

Grana

Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/sgra20

The elasticity of the exine

John Rowley & John Skvarla Version of record first published: 05 Nov 2010.

To cite this article: John Rowley & John Skvarla (2000): The elasticity of the exine, Grana, 39:1, 1-7

To link to this article: http://dx.doi.org/10.1080/00173130150503759

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <u>http://www.tandfonline.com/page/terms-and-conditions</u>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

The elasticity of the exine

JOHN R. ROWLEY and JOHN J. SKVARLA

Rowley, J. R. & Skvarla, J. J. 2000. The elasticity of the exine. - Grana 39: 1-7. ISSN 0017-3134.

We pressed pollen grain exines of ten genera with sizes ranging from about 20 to over 100 μ m in diameter past a piston in a close fitting cylinder. The clearance between piston and cylinder was about 20 μ m. Except for exines of *Betula* all the other pollen types were at least twice the clearance diameter and could be expected to be greatly deformed, crushed or fractured. Cracks were evident with the light microscope in some grains and a few were clearly deformed but most appeared intact, even exines of *Zea mays* at a diameter of 100–110 μ m. With scanning electron microscopy cracks were apparent in most of the large grains (*Zea, Lilium, Pinus, Crinum* and *Epilobium*) but not in the smaller grains (*Betula, Ephedra, Tulipa, Fagus* and *Typha*). We also found many exines within exines. In some cases, e.g., *Lilium*, the exines entered through apertures but in other grains such as *Zea* and *Pinus*, exines came in through cracks which had opened during acetolysis or centrifugation, then closed so tightly that the cracks were difficult to see with light microscopy. This opening and closing of cracks in exines means that the pollen grain exine is very flexible and resilient and capable of withstanding shock without permanent deformation. To regain their original form the exine components that were severely cracked, ruptured or partly separated must spring back together like the partly separated halves of a tennis ball.

John R. Rowley, Botany Department, Stockholm University, SE-106 91 Stockholm, Sweden; John J. Skvarla, Oklahoma Biological Survey and Department of Botany-Microbiology, University of Oklahoma, Norman, OK 73019-0245, USA.

(Manuscript accepted 1 November 1999)

Traverse (1986: in Palynos) reported finding sapotaceous tricolporate pollen grains inside the large monosulcate pollen of a palm. He suggested that as many as eight of the smaller pollen grains had invaded the large grain during centrifugation following acetolysis. He requested comments from readers. Salgado-Labouriau (1987) sent a letter to Palynos in reply. She had seen many large grass grains filled with tetrads of *Mimosa* pollen. When she turned the grains over she found that they appeared to be entire and without cracks. The small *Mimosa* grains were thought to have entered the large grass grains through their pores during acetolysis.

Our approach to these observations of pollen inside other pollen grains came about as a result of efforts by Belin and Rowley to release pollen grain-held antigens as a source of proteins in pollen of *Betula* (Belin & Rowley 1971). A so called "X-press" was one of the procedures tested. The Xpress is a freeze press designed by Edebo (1960). In its use pollen in buffer was frozen at ca -70° C in a cylinder of the press. Then with a hydraulic press the frozen mixture was forced through a small aperture in the cylinder. As seen by light microscopy most exines appeared to be intact following such treatment. Belin & Rowley (1971) found, however, using transmission electron microscopy (TEM), that most exines had been fractured and many exine fractures fitted together rather closely.

Our aim in this experiment was to see if pollen grains of a variety of sizes, like *Betula* in the experiment above, as well as sapotaceous and *Mimosa* pollen grains as previously mentioned, still appear more-or-less intact after great distortion and fracturing. Such information will be useful for explaining pollen exine elasticity.

MATERIAL AND METHODS

Pollen grains used in the experiments are listed below along with their size data. Pollen of *Lilium, Typha, Betula, Zea, Epilobium* and *Tulipa* were obtained from Allergon AB (Allergon AB, Sweden). *Fagus* and *Crimum* came from the Stockholm University campus and greenhouses. *Ephedra* pollen grains came from Qatar and *Pinus* from the University of Oklahoma campus (Table I).

The pollen grains were dry, except for those of *P. resinosa*, which had just been shed from living microsporangia. The pollen was put into phosphate buffer. After one hour under vacuum in the buffer the mixture was divided into two centrifuge tubes.

The contents of one of these tubes were:

- pressed by a piston down into a Dounce homogenizer tube and up out of the tube three times,
- dehydrated in 100% ethanol, covered by acetolysis acid mixture and heated to 100°C for 10 min,

Table I. Pollen used in our experiment.

The size indicated was after acetolysis.

Taxa	Average size (µm)
Lilium regale	35×95
Fagus sylvatica	42
Typha latifolia (tetrad)	42
Betula pendula	21 - 25
Zea mays	100 - 110
Epilobium angustifolium	85
Tulipa gesneriana	35
Ephedra foliata	$18 - 28 \times 35 - 54$
Crinum purpurascens	55×75
Pinus resinosa	50×75



 washed, centrifuged and examined by light microscopy and scanning electron microscopy (SEM).

The contents of the other tube were:

acetolyzed then centrifuged and washed in water, then pressed by the piston six times as above and then examined as above.

Photomicrographs were taken under oil at X40/Na 1.0 or X100/ Na 1.3 with planapochromatic objectives. In preparation for scanning electron microscopy (SEM) the exines were dried using a critical point drying alternative, hexamethyldisilazane (Chissoe et al. 1994), sputter-coated with gold/palladium (60/40) and examined with a JEOL 880 SEM equipped with a lanthanum hexaboride gun.

RESULTS

Except for *Epilobium* very few of the large exines, *Zea*, *Lilium*, *Crinum*, *Pinus*, *Fagus*, *Typha* and *Tulipa*, showed obvious distortion or cracks by LM after piston/cylinder treatment either before or after acetolysis. The large and gaping crack in the *Zea* exine (Fig. 1) was exceptional in LM observations. There were rare examples of exines broken of *Zea* and *Pinus* with the "halves" detached; part of a *Pinus* exine is in the *Zea* exine in Fig. 18. Exines of *Betula*, *Fagus*, *Typha* and *Ephedra* showed no obvious distortion, except for one *Fagus* grain of the several hundred *Fagus* exines seen with LM, which had an exine of *Betula* nested in it (Fig. 5). Most exines of *Epilobium*, however, were deformed to some extent (Figs. 3, 8 & 10).

Except for *Ephedra* (if passed between piston and cylinder lengthwise) and *Betula*, all the other exines could be expected to be compressed to at least half their diameter on being pressed between the piston and cylinder. Since relatively few exines were distorted these results show that exines are extremely flexible and elastic which allows them to recover their original form after compression and even after extensive fracturing.

In samples prepared for SEM, frequent cracks (fractures) occurred in the largest exines *Zea* (Figs. 6, 9), *Lilium* (Figs. 11, 12), *Pinus* (Fig. 7), and *Epilobium* (Figs. 8, 10) but rarely in the other exines. Exines of *Typha* (Figs. 7 & 9) were not distorted and the *Fagus* exine (Fig. 9) was not crumpled. The sacci were separated to some extent from the body of the *Pinus* exine in Fig. 7 but the exine was not distorted. The exines were severely crushed but sprang back to their original form.

Many exines were seen inside exines that had been pressed between piston and cylinder. The frequency of exines within exines was similar in acetolyzed and unacetolyzed exines. Those exines which contained exines were mostly the larger ones, i.e., Zea, Crinum and Lilium. The smaller (ca $20-40 \mu$ m) exines were mainly those of Betula although the larger (ca $40-80 \mu$ m) exines of Fagus, Pinus, Epilobium and tetrads of Typha were seen in Lilium. The aperture of Lilium is large enough to permit entry of these exines, and some exines of Lilium were clearly cracked (Figs. 11, 12). Probably all pollen grains of reasonable size with sulcate apertures would permit entry of other smaller exines, e.g., Crinum in Figs. 13–16. The pore of Zea is much too small for any of the grains in our mixture and grains within exines of Zea must have entered by a fracture in the exine. Many exines of Zea were flattened and showed concavities; exines of other species were frequently nested in such concavities (Fig. 3 and probably also one of the Betula exines in Fig. 5).

The through-focus Figs. 13-16 of a *Crinum* exine show that the *Betula* exine is inside the *Crinum* one. The through-focus series of *Zea* (Figs. 17-19) shows that a *Betula* exine and portion of a broken *Pinus* exine are within the *Zea* grain. The crack in the *Zea* grain that allowed entry of *Betula* and *Pinus* is essentially closed but shows as two bright parallel lines (Fig. 19).

DISCUSSION

Only when apertures are large do exines get into other exines without the inner or outer exines being fractured or cracked. This is certainly the case for *Lilium* and probably also for *Crinum*. Entry of exines into a grain like *Zea* with a very small aperture must be through a fracture or crack. We supposed that the exine on each side of an extensive crack could, on being pressed through a narrow space, separate like the halves of a broken tennis ball. Like the broken tennis ball the still connected halves of a broken exine could return to a nearly complete form although, of course, still cracked. Other exines might enter the cracked grain when the crack is open as during acetolysis, centrifugation or immediately following being pressed by the piston through the cylinder.

Recognition that exines flex between hydrated and dry conditions is apparent in the works of von Mohl (1835), Mirbel (1833), Fritzsche (1834) and Meyen (1828) between the years 1830 and 1839 (see text and references in Wodehouse (1935). The concept of harmomegathy (Wodehouse 1935) involves exine flexibility in its accommodation to volume changes. Payne (1972) found that the entire pollen wall, not only the apertures, functions in volume

Figs. 1-5. Light micrographs of acetolyzed or unacetolysed exines pressed between a piston and tight fitting (ca 20-µm clearance) cylinder. Bars = 10 µm (All photomicrographs are at the same magnification). (1) An extensive crack in *Zea*. (2) The exine of *Zea* is clearly cracked and has a large opening that partly shelters an exine of *Fagus (arrow)*. Two *Betula* exines (*arrowheads*) lie within the broken *Zea*. On passing the piston and cylinder the *Zea* exine must have been distorted to a diameter about that of the *Betula* exines. The exines of *Pinus (asterisks)* appear to be intact. (3) Three exines of *Betula* within a *Zea* exine (note pore, *circled*) and a tetrad of *Typha* (T) outside nested in an exine concavity. The exine of *Epilobium (star)* is greatly damaged. The exine of *Fagus (arrow)* and several of *Pinus* (one marked by an *asterisk)* and *Betula (arrowhead)* do not appear damaged. (4) Exine of *Pinus* within an exine of *Zea* enclosing three *Betula* exines. The arrow at the upper right points to a crack seen as a bright line on the surface of the *Zea* exine. This crack could be traced part way under the exine at lower focal levels. The greatly damaged exine at the lower left is *Fagus* overlaid by or partly enveloping an exine of *Betula (arrowhead)*. The *Pinus* exine appears to be intact.



regulation. Bolick (1981) measured forces involved in changes in exine form, such as bending. Heslop-Harrison (1979), in his work on the hydrodynamics of pollen, discussed the dilation of the cytoplasm and thus substantial expansion of grains, during their rehydration in the pregermination interval on stigmatic surfaces.

During microspore development to pollen grain maturity it is clear that in most species there is a certain degree of enlargement of the circumference of the exine. In Zea the volume increases ca 1,200 times from microspores in the tetrad at ca 10 µm in diameter to mature pollen at ca 120 µm. How this increase is accomplished with regard to the exine is not clearly defined, as far as we know. Stretching, which is an indication of plasticity, is mentioned in reports of exine development. Banerjee et al. (1965) showed that columellae were arranged in files of twos and threes under muri early in development of the reticulate exine of Sparganium longifolia. Later in development, as the microspores/pollen grains enlarged, the muri of the greatly enlarged reticulations were "supported" by a single file of columellae. Not only were the columellae uniserate rather than multiserate under the muri but they were also more widely separated than in younger stages (Banerjee et al. 1965: Figs. 12-17). Banerjee et al. show that exines can be stretched. The extent of stretching can be appreciated in their camera lucida drawings and measurements of separation of columellae throughout development. Columellar separation increases from 0.14 µm during tetrad time to 0.35 µm at maturity. Takahashi (1980) in work on development of the reticulate structure of Hemerocallis pollen agreed with the above interpretation.

A remarkable aspect of our experiment is the condition of the muri of the *Lilium* reticulum. Muri are supported by relatively slender and tall columellae about 0.5 μ m in height (Dickinson 1970, Southworth 1985, and Takahashi 1995). The large exines of *Lilium* were greatly compressed by our methods. In our SEMs we have selected a damaged aperture in Fig. 6 and a broken exine in Fig. 7 because exines of other grains were evident within them, but most exines of *Lilium* were without obvious damage especially with respect to the muri (Fig. 5). Even in Fig. 7 where the exine is broken the muri appear without modification up to the edge of the fractures. The exine structure that allows such damaging treatment without deformation is strong but plastic and flexible and certainly not brittle. The exine of *Epilobium angustifolium* seems to be an exception to the above situation since its exine was seen in many cases to remain deformed following compression between piston and cylinder. This result might be explained or even expected, perhaps, by the exceptional structural complexity of the onagraceous pollen exine as reported by Skvarla et al.1975, 1976, Keri & Zetter 1992, Praglowski et al. 1994, and Rowley & Claugher 1991.

Pollen exines may enter other pollen grains through large apertures. If the aperture is small, grains probably enter through breaks or cracks that are open during acetolysis, centrifugation or, in our experiment, when they are pressed by a piston past the close fitting cylinder.

Fractured pollen grain exines are like broken tennis balls. They can be almost completely separated by a fracture but the remaining "wall" will be enough to "snap" the components back to a very nearly intact-appearing form. The clearance of our piston/cylinder device is ca 20 mm, thus only *Betula* could be expected to be undamaged. Most of the exines, even the large ones (*Zea* and *Lilium*), appeared undamaged although they must have been greatly compressed (flattened). Thus our foremost conclusion is that the exine structure is very flexible, elastic and strong.

ACKNOWLEDGEMENTS

We thank W. F. Chissoe, University of Oklahoma and Samuel Roberts, Noble Microscopy Laboratory, for his assistance with the scanning electron microscopy. We wish to thank Dr Nina I. Gabarayeva for many helpful suggestions on an early draft of our manuscript.

REFERENCES

- Banerjee, U. C., Rowley, J. R. & Alessio, M. L. 1965. Exine plasticity during pollen grain maturation. – Journal of Palynology 1: 70–89.
- Belin, L. & Rowley, J. R. 1971. Demonstration of birch pollen allergen from isolated pollen grains using immunofluorescense and single radial immunodiffusion technique. – International Archives of Allergy and Applied Immunology 40: 754–769.
- Bolick, M. R. 1981. Mechanics as an aid to interpreting pollen structure and function. Review of Palaeobotany and Palynology 35: 61–79.
- Chissoe, W. F., Vezey, E. L. & Skvarla, J. J. 1994. Hexamethyl-

Figs. 6-12. Scanning electron micrographs of acetolyzed exines pressed between a piston and tight fitting (ca 20-µm clearance) cylinder. All micrographs are at the same magnification. (6. Bars in Figs. 6 and $7=10\mu m$) This Zea exine is extensively cracked but the crack is more or less closed. The pore is about 5 nm in diameter and is occluded by its operculum. Many exines were seen with the LM to be within Zea exines, in some cases cracks were apparent (Figs. 1, 2, 9 & 19) but in others cracks were either not apparent by LM (Fig. 3) or very difficult to see (Fig. 5). It is without question, that the exines had entered Zea via a crack but cracks as open as in Fig. 1 were very rare, as seen by LM. The elasticity of exine construction is indicated by the more-or-less closure of cracks that had been large enough to allow entry of any of the exines in our mixture. (7) Exines of Betula (arrowhead), Pinus (asterisk) and Typha (arrow). The Betula and Typha tetrad appear undamaged but both sacci of Pinus are fractured from the aperture region although still attached to the cap. (8) Exines of Epilobium, unlike those of the other nine genera, were frequently crushed or otherwise greatly distorted. The complex structuring of its exine apparently is not as flexible as the others. (9) Zea exine is crumpled and fractured. There are grains of Fagus (arrowhead) and Typha (arrow) adjacent to Zea and the sacci of Pinus are at the top and left margin of the figure. (10) The exine of Lilium shows the intact proximal face. A greatly damaged exine of Epilobium partly overlaps the Lilium exine. A portion of an Ephedra exine (arrow) shows at the upper left. (11) Because of the large size of the Lilium pollen aperture, exines of other grains could be seen within Lilium in SEM images. The exine apparent in the aperture is that of Pinus (asterisk). The Lilium aperture must have been wider during entry of the Pinus exine. (12) The Lilium exine is severely damaged and shows an exine of Fagus through the tear in its exine. There are adjacent exines of Betula and Fagus.



Figs. 13-16. Through focus LM series of a *Betula* pollen grain inside a *Crinum* grain. The *Crinum* grain is 1-sulcate and large (Table I). The grain of *Betula* may have entered the *Crinum* exine via the aperture but the *Crinum* exine may have been split open and then closed around the *Betula* exine. All magnifications are the same; Bars on Figs. 13 and $14=10 \mu m$. (13) A photomicrograph at a high focal level. Surface spines of *Crinum* and the two *Betula* exines at the top of the figure are in focus. (14) Optical section that is nearly medial. Spinules of *Crinum* are seen in profile. The *Betula* grain is in better focus, and the two *Betula* grains above are going out of focus. (15) Below the medial section of *Crinum*. The *Betula* exine is better focused inside the *Crinum* exine. The two *Betula* exines at the top of the figure are in focus. The two *Betula* grains above are now out of focus. These figures show that the *Betula* exine is within the exine of *Crinum*.



Figs. 17–19. LMs of a Zea exine with Betula and part of a Pinus exine within it. The saccus of Pinus (asterisks) at the top of Figs. 17 and 18 and the pointed tracheid (stars) at the bottom of Figs. 18 and 19 show that these micrographs are of the same Zea exine. Bars=10 μ m. (17) The focus is high with the pore and surface texture in evidence. (18) This medial focal level shows the Betula exine (arrowhead) and part of a Pinus exine (arrow). (19) The surface texture of Zea is apparent. The bright curved double line (circled) at the upper right in the Zea exine indicates the crack that opened allowing entry of the exines of Betula and Pinus.

disilazane as a drying agent for pollen scanning electron microscopy. – Biotechnic and Histochemistry 69: 192–198.

- Dickinson, H. G. 1970. Ultrasturctural aspects of primexine formation in the microspore tetrad of *Lilium longiflorum*. – Cytobiologie 1: 437–449.
- Edebo, L. 1960. A new press for the disruption of microorganisms and other cells. – Journal of Biochemical and Microbiological Technology and Engineering 2: 453–457.
- Fritzsche, C. J. 1834. Ueber den Pollen der Pflanzen und das Pollenin. – Poggendorf's Annalen der Physik und Chemie (Leipzig) 32 31: 481–492.
- Heslop-Harrison, J. 1979. An interpretation of the hydrodynamics of pollen. – American Journal of Botany 66: 737–743.
- Keri, C. & Zetter, R. 1992. Notes on the exine ultrastructure of Onagraceae and Rhododendroideae (Ericaceae). – Grana 31: 119–123.
- Meyen, F. J. F. 1828. Anatomisch-physiologische Untersuchungen über den Inhalt der Pflanzenzellen. – Hirschwald, Berlin.
- Mirbel, C. F. 1833. Recherches anatomiques et physiologiques sur le Marchantia polymorpha pour servir à l'histoire du tissu cellulaire de l'épiderme et des stomates. – F. Didot, Paris.
- Mohl, H. von. 1835. Sur la structure et les formes des grains de pollen. – Annales des Sciences Naturelles (Paris) 3: 148–180; 220–236; 304–346. pl.: 9–11.
- Payne, W. W. 1972. Observations of harmomegathy in pollen of Anthophyta. – Grana 12: 93–98.
- Praglowski, J., Nowicke, J. W., Skvarla, J. J., Hoch, P. C., Raven, P. H. & Takahashi, M. 1994. Onagraceae Juss. Ciecaeeae DC., Hauyeae Raimann, Epilogieae Spach. – World Pollen and Spore Flora 19. Scand. Univ. Press, Stockholm.
- Rowley, J. R. & Claugher, D. 1991. Receptor-independent sporopollenin. – Botanica Acta (Germany) 104: 316–323.
- Salgado-Labouriau, M. 1987. Letter with no title. Palynos 10 1: 1.
- Skvarla, J. J., Raven, P. H. & Praglowski, J. R. 1975. The evolution of pollen tetrads in Onagraceae. – American Journal of Botany 62: 6–35.
- Skvarla, J. J., Raven, P. H. & Praglowski, J. R. 1976. Ultrastructural survey of Onagraceae pollen. – In: The evolutionary significance of the exine. (ed. I.K. Ferguson & J. Muller), pp. 447–479. – Linn. Soc. Symp. Ser. No 1. Academic Press, London.
- Southworth, D. 1985. Pollen exine substructure. I. Lilium longiflorum. – American Journal of Botany 72: 1274–1283.
- Takahashi, M. 1980. On the development of the reticulate structure of *Hemerocallis* pollen (Liliaceae). Grana 19: 3–5.
- Takahashi, M. 1995. Three-dimensional aspects of exine initiation in *Lilium* longiflorum (Liliaceae). – American Journal of Botany 82: 847–854.
- Traverse, A. 1986. Would you believe? Palynos 9 (2): 8.
- Wodehouse, R.P. 1935 Pollen grains. McGraw-Hill, New York London.