollenin accumulation, which results in the appearance of the mature long alveolate pattern of the exine, is independant of SAPs and can be regarded as secondary accumulated sporopollenin. SAPS are seen in exine development of different species, for example – during supratectal gemma development in *Borago officinalis*. It is impossible to observe SAPs in mature exines because they are covered by sporopollenin, but if sporopollenin is partly eroded in the process of oxidation, SAPs become evident again. Thus, after treatment of mature pollen of *Lavatera arborea* (Malvaceae) with glacial acetic acid clusters of SAPs are observed on the surface of the columellae. Similar clusters of SAPs become evident on the surface of the columellae in mature exine of *Cabomba aquatica* (Cabombaceae) after degradation in potassium permanganate, and it is also possible to observe SAPs at the same sites during exine development of this species.

I suggest two stages in exine development which are realized by self-assembly: the formation of the glycocalyx and the accumulation of receptor-independant sporopollenin. The intermediate stage - the initial accumulation of receptor-dependant sporopollenin - is carried out under the control of SAPs.

Variation in the early development of monocot pollen

Nadot, S.[1]; Forchioni, A.[1]; Penet, L.; Sannier, J. & Ressayre, A.[2]

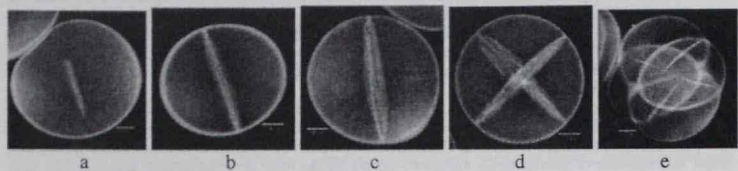
[1] Laboratoire Ecologie, Systématique et Evolution, Université Paris-Sud, 91405 Orsay Cedex (France).
[2] Station de Génétique Végétale, INRA, Ferme du Moulon, 91190 Gif-sur-Yvette (France).

Like many morphological characters, pollen morphology is the result of a compromise between selective forces acting on morphology and developmental constraints limiting the range of possible morphologies. To investigate what are the respective roles of development and selection in the resulting pollen morphology, we have chosen to study the characteristics of cell division during male meiosis, since it has been shown that aperture pattern is determined during the early stages of microsporogenesis. Comparing both developmental pathway and aperture pattern across higher plants will allow us to obtain insights into the way developmental constraints and selection interact to result in the observed pollen morphology.

Monocots are particularly interesting for our purpose. Although their pollen is predominantly monosulcate, other aperture types are found within this taxonomic group, such as the trichotomosulcate, zona-aperturate or di-aperturate types for example. In several species, monosulcate pollen occurs along with trichotomosulcate within individuals, a phenomenon called pollen heteromorphism. Beside the diversity observed in pollen morphology, variation is found in the post-meiotic stages of pollen development. The successive type of cell division, which is the most widespread, is probably the ancestral condition, but the simultaneous type also occurs in several groups, such as in Palms or in Lower Asparagales.

Our observations of the microsporogenesis pathway show that the way callose is deposited between the microspores varies across monocots. Centrifugal cell plates seem to be the common rule when cell division is successive (see figure below). When cell division is simultaneous, callose deposits among microspores can be either centripetal or centrifugal. In some species, only callosic cell plates divide the microspores whereas in other species extra callose deposition occurs in addition to the cell plates. We show that shape of the tetrad, the type of cell division and the way callose is deposited are essential in the determination of aperture type. We show that the variation observed in the post-meiotic developmental sequence of monosulcate pollen is constrained by these parameters.

The developmental sequence of microsporogenesis in *Wachendorfia paniculata* (Haemodoraceae), a species with successive cell division and monosulcate pollen



a: first cell division (centrifugal cell plate). b: dyad stage. c: second cell division (centrifugal cell plates). d: tetrad stage. e: tetrad of monosulcate pollen

Unique pollen features in Araceae

Weber, M.

Department of Ultrastructure Research and Palynology, Institute of Botany, University of Vienna, A-1030 Wien, Austria.

The pollen wall of *Callopsis volkensii* differs in several aspects from a classical pollen wall. As in other Araceae (WEBER et al. 1999) it lacks a sporopolleninous ectexine. The outermost wall layer is formed by a spongy endexine. Attached to the endexine are polysaccharidic spines. Different from expectation these spines are not removed from the pollen surface after acetolysis. This implicits a protecting layer, although no layer is visible after conventional staining procedures with uranyl acetate and lead citrate. Only after staining with thiocarbohydrazine and silver proteinate (a method for the detection of unsaturated lipids in osmium fixed material; ROWLEY & DAHL 1977) an electrontranslucent layer becomes visible, which covers the whole pollen surface, including the spines. From the staining properties, this ectexine-like layer resebles much more a cuticula than an ectexine.

ROWLEY, J.R. & DAHL, A. O. 1977. Pollen development in *Artemisia vulgaris* with special reference to glycocalyx material. **Pollen Spores** 19: 169-284.

WEBER, M., HALBRITTER, H., HESSE, M. 1999. The basic pollen wall types in Araceae. **Int. J. Plant Sci.** 160: 415-423.

Study of male sterility in *Taiwania cryptomerioides* Hayata

Chen, S. H.[1]; Chung, N. J.[2]; Wang, Y. N.[3]; Lee, C. L.[1] & Lee, Y. L.[1]

[1]Department of Life Science, National Taiwan University. 106 Taipei (Taiwan).
[2] Experimental Forest, National Taiwan University. 540 Nantou (Taiwan).
[3] Department of Forest, National Taiwan University. 106 Taipei (Taiwan).

The male sterility of an endemic cypress species to Taiwan, *Taiwania cryptomerioides* Hayata (Taxodiaceae) was studied with the aids of electron microscopic observations and histochemical staining. The ontogenic processes of fertile and sterile strobili collected from Hsitou Experimental Forest and Yeou-Shoei-Keng Clonal Orchard, National Taiwan University are compared.

The male strobili appeared in August. However, the sporogenous tissue became recognizable first after October. The microspore mother cells occurred near the end of December, while the meiosis in late February. By late March, the majority of male strobili contained separated microspores. The pollen grains matured and were shedded by the end of March.

The fertile pollen mother cells were filled with abundant vesicles. After first meiosis, no wall was deposited. Instead, an organelle band consisting of amyloplasts, vesicles, and oil droplets was formed in the equatorial zone of dyad. Subsequently, like land plants, the tetrad microspores were separated by the callose wall at the end of meiosis. After the degradation of the callose wall, the free microspores now contained a large number of vesicles and vacuoles and were enclosed by non-homogenously thickened intine and a thin synthesizing exine. The mature pollen grains contained abundant starch grains and were enclosed by a reduced ectexine, Ubish bodies, lamellate endexine and thick intine.

Both the fertile and sterile strobili appeared about the same time. Nevertheless, the development of the microspore mother cells in sterile one was retarded, when compared with fertile one. In the sterile strobili, both the dyads and tetrads could be found in the same pollen sac, suggesting that the development of microspore mother cells was not synchronous.

At the early ontogenic stages, both the fertile and sterile strobili did not show a significant difference in morphology. A remarkable difference between them was first observed after meiosis. In sterile one, many microspores gathering together. As a result, Siamese-twins or quad became visible after dissolution of the callose wall, associated with intertwining of the exine walls with each other in the proximal faces. Moreover, more than two nuclei could be found in those microspores without intine. At the pollen shedding stage, a dramatic collapse of these structures was observed, giving rise to numerous remains in the microsporangia.

The sterile pollen was characterized by (1) failure development in pollen wall, (2) more than two nuclei, (3) reduced and degenerateive cytoplasm, (4) collapse after meiosis. The present study indicates that the abortion in pollen development should have occurred before the meiosis. Whether such abortion is related to a meiotic abnormality, chromosomal deficiency or the expression of sterility gene is worth of further study

Pollen carbohydrate and water content during development, presentation and dispersal

Nepi, M.; Guarnieri, M. & Pacini, E.

Dipartimento di Scienze Ambientali, Università di Siena, Via P.A. Mattioli 4, 53100 Siena, Italy.

During pollen development, soluble carbohydrates of sporophytic origin may be consumed immediately, polymerized to form starch reserves or intine or transformed in other molecules. In mature pollen there are three different types of carbohydrates: (1) polysaccharides such as starch in amyloplasts or polysaccharides as pectins or callose in cytoplasmic vesicles, (2) disaccharides such as sucrose, (3) monosaccharides such glucose and fructose. Pollen grains can be starchy or starchless and with high or low amounts of sucrose and monosaccharides. Cytoplasmic carbohydrates and sucrose are involved in protecting pollen viability during exposure and dispersal. When pollen is ready for dispersal water content may varies from less than 5% to greater than 50%, depending on the species. During presentation and dispersal water content may: a. remain constant; b. decrease and to become constant; c. decrease sharply. In cases a. and b. the pollen remains viable; in case c., it dies. According to its water content at dispersal, pollen is classified as: partially hydrated (PHP) if water content is over 30%, partially dehydrated (PDP) if less than 30%. Both types have been found even in systematically contiguous groups or the same genus. PHP has the advantage of germinating quickly (5-45'), but the disadvantage to loose water quickly, to desiccate and dies unless it has biochemical or anatomical devices to retain water.

During presentation and dispersal pollen may experience wide variations in temperature and relative humidity. Turgor pressure of pollen may adjust to environmental changes by polymerization / depolymerization of carbohydrates; their interconvertion is also responsible for water retention and preventing ice crystal formation when temperature go below zero. PDP is characterized by carbohydrate interconvertion and survives better than

PHP, which rarely has these mechanisms. Pollen morpholohy is related to water content. PDG have furrows, whilst PHG are devoid of these harmomegathic device.

FRANCHI, G. G., NEPI, M., DAFNI, A. & PACINI, E. 2002. Partially hydrated pollen: taxonomic distribution, ecological and evolutionary significance. **Plant Systematics and Evolution** 234: 211-227.
NEPI, M., FRANCHI, G. G. & PACINI, E. 2001. Pollen hydration at dispersal: cytophysiological features and strategies. **Proptoplasma** 216: 171-180.

The extracellular lipids and lipid-binding proteins of pollen grains - their biosynthesis and roles in pollination

Murphy, D. J.

Biotechnology Unit, School of Applied Sciences, University of Glamorgan, CF37 1DL, Wales, United Kingdom.

The extracellular matrix of the pollen grains of many entomophilous plants is covered with a sticky substance known as the tryphine, or pollen coat. We have characterised the pollen coat components in plants of the Brassicaceae, including rapeseed (*Brassica napus*) and *Arabidopsis thaliana*. The pollen coat in these species is mostly made up of a unique mixture of lipids that includes acylated compounds such as sterol esters and phospholipids. These lipids are characterised by their unusually high degree of saturation – they typically contain 70-90% saturated acyl residues such as myristate, palmitate and stearate. The major sterol components of the pollen coat are acyl esters of stigmasterol, campesterol and campestdienol respectively. The second major pollen coat component is a group of proteins that we term pollenins. The pollenins are derived from larger proteins, called oleo-pollenins, that are synthesised in the anther tapetum. Oleo-pollenins contain an N-terminal domain that is similar to the central hydrophobic domain of seed oleosins. This oleosin-like domain is removed by a specific peptidase after the oleos-pollenins are released into the anther locule following tapetal apoptosis. The mature pollenins are made up of a diverse series of repetitious motifs that are characteristic of structural proteins, rather than enzymes. In this talk, I will describe the mechanism and localisation of the biosynthesis of the pollen coat protein and lipid components and their relocation to the pollen wall. I will then discuss the function of the oleosin-like domain as a novel targeting signal. Finally, I will also discuss the possible roles of pollenins and the pollen coat lipids in adhesion to insect vectors and in pollen-stigma interactions both during and following fertilisation.

Pollen ontogeny in the Nymphaeales

Osborn, J. M.; Schwartz, J. A.; Gutman, B. L.; Melroe, N.A.; Ingraham, A. M.; Taylor, M. L.; Strandquist, J.N.; Hudson, P. J.; Miesner, J.M.; Schoenekase, A. N.; Thiemann, T. & Doores, A. S.

Division of Science, Truman State University, 100 East Normal Street, Kirksville, Missouri 63501-4221, USA.

The Nymphaeales, or water lilies, have a cosmopolitan distribution in fresh water habitats and comprise two families and eight genera: Nymphaeaceae (*Victoria, Euryale, Nymphaea, Ondinea, Barclaya, Nuphar*) and Cabombaceae (*Cabomba, Brasenia*). Water lilies are widely regarded to be among the most primitive flowering plants, as recent phylogenetic studies have consistently indicated that either *Amborella*, or *Amborella* plus Nymphaeales is the sister group to the remaining angiosperms. Although studies of pollen development provide important data for assessing phylogeny, little is know about these characters in the Nymphaeales.

In this presentation, data on pollen and another ontogeny will be described and reviewed for all genera of Nymphaeales, with emphasis on the taxa that have been studied with combined scanning electron, transmission electron, and light microscopy. Anthers at the sporogenous tissue, microspore mother cell, tetrad, free microspore, and mature pollen grain stages will be documented. Events including the deposition of a microspore mother cell coat, a callose 'special' wall, a primexine, and the sporoderm layers will be discussed.The tetrad stage proceeds rapidly, and the tetrads of most genera are of the tetragonal type. In addition to this tetrad configuration, several genera also co-produce tetrahedral tetrads. The mature pollen grains of most genera occur as monads; however, in

Victoria grains are held together in permanent tetrahedral tetrads. In *Euryale*, the production of pollen monads is typical, but the formation of permanent tetrads and dyads has also been observed. Significant exine deposition, including formation of the columellae and tectum occur during the tetrad stage. Pollen of all genera has a columellar infrastructure, with variation occurring in columellae size and ultrastructure. Development of a series of prominent tectal/columellar microchannels occurs in *Cabomba*. Exinous microchannels are also produced in the pollen of *Nuphar* and *Victoria*. The endexine lamellae and a foot layer form during the free microspore and early pollen grain stages. Pollen grains of only two genera possess 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 scultural rods of *Cabomba*, or the minor tectal ornamentation of other water lilies. The principal characters of anther ontogeny discussed will include the number and size of anther wall layers, changes in tapetum morphology, and timing of tapetum dissociation. An amoeboid tapetum has been observed for the first time in several nymphaelean genera.

Water lilies are also characterized by diverse pollination mechanisms, including beetle, bee, fly, and wind syndromes, as well as cleistogamy. Because pollination syndromes have been well documented within the Nymphaeales, the group provides as excellent system to investigate functional correlations between pollen developmental characters and pollination ecology. In addition, given the basal position of Nymphaeales, the group provides the opportunity to investigate the early evolution of pollination ecology and associated reproductive characters within angiosperms. For example, *Cabomba caroliniana* is fly-pollinated and mature pollen grains are coated with copious amounts of tapetally derived pollenkitt, that is stored within the exinous microchannels. The ontogenetic timing and ultrastructure of these microchannels appear to be adaptation for pollination. The adaptative significance of such developmental characters, along with their importance in examining systematic and phylogenetic relationships of the Nymphaeales will be discussed.

Session a2

SIGNALLING IN POLLEN DEVELOPMENT, STRESS-INDUCED MICROSPORE EMBRYOGENESIS AND POLLEN GERMINATION

Signal transduction by MAP kinases in pollen germination

Heberle-Bors, E.[1] & Wilson, C.[2]

[1]Max-Perutz-Laboratories, Vienna Biocenter, University of Vienna.
[2]Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione; Unita Nutrizione Sperimentale.

Mitogen-activated protein (MAP) kinases are highly conserved in eucaryotes and have been implicated in the transduction of a variety of extracellular signals involved in development and stress response. Downstream targets include transcription factors but also other cellular structures such as the cytoskeleton. MAPKs are part of a three-partite signalling module consisting of a MAPKKK (or MEK2), a MAPKK (or MEK) and a MAPK. Eucaryotic genomes contain gene families for all three members of the module, including plants.

We have isolated a number of MAP kinase genes from tobacco and alfalfa. The tobacco MAP kinase NTF4 accumulates in mature, dry pollen as an inactive kinase. Upon rehydration the NTF4 kinase is rapidly and transiently activated, and is again inactivated well before pollen tube emergence (Wilson et al. 1997). Water alone is sufficient to activate this kinase. NTF4 or a very similar MAPK is also involved in hypoosmotic stress signaling in somatic cells (Cazale et al. 1999).

We have now found that the highly related MAP kinase SIMK is also expressed and activated in pollen by hydration and that both MAPKs are regulated by the MAPKK MEK2. Transient transformation of pollen with a kinase-negative mutant version of MEK2 indeed inhibited pollen germination (Voronin et al. 2004).