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Uncommon pollen walls: reasons and consequences*)

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The mature pollen wall of gymnosperms and angiosperms consists in principle of two fundamentally different layers, the complex, thick sporopolleninous exine and the homogeneous, thin, single-layered pectocellulosic intine. In angiosperms, the typical exine is usually formed by a tectum, columellae, a foot layer, and an endexine. An exine reduction (minimally up to the complete absence) occurs in many unrelated seed plants, without consequences for pollen viability. The intine sometimes also deviates from its common form, being either extremely thick or appearing two- or even three-layered. Environmental factors or developmental constraints are highlighted as being responsible for the various deviating exine and intine forms.

Pacini E. & Hesse M, 2012: Unkomplette Pollenwand – Gründe und Konsequenzen.
Die fertige Pollenwand der Gymnospermen und der Angiospermen besteht im Prinzip aus zwei fundamental verschiedenen Lagen, aus der komplexen, dicken und sporopolleninhältigen Exine, und der homogenen, dünnen, einschichtigen und überwiegend zellulosehältigen Intine. Bei Angiospermen ist die typische Exine aus einem Tectum, aus Columellae, aus einem Foot Layer und zumeist noch aus einer Endexine geformt. In vielen, nicht miteinander verwandten Angiospermen (seltener bei Gymnospermen) is die Exine mehr oder weniger stark reduziert, was allerdings keinen Einfluß auf die Keimungsfähigkeit des Pollens hat. Auch die Intine weicht manchmal von ihrer üblichen Ausbildung ab, ist entweder auffallend dick oder zwei bis dreischichtig. Sowohl Umweltfaktoren als auch embryologisch und entwicklungsgeschichtlich bedingte Hemmungen sind für die abweichenden Exine- und Intineformen verantwortlich.

Key words: pollen, intine, exine, callose, harmomegathy, water content, environmental constraints.

Introduction

A major challenge encountered by the first land plants was to protect their spores from dehydration and damage by UV irradiation. The protection of the gametophyte (the male germ plasma) against desiccation and UV radiation damage from anther opening to the transport of spores and pollen grains was met by the presence of a specialized bi-layered outer cell wall, the sporopollenin-containing exine and the pectocellulosic intine (Blackmore et al. 2007).

The question arises why so many plant propagules (especially fern and moss spores, pollen grains) have usually sporopollenin within their outermost wall layers.

The (probably) simple answer is that the "invention" and production of sporopollenin was a prerequisite for becoming a land plant in the Silurian. It was Fritzsche (1837) who recognized first the presence of two walls around the cytoplasm of spores and pollen grains, the exine and the intine, and used these topographical names because of their position in respect to the cytoplasm. The intine, a pectocellulosic wall, shows preferably in its role to pollen germination more structural complexity than the common pectocellulosic walls, e.g. in leaves, simply do. The exine is unique in its chemistry and physical properties, its history is of interest: The spores of some green algae, spores of early land plants (mosses and ferns) and pollen grains of the seed plants have as a common feature in their walls the presence of a very peculiar biopolymer molecule, sporopol-

*) Dedicated to Doz. Dr. Franz Speta of his 70th birthday.

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lenin, This molecule is characterized by its resistance to fungal, microbial and chemical attacks and its elasticity (BLOCKER et al. 1999). Sheets of sporopollenin appear first inside the pectocellulosic wall of vegetative cells of different green algae living in fresh water as *Tetrahedron*, *Scenedesmus* and *Pediastrum* (BLOKKER et al. 1999), in order to survive temporal lack of water and to facilitate dispersal, probably to acquire one of the mechanisms allowing to tolerate low relative humidity (BUZER et al. 1985).

The exine of seed plants consists preferably of this biopolymer sporopollenin, and is usually constructed in a complex manner within an elaborated ektexine and a usually homogeneous endexine (fern and moss spores often have an additional layer on top of the exine, the non-sporopollenin perine or perispore, and are not considered here). The exine is surely not brittle, but to a certain degree elastic, and, as being outside, it has to accommodate changing osmotic pressure of the cytoplasm during hydration or dehydration, with the increase in volume of the cytoplasm during water uptake or vice versa decrease of volume during water loss (the rather complex mechanism, and its consequences is called harmomegathic effect). Harmomegathic movements act during pollen development, before and during pollen dispersal, hydration and early germination stages. This elasticity allows a limited loss or influx of vapour water due to relative humidity variations during pollen presentation. Angiosperm pollen may have different water contents at dispersal. FRANCHI et al. (2002) show variations from 1.7% as in *Dasylirion acrotrichum* (Agavaceae) to as high as 67.7% as in *Fuchsia coccinea* (Onagraceae).

Therefore two categories of pollen grains were envisaged: partially hydrated grains (PHG) with a water content higher then 30%, the recalcitrant pollen, and partially dehydrated (PDG) with less than 30% (NEPI et al. 2001, FRANCHI et al. 2002).

There exists an astonishing amount of cases, where the common pollen wall strata, the exine and the intine, are chemically and structurally modified to a more or less great extent.

The aim of this article is to enumerate the sporoderm modifications occurring in seed plants (when the exine is reduced, discontinuous or even absent, or if and when additional walls appear), and to elucidate the potential reasons of this diversifications.

Material and Methods

Preparation of pollen for TEM

For TEM-investigations, mature anthers were fixed for 20 hours in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and after washing in buffer and distilled water, postfixed overnight in 2% osmium tetroxide (OsO_4) and 0.8% potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) 2:1 at 4°C (WEBER et al. 1999). After fixation and washing in distilled water the material was dehydrated in 2.2-dimethoxypropane (DMP) and acetone and embedded in Spurr's low-viscosity epoxy resin. Sections of about 70–90 nm were made on a Reichert-Jung Ultracut S microtome with a diamond knife and transferred to copper and gold grids. Ultrathin sections were post-stained in 2% alcoholic UA-PB. Specimens were examined with a ZEISS EM-900 transmission electron microscope.

Light microscopy and histochemistry of pollen wall strata (for technical details see PACINI et al. 1999). Callose can be stained with Aniline blue, pectocellulosic walls can be stained with the fluorochrome calcofluor. The exine, formed by sporopollenin shows autofluorescence, which can be enhanced by the use of fluorochromes as Auramine O.

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Outside the plasmamembrane of developing pollen, according to the developmental stage, it is possible to observe walls made by different kind of substances. Polysaccharide walls are of two types: a) the pectocellulosic walls of microspore mother cells, intine and the wall separating vegetative from generative cell. These walls can be stained with the PAS for total insoluble polysaccharides having beta glucans, which can be also demonstrated with the fluorochrome calcofluor. b) the callosic wall of the microspore mother cells and microspores. A thin callosic wall sometime appears temporary just after cytokinesis of the first haploid mitosis. It can be stained with Aniline blue and other fluorochromes. On the other hand the exine, formed by sporopollenin, shows autofluorescence and this fluorescence can be enhanced by the use of fluorochromes as Auramine O. It is possible to combine PAS test with Auramine in order to better differentiate exine and intine in the same section (Nepi & Franchi 2000).

A Note on Terminology

The concepts of PHG = partially hydrated pollen grains, and PDG = partially dehydrated pollen grains versus pollen condition in dry state and in turgescent (fully hydrated) state pollen state should not be confused or merged. PHG and PDG mean the physiological status of living pollen just shed from open anther – independently from the shape. The harmomegathic effect of pollen refers to pollen shape under different status of turgescence (pollen in dry or in turgescent state), and only indirectly to the physiological status.

Results and Discussion

We refer in this paper only to pollen walls outside of apertures. The complex development-depending apertural wall conditions are not considered, as well as the conditions inside of compound pollen (in those cross walls often a reduced or even absent exine is present, Knox & Mc Conchie 1986, Dannenbaum & Schill 1991, Pacini & Hesse 2002).

Exine origin

Actually there is a consensus of opinion that the early exine is formed by the microspore, while it is completed by the (sporophytic) tapetum (Blackmore et al. 2007). In exineless pollen the tapetal functions are restricted only to the nutrition of the microspore/pollen grain and the formation of substances responsible for pollen clumping.

Exine and intine develop centripetally. The exine starts to be formed first and appears as primexine inside a mould of carbohydrates at the end of the meiotic process beneath the callosic wall separating the four microspores (Heslop-Harrison 1963). An interesting aspect is the primexine as reason for presence or absence of exine. Primexine is absent twice: in submerged hydrophilous taxa (usually exineless) and in Araceae/Aroideae, devote of sporopollenin exinous ornamentation features. In both cases the primexine looks to be a prerequisite of a sporopollenin exine (Anger & Weber 2006). The genuine exine starts with the formation of the common tectum (in tectate pollen grains) or of bacula/columellae (in intectate pollen), followed usually by a foot-layer. The endexine (if present) is the last exine layer to be formed. Only then the pectocellulosic intine is added.

The exine is patterned by the gametophyte with the contribution of the sporophyte, i.e. the anther tapetum (Blackmore et al. 2007). The intine, instead, is formed later when exine is already completed at mid microspore stage, when pollen continues its growth in volume. Intine formation always precedes the first haploid mitosis and is formed completely by the gametophyte. Just before or after its completion the thin wall between veg-

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etative and generative cell grows centrifugally and is attached to the intine (PACINI and JUNIPER 1984, figs 31 and 32)

The intine is more affected by environmental constraints than the exine, because it represents the wall typical of all plant cells. Exine is elastic and less affected *per se* by the environment, being outside it represents a sort of interfacies between the environment and the grain.

The starting point for our journey through the steps of exine reduction is the common ektexinre tectate-columellate (T-C) condition, continuous over the pollen grain (except the apertures), with or without foot layer, and mostly with an endexine. The intine is always thin and inconspicuous, except of the apertures.

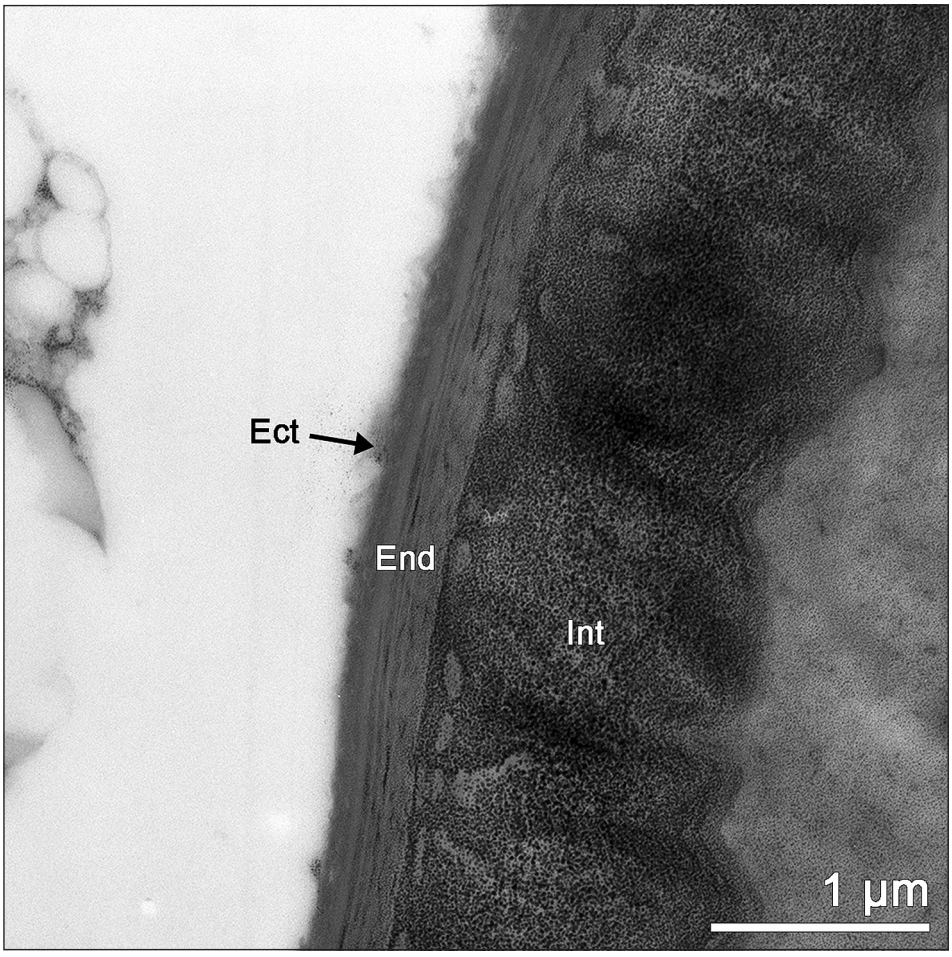


Fig. 1: *Gearum brasiliense* (Araceae). Pollen wall with a thin, smooth ektexine and a moderately thick, lamellated endexine; below, a conspicuously thick, elaborated intine. – Abb. 1: *Gearum brasiliense* (Araceae). Pollenwand mit einer dünnen, glatten Ektexine und einer durchschnittlich dicken, lamellierten Endexine, darunter eine auffallend dicke und strukturierte Intine.
Ect = ectexine, End = endexine, Int = intine.

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Different degrees of exine (more precisely, of the ektexine) reduction exist (rudimentary exines). In all cases the intine is very thick everywhere and channelled, i. e., crossed by tubules of plasmamebrane.

1. A continuous ektexine, but no T-C structure, only cross-linked mostly spiny plates is the result of a convergence (in Laurales and Zingiberales, Stone 1987). The exine of *Persea* is reduced to spines and gemmae with underlying skin-like foot-layer (Gabarayeva et al. 2010).

2. Continuous (ekt-)exine, without any structure, only a skin-like layer (*Strelitzia*: Hesse & Waha 1983, and *Ravenala*: Rowley et al. 1997).

3. Discontinuous ektexine (with various modifications):

a) consisting of separated elements with a tectate-columellate condition in a reduced form (*Enhalus*, Tanaka et al. 2004).

b) isolated granules, not connected at their base (*Ottelia*, *Heliconia,* Stone 1987, or *Vallisneria,* Furness & Banks 2010).

c) clavae connected at the base (*Blyxa*, Tanaka et al. 2004)

d) isolated spines formed by polysaccharides, not by sporopollenin, e.g. *Arum* (Pacini & Juniper 1983) or *Sauromatum* (Weber et al. 1999).

4. exine absent, intine only (*Najas, Heterozostera, Phyllospadix, Thalassia, Posidonia*, *Halophila, Marantachloa, Calathea*, submerse *Callitriche* (Furness & Banks 2010, Tanaka et al. 2004, Furness & Rudall 1999). The genus *Callitriche* includes species living in various environments characterized by different degree of water availability, these species show a progressive exine reduction and the exine is totally absent in submerged species (Osborn et al. 2001), see the special chapter on underwater pollination.
   Aside from the ektexine a quite different case is the potential reduction of the endexine. The endexine was up to recently interpreted as always forming a compact continuum around the intine. In contrast, the endexine is sometimes discontinuous, or lamellar (in angiosperms! In gymnosperms this is a common condition) or spongy, or even only present in apertures (Weber & Ulrich 2010). Sometimes an endexine is not easily to detect (Weber & Ulrich 2010). The endexine is definitely lacking in several families, e.g. in Poaceae; in *Persea* and probably in other Lauraceae there is no endexine (Gabarayeva et al. 2010).

The Tables 1 and 2 show different patterns for exine reduction and exine disappearance with some examples. In Table 2 the taxa belonging to the classes 1 and 2 are often encountered in the same family and are not always linked to a wet environment. Members belonging to the classes 3, 4 and 5 generally live in wet environments or if in temperate regions their pollination occurs when the environment has a high relative humidity as during the night.

Functional aspects:

In sharp contrast to the most common pollen wall type, i.e. with an elaborated (ekt-)exine, stands the convergently developed moderately or highly reduced or even absent exine. Highly reduced exines probably are of advantage in pollination ecology of taxa living in humid, moist or even wet environments. The advantage consists in a rapid germination due to the short rehydration time.

Tab. 1: Different cases of reduction and absence of an exine in some selected Angiosperms. – Tab. 1: Beispiele für Reduktion oder Fehlen einer Exine bei Angiospermen.

Exine					
Continuous tectate columellate and interrupted only in the /apertural regions	***Continuous, but a skin-like exine only, intine commonly thick and channelled***	***discontinuous in some linear areas, intine commonly thick and channelled***	***totally or partially composed by small plates, spines, rods or granules united at the base to form a continuous thin layer, intine commonly thick and channelled***	***discontinuous because formed by isolated plates, rods or spines, intine commonly very thick and crossed by tubules, which entrance is sealed during pollen ripening***	***absent***
pollen grains commonly spherical at dispersal	Pollen grains spherical at dispersal	pollen grains commonly spherical at dispersal	pollen grains commonly spherical at dispersal	pollen grains commonly spherical at dispersal	pollen grains spherical or extremely elongated at dispersal
Examples*:* – this is the more common type of pollen, especially in temperate regions In contrast to amphibious species of *Callitriche* (see third column) – terrestrial taxa of *Callitriche* species – Callitrichaceae (Martinsson 1993, Cooper et al 2000)	**Examples:** *Strelizia reginae* (Kronestedt – Robards and Rowley 1989)	**Examples***:* – Some *Crocus* species – Iridaceae (Chichiricco 1999) – *Tapeinochilos ananassae* (Stone et al. 1981)	**Examples**: Amphibious taxa of *Callitriche* species (Martinsson 1993, Cooper et al 2000) – *Trillium kamtschaticum* (Takahashi 1987) – *Tetrasynandra laxiflora* – Monimiaceae (Sampson 1997) – *Globba atrosanguinea* – (Furness and Rudall 1999) – *Ruscus streptophyllos* – (Furness and Rudall 1999)	**Examples**: – *Canna generalis* (Rowley and Skvarla 1986) – *Heliconia* sp. – Zingiberales (Kress et al. 1978, Stone et al. 1979): exine is discontinuos at the distal pole but continuous and thin at the proximal one. – *Zingiber spectabile* – Zingiberaceae (Theilade and Theilade 1996) – *Stylochaeton zenkeri* – Araceae (Hesse et al. 2001)	**Examples**: A. Spherical – Obligately submersed taxa of the genus *Callitriche* (Martinsson 1993, Cooper et al. 2000) – *Halophila* sp.– Hydrocharitaceae (Pettitt 1981) pollen grains are released inside a mucilagineous moniliform tube, analogous to the pollenkitt B. extremely elongated – *Amphibolis antarctica* . Cymodoceaece (Pettitt et al. 1984)
PDG or PHG Pollen viability decreases rapidly or slowly, according to water content	**PHG Pollen viability decreases rapidly**	**PHG Pollen viability decreases rapidly**	**PHG Pollen viability decreases rapidly**	**PHG Pollen viability decreases rapidly**	**PHG Pollen viability does not decrease rapidly**

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Tab. 2: Flush diagram showing the developmental patterns leading to pollen having different wall layers and water content. – Tab. 2: Entwicklungsgeschichtliche Voraussetzungen für unterschiedliche Wandschichtungen und unterschiedlichen Wassergehalt.

callosic wall and primexine				
Present exine build up is completed at mid microspore stage, when intine start to be formed			Absent Neither callose nor primexine is formed	
1 continuous exine, often with 3 or even more furrows and pores PDG	**2** continuous exine devoid of furrow/s, pores one or many, +/- protruded at dispersal PHG	**3** different degrees of exine discontinuità PHG	**4** modified exine present mostly PHG	**5** exine absent obligatory submerged species PHG
this is the more common pattern in many angiosperm families, especially in temperate regions	1. with 1 pore, Poaceae 2. with 3 pores, some *Urtica* species 3. with many pores, e.g., Caryophyllaceae	1. multiaperturate grains - some amphibious *Callitriche* species 2. poreless grains - some Lauraceae - *Helicon*ia (Stone et al. 1979)	pollen ornamentations present but not made by sporopollenin Some Araceae, e.g. *Arum* (Anger & Weber 2006)	1. Monocots submerged sea-grasses 2. Dicots *Callitriche truncata*

An exine reduction is very often correlated with inaperturate (omniaperturate) pollen. Stone (1987) mentioned neoteny and the omniaperturate condition as triggering factor for the convergent evolution of exine reduction in a high number of angiosperm orders. The high number of monocot orders with omniaperturate pollen might be a further factor to explain the convergent evolution (Furness & Rudall 1999, Furness & Banks 2010).

In contrast to the ektexine, functional aspects of a reduced or modified endexine remain speculative.

In seed plants exine and intine usually closely adhere and stay together from their origin until pollen germination.In contrast, in some gymnosperms, e.g., in Cupressaceae or in *Ephedra*, but also in distinct angiosperms (some Araceae), the exine is shed before pollen germination, and the intine, enclosing the protoplast, is remaining (Duhoux 1982, Danti et al. 2011, El Ghazaly et al. 1998, Weber & Halbritter 2007).

Sporoderm and water content

Pollen wall stratification is a specific feature of a taxon, but it also reflects the reproductive ecophysiology of the species. It s deeply influenced by the environment and modality of pollination: Pollen water content, pollen wall stratification and cytoplasmic carbohydrates are responsible to keep pollen viable during presentation and dispersal (Table 2).

As a consequence of exine reduction and discontinuity such pollen is not dispersed in a desiccated state, pollen grains are always partially hydrated at dispersal and its

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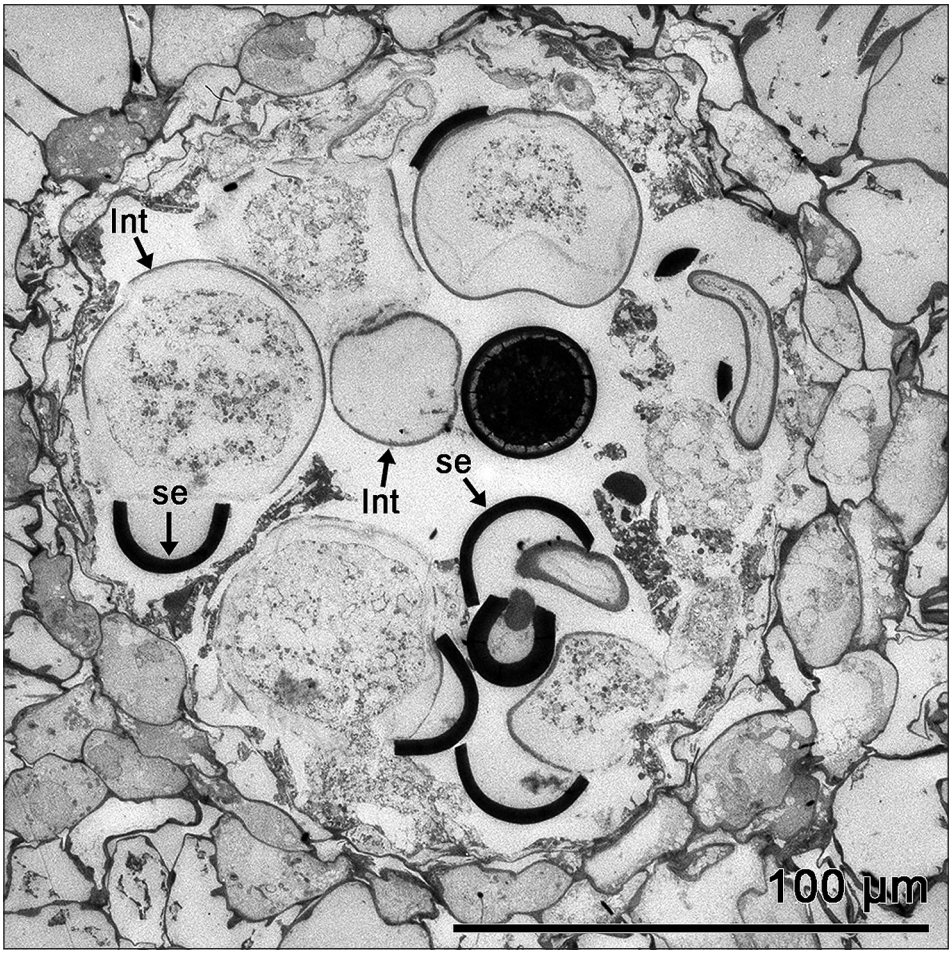


Fig. 2: *Cryptocoryne ciliata* (Araceae). Cross section through a pollen sac, with pollen grains. Some grains appear with broken exines or have shed their exine. In such cases, exine-less pollen grains remain, covered only by the intine. – Abb. 2: *Cryptocoryne ciliata* (Araceae). Querschnitt durch einen Pollensack mit einigen Pollenkörnern. Bei einigen ist die Exine zerrissen oder sie haben ihre Exine ganz abgeworfen: es bleiben dann exinelose Pollenkörner übrig, die nur von einer Intine umhüllt sind.
Se = shed exine, Int = intine.

mean life is very short, for example, less than 24 h in the tropical *Heliconia* (PEDERSEN and KRESS 1999). This looks to be valid also for many Aroideae pollen without a sporopollenin ektexine, which have only a polysaccharide outer layer.

The main question: has pollen to desiccate or not to desiccate? Desiccation is a strategy to keep pollen viable longer, but the lack of desiccation is a way to have a fast germination because rehydration time is shorter (FRANCHI et al. 2011).

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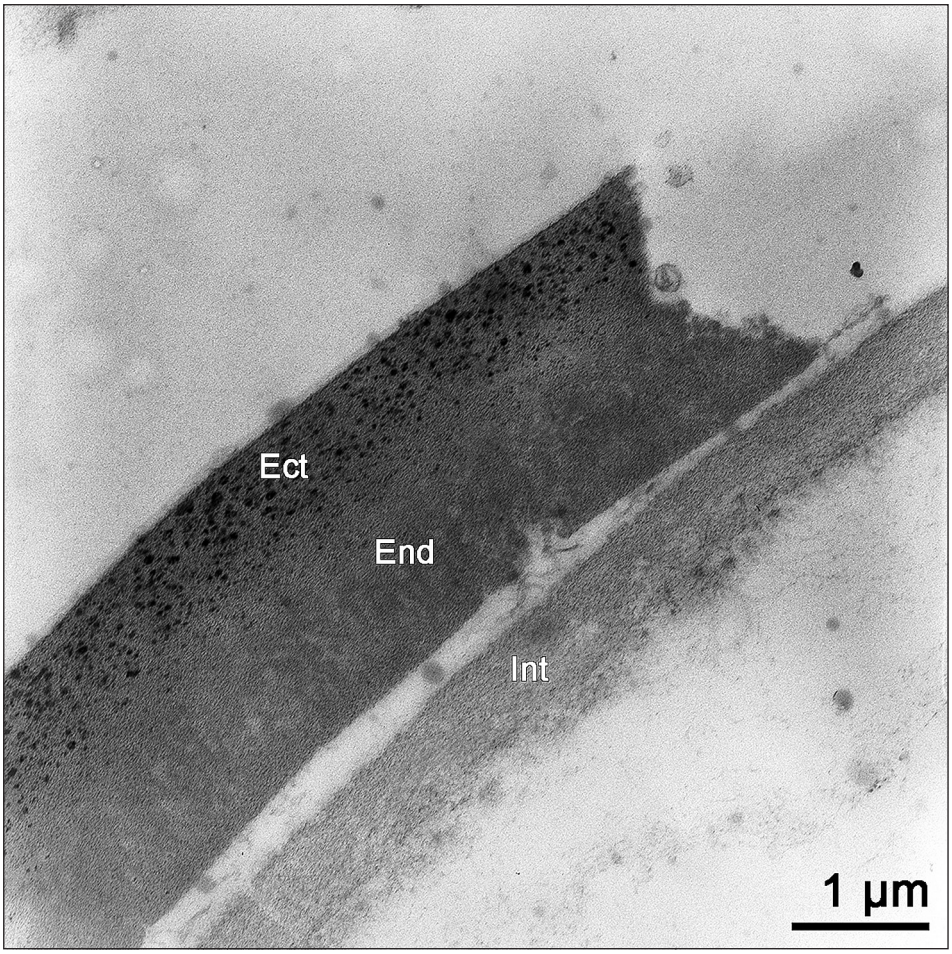


Fig. 3: *Cryptocoryne ciliata* (Araceae). Detail of a pollen grain with a ruptured exine. A part of the pollen grain lacks the exine – only the intine remains. – Abb. 3: *Cryptocoryne ciliata* (Araceae). Ausschnitt eines Pollenkorns mit einer zerrissenen Exine. Einem Teil des Pollenkorns fehlt die Exine, nur die Intine ist übrig.
Ect = ectexine, End = endexine, Int = intine.

A closer look on ecological and developmental constraints may provide insights in functional consequences.

The quite unusual pollen of water-pollinated plants

Taxa with surface water pollination have no or only slight exine reduction.

Exineless pollen adapted for surface water pollination occurs convergently in three unrelated angiosperm orders, namely in the Alismatales (two or three times independently: Ackerman 1995, Tanaka et al. 2004, Furness & Banks 2010), in some species of the eudicot *Callitriche* (two times independently, Osborn et al. 2001), and in the Basal Angiosperm *Ceratophyllum* (Takahashi 1995).

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ACKERMAN (1995) in his review on the convergence of filiform pollen morphologies in sea grasses with underwater pollination (hypo-hydrophily) reports the extreme reduction or even absence of exine in the sea grass families: Zosteraceae (including Aponogetonaceae and Posidoniaceae), Cymodoceaceae and (in part) Hydrocharitaceae.

Exine reduction up to the complete absence of a sporopollenin exine facilitates pollen germination under the underwater realm condition. But exine reduction is not the only feature shared by plants with underwater pollination. Similar or identical pollen characters in taxa adapted to specialised pollination ecology include the inaperturate condition, the absence of exine (in alismatid seagrass pollen, in *Callitriche* and in *Ceratophyllum),* a thread-like shape (only in alismatid seagrass pollen, but not in *Callitriche* and *Ceratophyllum*).

The absence of exine in marine monocots is marked also by the lack of developmental stages otherwise common to all pollen (BLACKMORE et al. 1987), i.e. the absence of a primexine and the lack of a callosic wall layer during/after meiosis (e.g. *Ceratophyllum*, TAKAHASHI 1995) separating the four microspores during the meiotic process, both responsible for exine formation and aperture patterning.

Reproductive strategy does not provide sufficient information to understand this convergence.

COX and HUMPHRIES (1993) raised the question if filiform pollen represents really an evolutionary convergence or if all such taxa descend from a common ancestor having filiform pollen. They express the interesting idea that filiform pollen in seagrasses may be seen as preadaptation for water surface or underwater pollination.

There is a caveat for the premature conclusion that underwater pollination results in elongated or filiform pollen. ACKERMAN (1995) has discussed various strategies followed by taxa with underwater pollination. Underwater pollination (hypo-hydrophily) does not necessarily result in elongated or even filiform pollen, as demonstrated by the presence of spherical pollen in *Callitriche* (Lamiales, COOPER et al. 2000, OSBORN et al. 2001) and *Ceratophyllum* (Ceratophyllales, TAKAHASHI 1995), or spherical pollen grains encaged in slime

According to PHILBRICK & OSBORN (1994) pollen of the obligately submersed *Callitriche hermaphroditica* is not filiform, as might be expected, but spherical. Nevertheless, pollen is functionally filiform, because pollen germinates within the locule, and a tangled mass of pollen tubes results.

The Araceae, an example for ecological and developmental constraints:

Compared to the sea-grasses, the Araceae show quite another pollen adaptations to pollination modes: no filiform exine-less pollen, but pollen adapted for the aroid-typical spathe-spadix construction and the unique aroid pollination ecology. The Araceae comprises 105 genera with c. 3.300 species, traditionally arranged in several subfamilies (MAYO et al. 1997). Two basic types of pollen walls can be distinguished in Araceae, each with two subtypes. The two main types are characterized by presence (type 1) or absence (type 2) of an acetolysis-resistant ektexine.

The extreme exine modification is an adaptive prerequisite of the specialized pollination biology in many Aroideae. The spathe often forms a more or less closed, even constricted tube around the spadix, pollinating insects get imprisoned for some time (special devices of kettle traps and trap pollination). In Aroideae the exine reduction together with the un-

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stable, easily rupturing and destroyable non-sporopollenin outer exine layer facilitate the pollen tube formation and are adapations to a short pollination time-window.

Examples for reduced exine wall layers in Araceae: 1. polysaccharide outermost exine layer (sometimes absent). Weber et al. (1999) observed the basic pollen wall types in 60 species of Araceae, and found that in very many Aroideae a non-ektexinous pollen ornamentation is present. 2. ektexine and intine only in *Montrichardia* 3. endexine and intine only in *Zantedeschia*, *Taccarum* and *Cryptocoryne* (Fig. 2,3). 4. a sporopollenin thin smooth ektexine and an endexine in *Gearum* (Fig. 1).

The central questions, are the filiform, exine-less pollen in seagrasses, and the sporopollenin absence in Aroideae due to constraints during pollen development or to functional requirements in pollination ecology? The aberrant pollen forms in seagrasses and in Aroideae are caused by different, singular developmental constraints as well as from unique functional pollination ecology factors. Both factors (developmental constraints, functional requirements from pollination biology) play a significant role.

The taxoid pollen with a three-layered intine versus inaperturate monocot pollen with an extremely thick, homogeneous intine

The role of uncommon intine configurations is to store water and to protect cytoplasm against desiccation.

Intine can be uniformly thick as in the case of inaperturate pollen of angiosperms, or in gymnosperms with taxoid pollen where each pollen grain has a small exine perforation with a protrusion named papilla (Uehara and Sahashi 2000) or bulge (Danti et al. 2011), sometimes covered by an operculum (Duhoux 1982). *Cupressus* pollen shows two or even three intines (Chichiricco & Pacini 2008). Another peculiarity of taxoid pollen is given by the intine which is folded beneath the exine, and this probably explains why these walls separate upon rehydration. An angiosperm example is *Montrichardia* (Araceae) where intine is folded and the exine is shed after water uptake by the intine (Weber & Halbritter 2007).

When pollen rehydrates in *Taxus* the enormous increase in volume of intine and cytoplasm determines exine breaking in two halves: This process is starting from the papilla representing a weak point. The two parts of exine are sometimes released together or in two parts. Exine expulsion is rapid, a matter of few seconds (Duhoux 1982).

The intine of dry taxoid pollen (e.g., *Taxus*, *Cryptomeria*, *Cupressus*) can occupy even 70 % to 80 % of the total volume (Pacini et al. 1999). *Juniperus communis* pollen kept for 24 h at 60° decreases in volume but the decrease percentage is higher in the intine rather than in the cytoplasm (unpublished data). This suggests that one of the possible functions of the taxoid intine is to keep water within the cytoplasm. In fact *Juniperus* pollen is longer viable at low relative humidity and low temperature.

The enormous thickness of intine in some gymnosperm pollen of the taxoid type and the uniform intine thickness of poreless Heliconiaceae, Lauraceae (Kress 1986 and examples therein) can be confidentially thought as an external reservoir to keep the vegetative cell cytoplasm fully hydrated during dispersal. In the case of submarine pollination, pollen does not become dehydrated (it is impossible) and there is no change in volume before and during dispersal. As a consequence this pollen has only an intine, whilst an exine is lacking. Water is also stored inside the thick intine of in- or better omniaperturate grains of surprisingly many taxa in basal dicots as, e.g., Lauraceae and especially in monocots

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as Musaceae (FURNESS & RUDALL 1999). These are PHG and the presence of a thick intine is a way to maintain viability.

The case of a callosic wall in mature pollen

The presence of a callosic wall and their precursors in the cytoplasm of ripe angiosperm pollen grains seems a requisite for a rapid germination, nevertheless this was so far demonstrated in only few taxa.

Three walls: exine, intine and a thin callosic wall was observed in PHG of some grasses (HESLOP-HARRISON 1979), in *Cucurbita pepo* (NEPI et al. 1995) and in *Lavatera arborea* (NEPI & PACINI 1999), in all these cases some callosic reserves for the build up of new wall at germination were detected also in the cytoplasm.

A callosic wall is always formed before pollen germination, either in vivo or in vitro, it becomes continuous with that of the pollen tube when it is emitted (CRESTI et al. 1977).

The presence of this wall in partially hydrated grains depends on the fact that these grains commonly germinate few minutes after pollen landing and this is possible because rehydration is shorter and a thin callosic wall is already present. *Parietaria judaica* is an exception of this rule because pollen is partially hydrated but devoid of the callosic wall at dispersal (FRANCHI et al. 2002), but callose is formed before germination (FRANCHI et al. 2006). A callosic wall in ripe pollen is present in the mutant *raring-to-go* of *Arabidopsis* where anther and pollen desiccation do not occur and pollen grains start to emit tubes within the anther simulating cleistogamy (JOHNSON & MCCORMICK 2001).

Polarization and wall stratification of saccate gymnosperm pollen are of interest in term of pollen diversity. Conifers have often saccate pollen grains, with some modifications in their walls. In this case LM histochemistry becomes more important than EM into establishing wall stratifications and their chemical nature (PACINI et al. 1999).

The aerial sacs of the distal part of *Pinus pinaster* pollen are delimited only by the exine, with branched ornamentations in the inner side. The distal part of the corpus has a thin exine and intine, from where the pollen tube will be emitted. Instead, the proximal part of the central pollen body (the corpus) has four walls: exine, a thin intine layer, a callosic wall and another thick intine wall, continuous with the intine surrounding the tube cell (PACINI et al. 1999). MARTENS et al. (1967) described for the first time the presence of the callosic wall in *Pinus sylvestris* pollen, ROWLEY et al (2000) showed some ultrastructural images of this wall development but they both did not observe the two external pectocellulosic layers and called the whole wall complex "outer intine". The callosic wall of the proximal part of *Pinus* pollen has probably another meaning as in angiosperms: its presence could be linked to the pathway of water for rehydration because *Pinus* pollen lands and adheres to the pollination drop surface with the pole between the two wings (LESLIE 2008).

Conclusions

Pollen is the spermatophytic male gametophyte dispersed in order to reach the pollination drop in Gymnosperms or the stigma in Angiosperms. Independently of the water percentage at dispersal pollen grains always dehydrate before to be released and rehydrate on the female counterpart. Pollen walls are flexible and elastic, programmed for dehy-

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dration before and rehydration at the end of dispersal. Structural differences may occur in pollen as a response related to different aspects of pollination.

Intine, being a modification of a common pectocellulosic wall is always present, exine is discontinuous or lacking in pollen dispersed with high water content or living underwater. In some cases a third (callose) wall is already present beneath the intine in order to allow a quick emission of the pollen tube. The possible function of a polysaccharide-protein wall outside the exine in some members of Araceae is up to now widely speculative, perhaps related to a limited life-time of such pollen.

To summarize: Modifications of pollen walls are always due to the environment they must cross, the site where they land (pollination drop or the different types of stigma surface) and the programmed time for dispersal.

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