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The Fine Structure of the Pollen Wall in *Strelitzia reginae* (*Musaceae*)

By

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Abstract: The pollen wall of *Strelitzia reginae* (*Musaceae*) consists of a nearly unsculptured, very thin, highly reduced, but coherent exine, and a thick intine (with an outer, channeled layer and an inner, largely homogeneous layer). After short, incomplete acetolysis the exine covers the remaining, severely shrinked protoplast as a folded, but unaltered "skin", while the intine has totally disappeared. After extended acetolysis only the coherent, skin-like exine remains. Thus, the term "exine-less pollen" sometimes used for similar sporoderm structures in other genera of *Zingiberales* is misleading and should be substituted by the term "skin-like exine". Surprisingly, the peculiar pollen wall ultrastructure of *Strelitzia* and some other *Zingiberales* is very similar to that of some genera of the *Laurales*, an example for convergent evolution within the angiosperms.

The fine structure of the angiosperm pollen wall does not always consist of a highly elaborated exine and a rather simple intine. The statement by STONE & al. (1979) that "the biology of pollen becomes curiouser and curiouser, as new techniques and insights are brought to bear" seems to be very appropriate in the light of some recent findings.

It is well known that the pollen of some *Zingiberales* genera is devoid of a conspicuous exine (ERDTMAN 1952): The exine consists only of a very thin, either smooth or spinulous exine and a thick, structurally complex intine (*Canna:* SKVARLA & ROWLEY 1970; *Heliconia:* KRESS & al. 1978, STONE & al. 1979). The present paper describes another example with such peculiar sporoderm in the Zingiberalean family *Musaceae: Strelitzia reginae*.

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Amazingly, this peculiar sporoderm fine structure is not restricted to the monocotyledonous *Zingiberales*, but is also found in the dicotyledonous *Laurales* (KUBITZKI 1981, ROWLEY & VASANTHY 1980, HESSE & KUBITZKI 1983). Moreover, a skin-like exine (but with a rather "conventional" intine) is to be found in some hydrophilous seagrasses (PETTITT 1976, PETTITT & JERMY 1975). Probably some of the peculiar sporoderm characters are correlated with the pollination ecology of these genera.

Material and Methods

Anthers with mature pollen grains of *Strelitzia reginae* AITON (*Musaceae*), cultivated in a greenhouse of the Botanical Garden of the University of Vienna, were fixed in 6 % glutaraldehyde (SÖRENSEN-buffer, pH 7.2). Acetolysis was done according to the slightly modified method by AVETISSIAN (cited by BROWN 1960) which allows to prepare non-fossil pollen on the slide. After short acidification (glacial acetic acid) some droplets of the acetolysis fluid and a cover glass are placed over the pollen grains. Then the slide (at best a hollow-grounded-slide) is heated for few minutes, while the acetolysis process can be watched under the microscope and stopped when required. After cooling and washing the material thoroughly with glacial acetic acid or absolute alcohol, the specimens are ready for further preparation.

For SEM investigations the material was either air-dried or critical-point dried after the dehydration in ethanol according to SITTE (1962), and coated in a sputter coater. For TEM studies the material was partly poststained with OsO_4 and/or with alcoholic uranyl acetate, and after dehydration embedded in "SPURR low viscosity resin".

Results

The sporoderm of the fresh, living *Strelitzia* pollen grain is translucent, which can be easily demonstrated by squeezing the pollen grain: The opaque protoplast slips out, and the fragile, but homogeneous sporoderm remains. It is impossible to discriminate the glossy exine from the also translucent intine at this level of investigation.

The transparency of the sporoderm can be demonstrated even better in fixed material. Especially after staying in absolute alcohol for 24 hours before critical-point-drying the protoplasts are severely shrinked, while the pollen wall itself remains unaltered with respect to its dimensions (Fig. 1*a*). The surface of the nearly spherical grains is often covered by some tapetal residuals (mostly pollenkitt, which causes some pollen stickiness; Fig. 1*b*). Threads are in close neighbourhood to the grains (Fig. 1*c*); by no means should they be identified with the so-called "viscin threads". The grain surface is not

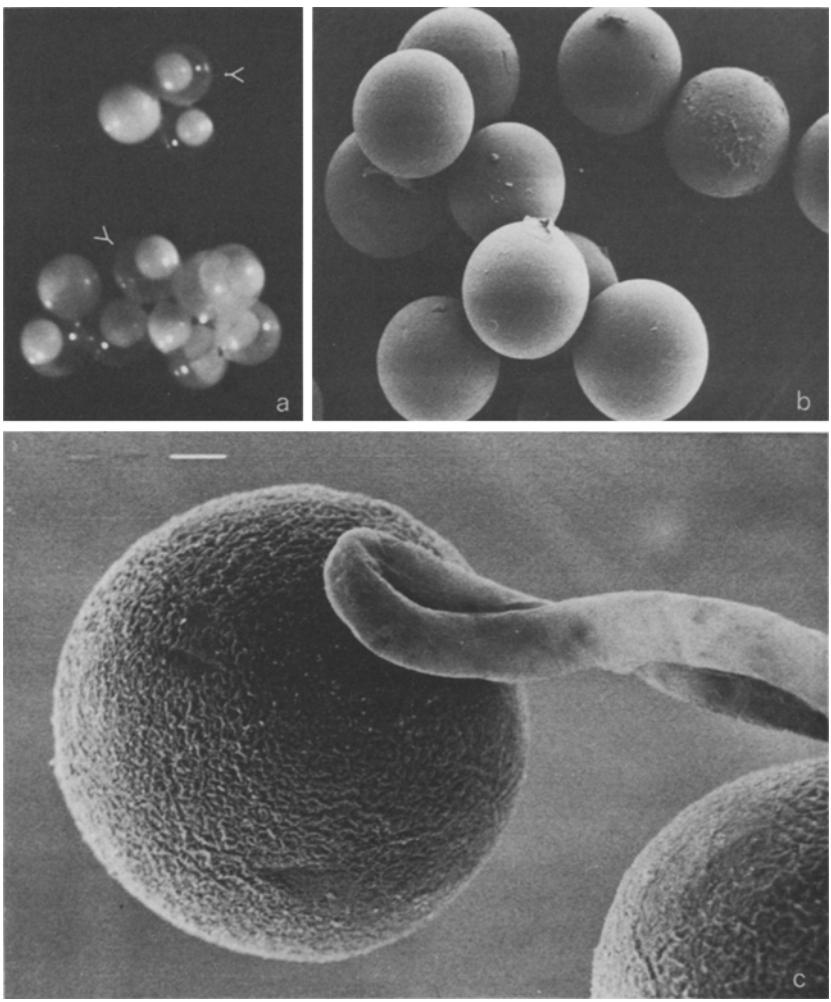

Fig. 1. Non-acetolysed, critical-point-dried pollen grains of *Strelitzia reginae* in light (*a*) and scanning electron microscope view (*b* and *c*). In *a* most of the protoplasts are severely shrinked and so the translucent pollen wall is to be seen (white arrows) (90×, dark field illumination). Pollen grains may be naturally without (*b*, 170×) or with threads (*c*, 700×, bar = 10 μm). The smooth, tube-like threads only slightly adhere to the wrinkled pollen surface

totally smooth, as one might suppose from low power micrographs, but appears slightly wrinkled (Figs. 1, 2, 5, and 6).

Ultrathin sections of non-acetolysed pollen grains reveal a thin, ± homogeneous, nearly unsculptured, but strictly coherent outer layer, the **exine,** covering a thick, complex inner layer, the **intine** (Figs. 2 and 3). The exine seems to be strictly homogenous in radial sections only, but in oblique sections this layer often splits up (because of "tangential" microtoming, Fig. 2), demonstrating that this layer consists in reality of several exine lamellae.

Beside the acetolysis (see below) some staining experiments prove that the outermost, thin layer is the exine. First, after OsO_4-staining only, its electron density is of the same order as in other sporopollenin-containing structures. Further on, after staining with uranyl acetate only, the exine layer differs clearly with respect to its electron density from the thick inner layer, the intine: cellulosic structures often stain heavily after uranyl acetate impregnation, while sporopollenin structures do not. Finally, after acetolysis only the thin, outer layer remains.

The threads are no part of the pollen wall, they are not fused with the exine, they only adhere by some lipid droplets on the sporoderm surface (Fig. 2*b*). The wall of these threads shows a consistency and electron density quite similar to cellulosic wall layers. Moreover, these layers lack sporopollenin, because the threads are completely dissolved after acetolysis. At the time of anther dehiscence the threads are partly filled with material of degenerated cytoplasm, e.g., with some senescent mitochondria and nuclei. Later on, one finds only "empty", highly vacuolized "threads", consisting solely of cell walls.

The *Strelitzia* pollen grains are inaperturate (*sensu* Skvarla & Rowley 1970) offering an infinite number of sites for pollen tube initiation. Thus the approx. 0.3 μm thick exine completely covers the complex, inner sporoderm layer, the intine, which is completely destroyed by acetolysis. It consists of two different sublayers, I_1 and I_2 (Fig. 2*a*). The rather thin, homogeneous, slightly lamellated I_2 borders the cytoplasm, while the fibrillar I_1, the dominating part of the intine, is channeled throughout. These strictly radially orientated "channels" (or "lacunes" after Le Thomas 1980) are subdivided by numerous fragile, tangentially orientated partition walls (Fig. 3) producing numerous chambers (or ventricles). The channel walls bulge into these chambers which are partly filled with granular and/or lipid material.

Already after short, incomplete acetolysis the intine is completely destroyed, while the shrinking cytoplasm is severely vacuolized and

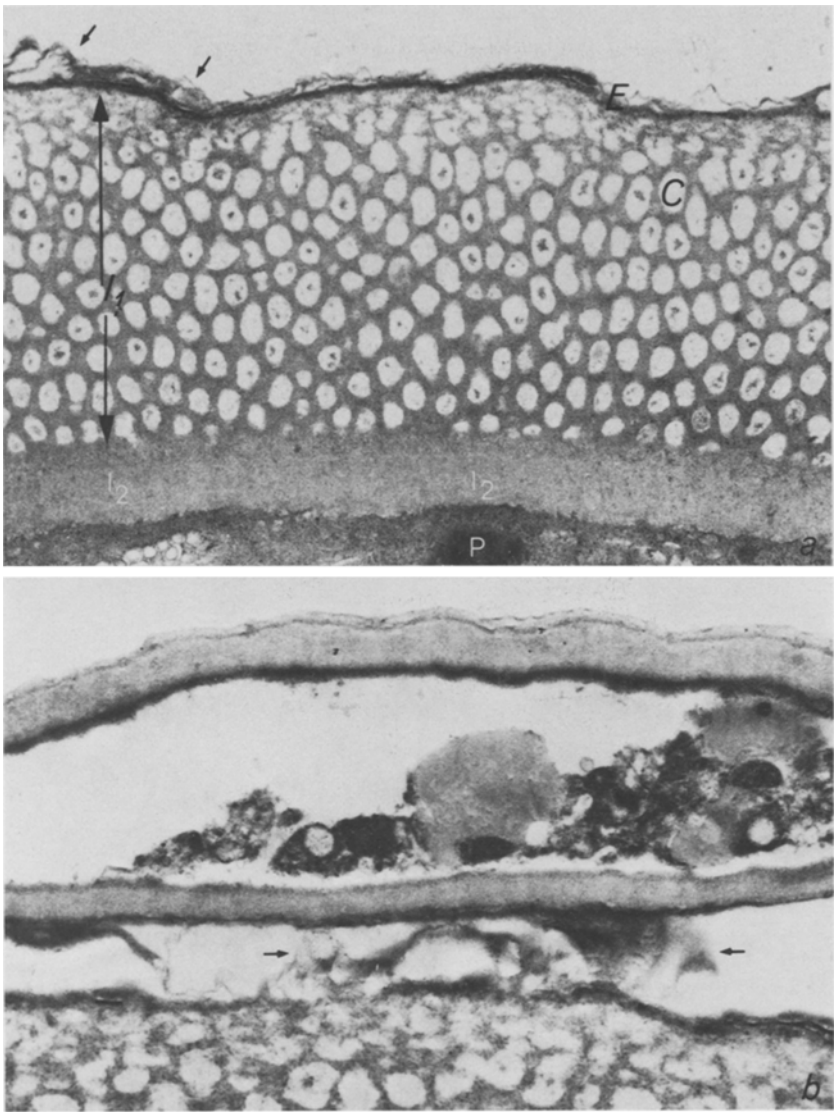

Fig. 2. Oblique sections of the pollen wall of *S. reginae* without acetolysis. *a* After OsO_4-staining the exine (E) is highly electron dense (cf. Fig. 3), thin, unsculptured and coherent, but is partly splitted up into some sublayers (arrows). The intine consists of an outer, thick, channeled layer ($\leftarrow I_1 \rightarrow$), and an inner, homogeneous layer (I_2) adjacent to the protoplasm (P) (8000×). *b* A single thread adheres only by some lipid, highly viscous material (probably pollenkitt) to the partly splitted exine (arrows). The structure and electron density of the thread wall is dissimilar to the exine, but resembles to a cellulosic cell wall. Condensed material, perhaps degenerated cytoplasm, partly fills the thread (15000×)

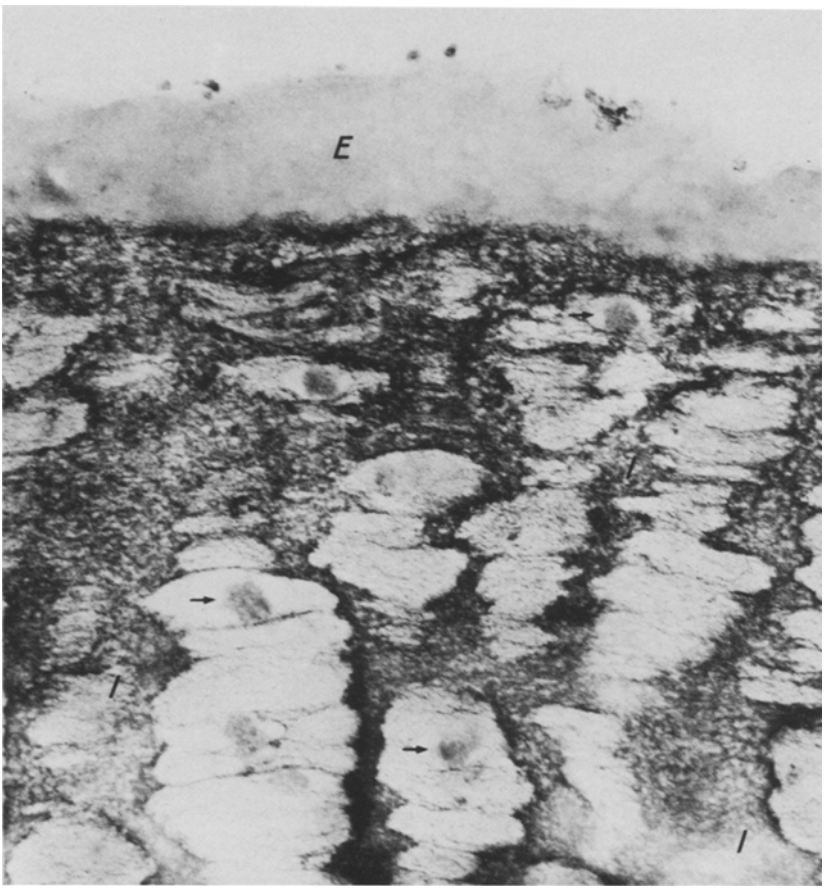

Fig. 3. Radial section of the non-acetolysed pollen wall of *S. reginae*. The exine (E) consists only of an unsculptured, approximately 0.3 μm thick layer (its low electron density is due to the staining with uranyl acetate only), covered by some tapetal particles. The complex, highly electron dense part of the intine (I) is channeled (the inner layer of the intine is not shown): The channels are radially orientated and chambered by thin partition walls. Some of the chambers include granular or lipid material (arrows) (60 000 ×)

artificially altered, but still visible (Fig. 4). In ultrathin sections the exine remains unaltered with respect to its thickness and structure. But as there is no support of a turgescent cytoplasm below, the exine mostly covers the remaining cell body as a loose skin. In such preparations one often can find "naked", artificially altered protoplasts. Evidently the exine has cracked during acetolysis, and the shrinked protoplasts have

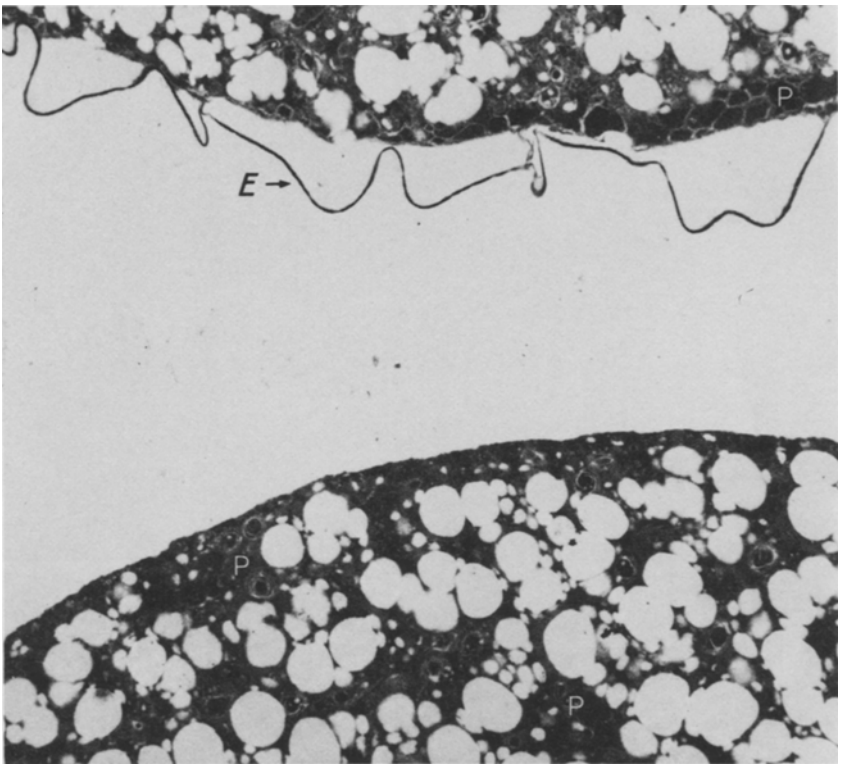

Fig. 4. Shortly acetolysed, OsO_4-stained pollen grains of *S. reginae*. The artificially vacuolized, but still remaining protoplasts (P) are either naked (below) or covered by the folded, but unaltered, skin-like exine (E); the intine has completely disappeared (4 000 ×)

slipped out. Thus, one observes (a) loose, but superficially unaltered exine-"skins" covering the remaining protoplasts, (b) naked protoplasts without exine, and (c) pollen grain-"ghosts" (the exine only), which are collapsed and totally empty. Sometimes there are also protoplasts partly covered by their exine skins (Fig. 5). Especially after air-drying the loose nature of the skin-like exine can be seen very well.

After complete acetolysis nothing is left of the intine and of the cytoplasm, only the thin exine remains. The latter in general is very sensitive to cracking (Fig. 6): Already the short exposure to a high energy primary electron beam irradiation results in the sudden bursting of the exine (whereas a "conventional" elaborated and thick exine is mostly resistent to such rough treatment).

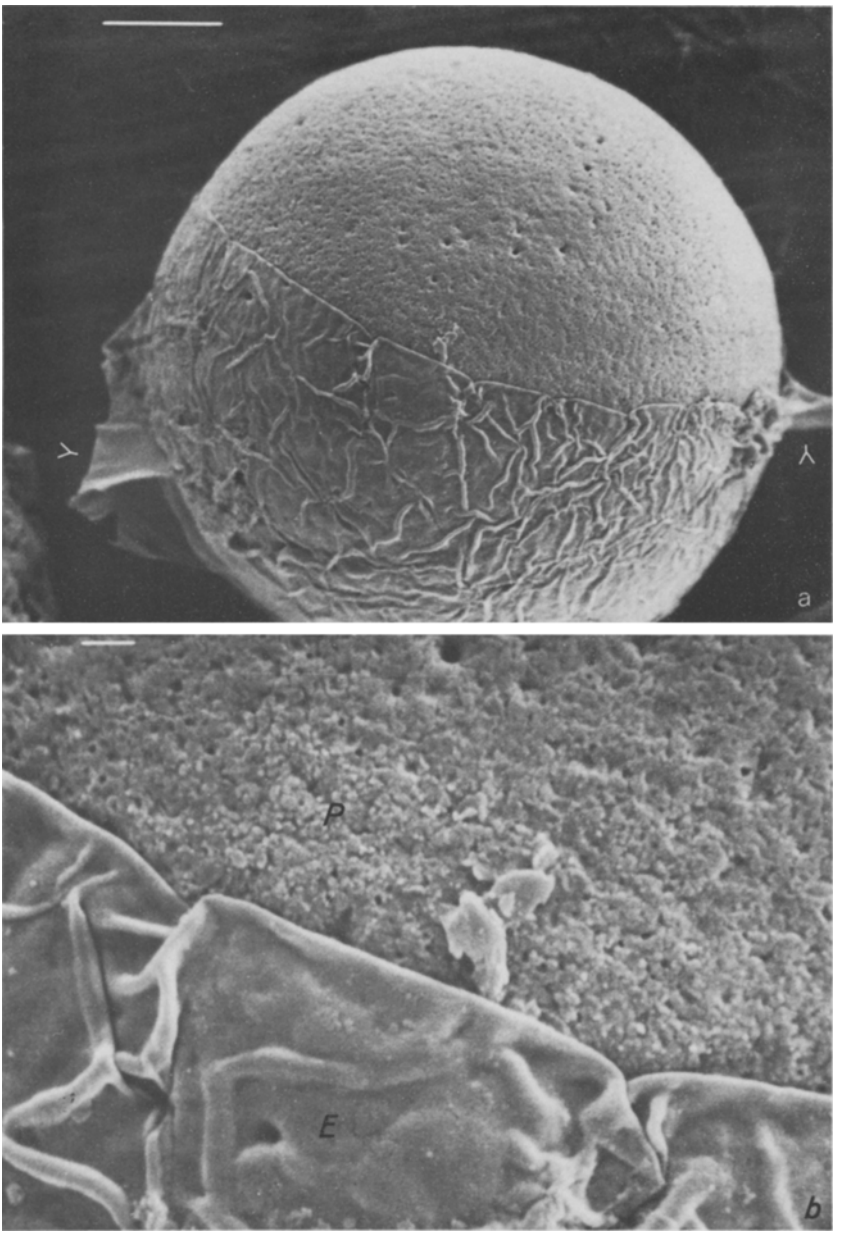

Fig. 5. SEM micrographs of shortly, incomplete acetolysed, intentionally air-dried *S. reginae* pollen demonstrate at best the looseness of the skin-like exine. *a* The folded exine accidentially covers only partly the remaining protoplasm and is shredded (arrows) (1 600 ×, bar = 10 μm). *b* The exine (E) has shrinked, the intine is completely disappeared, the vacuolated remaining protoplast (P) is bare (7 000 ×, bar = 1 μm)

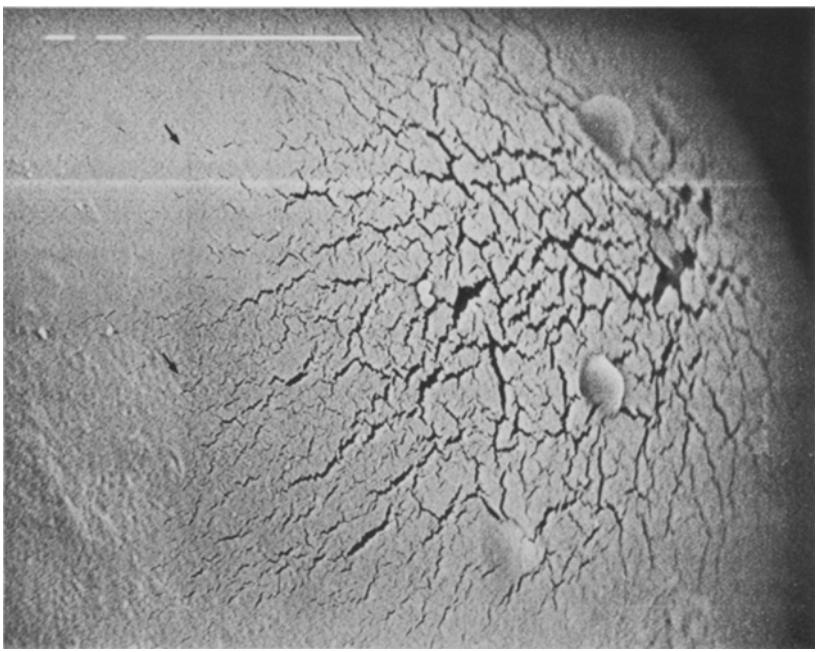

Fig. 6. The skin-like exine of a critical-point dried, although sputter-coated pollen grain of *S. reginae* is bumpy and cracked (right) by only short exposure to the high energy electron beam irradiation. The cracking stops at that exine part, which was exposed only to low power electron beam irradiation (left). The vertical line (arrows) indicate the boundary of the high energy beam square, while the bright horizontal line is due to charging (2 800 ×, long bar = 10 μm)

Discussion

The peculiar fine structure of the pollen wall, i.e., the bipartition into a thin, skin-like exine and a thick, complex intine, is not restricted to *Strelitzia reginae*, but also occurs in some other genera of the monocotyledonous *Zingiberales: Canna* (Skvarla & Rowley 1970), *Heliconia* (Kress & al. 1978, Stone & al. 1979), and—with some reservation— in *Tapeinochilos* (Stone & al. 1981); moreover it appears in some genera of the dicotyledonous *Laurales* (Kubitzki 1981, Hesse & Kubitzki 1983)[1]. Beyond striking similarities in sporoderm fine

[1] Already Wodehouse (1932) has observed this striking similarities between *Laurales* and *Zingiberales* and has wondered about that remarkable example of convergence (see also Skvarla & Rowley 1970).

structure there are also some differences not only between, but also within the orders.

The **intine** always consists of two characteristically formed sublayers. The inner layer (I_2) is much thinner than the outer, dominant layer (I_1). The inner layer is either totally (*Heliconia*, *Strelitzia*) or partly homogeneous (*Tapeinochilos* in the nonaperturate region), or exhibits numerous cytoplasmic inclusions (*Canna*, *Tapeinochilos* in the spiraperture region). The outer layer of the intine is often channeled throughout in *Canna* and *Strelitzia*; in contrast, the channels in *Tapeinochilos* appear only in the aperturate region, while in *Heliconia* they are normally restricted to the distal (= "aperturate") hemisphere of the pollen grain [the mature intine of the proximal (= non-aperturate) face of the pollen grain is solid and only slightly stratified]. This is further proof for the view that the whole surface of *Canna*, but also of *Strelitzia* pollen may act as a single aperture. One can also say (SKVARLA & ROWLEY 1970), that their pollen wall offers an infinite number of sites for pollen tube initiation (cf. also in this respect the distal hemisphere of *Heliconia*!).

The intine channels (or "lacunes" according to similar features in some *Annonaceae*, LE THOMAS 1980) are mostly radially orientated. The channel walls are commonly sculptured, but only in *Strelitzia* are the channels segmented. In their dimensions these channels differ strongly: by far the smallest occur in *Tapeinochilos* (resembling the "common" cytoplasmic intine inclusions), while in *Heliconia*, *Canna*, and *Strelitzia* they are much larger. Curiously enough, an intine very similar to the one described for the three last mentioned *Zingiberales* genera also has been found in some *Laurales* genera, e.g., *Persea*, *Nectandra*, *Cinnamomum*, and—slightly more different—also in *Gomortega* (HESSE & KUBITZKI 1983). In both orders the channels are partly filled with some protein or lipid material. These channels (or "lacunes") may act as deposits for material involved in pollen germinating or incompatibility processes (STONE & al. 1979, HESSE & KUBITZKI 1983).

The **exine** may be coherent or incoherent, but is always very thin. Especially the exine of inaperturate (*sensu* SKVARLA & ROWLEY 1970[1]) pollen has beeen reported to be coherent, though as a very thin layer. This is the case in some hydrophilous seagrasses from the orders *Hydrocharitales* and *Najadales* (PETTITT & JERMY 1975, PETTITT 1976, 1980), but also in the *Zingiberales* genera *Tapeinochilos* (without regard

[1] It would perhaps be better to term such grains "omniaperturate", but unfortunately this term is given away already to pantotreme grains (STRAKA 1975).

to the spiraperture region), in *Heliconia* (but only in the proximal, i.e., the non-aperturate hemisphere; the distal hemisphere represents a single, large aperture with a perhaps incoherent spinulous exine), and in *Strelitzia* (the whole pollen surface). The coherent exine in general is very thin; but in *Strelitzia* it is approximately 3-4 times[1] thicker than the exine located on the proximal hemisphere of *Heliconia* pollen (ca. 0.3 µm *versus* 0.08 µm, KRESS & al. 1978), while sometimes it is apparently extremely thin (e.g., in the distal hemisphere of *Heliconia* pollen, see below).

In contrast, there are reports on incoherent (most probably highly reduced) exines, e.g. for *Canna* (SKVARLA & ROWLEY 1970), *Ruppia* (PETTITT & JERMY 1975) and *Heliconia* (the distal hemisphere only! KRESS & al. 1978). In my opinion, most of these pollen grains are in fact covered by a very thin exine layer: The coherent, highly electron dense, line-like layer covering the distal hemisphere in *Heliconia* (Fig. 18 in KRESS & al. 1978, Fig. 31 in STONE & al. 1979) should perhaps be interpreted as an exine, as in other genera. Such a view is first supported by the observation of the remaining coherent pollen grain "skin" after acetolysis: this "skin" obviously contains sporopollenin, which is only found in the exine and not in the intine. Further proof for this opinion is given by the staining behaviour of this thin layer according to the staining behaviour of sporopollenin-containing algal cell walls (BURCZYK & HESSE 1981): OsO_4 in combination with uranyl acetate demonstrates a very thin sporopollenin layer in certain algae as well as in our pollen grains.

Nevertheless, the general interpretation of such heavy staining coherent surface lines as exine remains controversial: KRESS & al. (1978) do not accept the faint, but heavy staining coherent *Heliconia*-layer as a sporopolleninic exine; PETTITT (1981) rejects his former view (1976), that such thin electron dense layer in *Thalassodendron ciliatum* (*Cymodoceaceae*) represents an exine, while the nature of the "line-like" layers in some other sea-grasses is left undecided (PETTITT 1980). But if evidence is available, both on a remaining coherent exine-"skin" after acetolysis and a significant staining behaviour of ultrathin sections, we should, in my opinion, accept the interpretation of even the faintest layer as a sporopolleninic exine! In fact, such conditions can be found not only in *Strelitzia*, but also in *Persea* and especially in *Gomortega* (*Gomortegaceae*, *Laurales*): Its exine, reputedly discontinuous according to TEM-investigations, has been confirmed as coherent (HESSE & KUBITZKI 1983). In analogy, the pollen grains of *Amphibolis* and

[1] Most probably because the *Strelitzia* exine consists of several, ca. 0.1 µm thick lamellae.

Thalassodendron (Pettitt & al. 1978, Pettitt 1981)—though "exineless" *sensu* Kress & al. (1978)—exhibit a very faint layer as pollen wall coating, perhaps also an exine-"skin".

In short, two conclusions should be drawn: (1) The term "exineless" used for such exines like in *Heliconia* (Kress & al. 1978, Stone & al. 1979) apparently is misleading, because there indeed exists a real, although faint exine; if such an exine forms a "skin" after acetolysis like e.g. in *Strelitzia* or *Persea*, it should be called a "skin-like" exine. (2) In my opinion an exine cannot consist only of some isolated spines without any basal connection; this would cause a considerable pollen wall instability (and a stable sporoderm is one of the most important prerequisites for successful pollen transport!). Even the faintest coherent sporopolleninic layer would act as such a stabilizing and protective coat. Thus, in my view, no exine can be really incoherent, and all its "single elements" should be linked.

The **possible function** of such peculiar sporoderms with a skin-like exine and a thick, channeled intine should be discussed. Coherent, thin exines are to be found typically in hydrophilous species belonging to the *Hydrocharitales* and *Najadales* (Pettitt & Jermy 1975, Pettitt 1976, 1981), but they also occur—evidently as a sign for convergent evolution—in some *Laurales*, which are by no means hydrophilous (Hesse & Kubitzki 1983), and also in the *Zingiberales*, e.g., in the bird-pollinated genus *Strelitzia*. Thus, the peculiar sporoderm of these taxa is neither a direct adaptation to hydrophily, nor to bird- or insect pollination. Because the large intine channels apparently contain proteins for incompatibility processes, a thick, elaborated exine might perhaps prevent such processes; if so, the reduction of the exine could be useful.

In contrast to the questionable functional importance of the sporoderm, the **"threads"** of *Strelitzia* apparently represent an adaptation to bird-pollination. The exactly spherical, smooth *Strelitzia* pollen grains are connected by threads, which become most probably entangeld with the bird's rostrum or feathers during flower visits. These *Strelitzia* threads are by no means to be confused with the "viscin threads" in the *Ericaceae*, *Onagraceae* and in some *Caesalpiniaceae*, because they derive from epidermal cells, are lacking sporopollenin and are not fused with the exine itself (Palla 1891, Kronestedt & Bystedt 1981, Hesse 1981).

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References

BROWN, C. A., 1960: Palynological Techniques. — Baton Rouge: published by the author.

BURCZYK, J., HESSE, M., 1981: The ultrastructure of the outer cell wall layer of *Chlorella* mutants with and without sporopollenin. — Pl. Syst. Evol. **138**, 121—137.

ERDTMAN, H., 1952: Pollen Morphology and Plant Taxonomy **1**, Angiosperms. — Stockholm: Almquist & Wiksell.

HESSE, M., 1981: Viscinfäden bei Angiospermen — homologe oder analoge Gebilde? — Mikroskopie **38**, 85—89.

— KUBITZKI, K., 1983: The Sporoderm ultrastructure in *Persea*, *Hernandia*, *Nectandra*, *Gomortega*, and some other Lauralean genera. — Pl. Syst. Evol. **141**, 299—311.

KRESS, W. J., STONE, D. E., SELLERS, S. C., 1978: Ultrastructure of exineless pollen: *Heliconia* (*Heliconiaceae*). — Amer. J. Bot. **65**, 1064—1076.

KRONESTEDT, E., BYSTEDT, P.-A., 1981: Thread-like formations in the anthers of *Strelitzia regina*. — Nord. J. Bot. **1**, 523—529.

KUBITZKI, K., 1981: The tubular exine of *Lauraceae* and *Hernandiaceae*, a novel type of exine structure in seed plants. — Pl. Syst. Evol. **138**, 139—146.

LE THOMAS, A., 1980: Ultrastructural characters of the pollen grains of African *Annonaceae* and their significance for the phylogeny of primitive Angiosperms. — Pollen et Spores **22**, 267—342.

PALLA, E., 1891: Über die Entwicklung und Bedeutung der Zellfäden im Pollen von *Strelitzia reginae*. — Ber. Deutsch. Bot. Ges. **9**, 85—91.

PETTITT, J. M., 1976: Pollen wall and stigma surface in the marine angiosperms *Thalassia* and *Thalassodendron*. — Micron **7**, 21—32.

— 1980: Reproduction in Seagrasses: nature of the pollen and receptive surface of the stigma in the *Hydrocharitaceae*. — Ann. Bot. **45**, 257—271.

— 1981: Reproduction in Seagrasses: pollen development in *Thalassia hemprichii*, *Halophila stipulacea* and *Thalassodendron ciliatum*. — Ann. Bot. **48**, 609—622.

— JERMY, A. C., 1975: Pollen in hydrophilous angiosperms. — Micron **5**, 377—405.

— DUCKER, S. C., KNOX, R. B., 1978: *Amphibolis* pollen has no exine. — Helobiae Newsletters **2**, 19—22.

ROWLEY, J. R., VASANTHY, G., 1980: Sporopollenin accumulation on the surface coating of *Cinnamomum* pollen and its influence on exine resistance. — Abstr. 5th Intern. Palyn. Conf. Cambridge, p. 346, London 1980.

SITTE, P., 1962: Einfaches Verfahren zur stufenlosen Gewebe-Entwässerung für die elektronenmikroskopische Präparation. — Die Naturwissenschaften **49**, 402.

SKVARLA, J. J., ROWLEY, J. R., 1970: The pollen wall of *Canna* and its similarity to the germinal apertures of other pollen. — Amer. J. Bot. **57**, 519—529.

STONE, D. E., SELLERS, S. C., KRESS, W. J., 1979: Ontogeny of exineless pollen in *Heliconia*, a banana relative. — Ann. Missouri Bot. Gard. **66**, 701—730.

— — — 1981: Ontogenetic and evolutionary implications of a neotenous exine in *Tapeinochilos* (*Zingiberales*: *Costaceae*) pollen. — Amer. J. Bot. **68**, 49—63.

STRAKA, H., 1975: Pollen- und Sporenkunde. — Grundbegriffe der modernen Biologie **13**. — Stuttgart: Gustav Fischer.

WODEHOUSE, R. P., 1932: Tertiary pollen. I. Pollen of the living representatives of the Green River flora. — Bull. Torrey Bot. Club **59**, 313—340.

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