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Spore movement driven by the spore wall in an eusporangiate fern

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Abstract

We report the ejection of spores on sporangium dehiscence in the eusporangiate fern genus *Angiopteris* (Marrattiaceae, Marattiales). Using normal and high-speed video we document movement of the spores and using light and electron microscopy we study the structural changes associated with the movement. The sudden and spontaneous movement covering distances of up to several mm cannot be ascribed to action of the sporangium. We find that cavitation between exospore and perispore wall is the most likely responsible mechanism. We suggest that spore ejection by movement of a spore wall layer may have driven the evolution of an elaborate multilayered spore wall in ferns.

Keywords: *Angiopteris*, *Marrattiales*, *spore movement*, *spore wall*, *spore dispersal*

Explosive ejection of microscopic particles (spores or pollen-grains) is well-documented in fungi (Ingold, 1965; Trail et al., 2005; Pringle et al., 2005; Trail, 2007; Yafetto et al., 2008), but plays a much more limited role in green plants (Ingold, 1939; Edwards et al., 2005; Taylor et al., 2006). Of the various mechanisms that are involved (see Ingold, 1939), release of tension in cell walls through cavitation is perhaps the most widely known mechanism, as it can easily be observed in the sporangium of leptosporangiate ferns (Steinbrinck, 1897, 1902). In eusporangiate groups a similar mechanism has been observed in the genus *Selaginella* (Goebel, 1901; Steinbrinck, 1902; Koller & Scheckler, 1986). Spore movement has also been observed in the eusporangiate fern genus *Angiopteris* and has been ascribed to movement of the sporangium wall as well (Goebel, 1930, p. 1343).

Here we present new observations on the movement of spores during the dehiscence of sporangia of *Angiopteris*. We investigated the behaviour of the spores both in the context of the sporangium and after isolation, we studied

dehydration/rehydration behaviour under a variety of conditions and we made a structural investigation of the spore wall layering. We conclude that it is not the sporangium wall but cavitation-related movement of the wall layers of the spore itself that is the most likely responsible mechanism for this behaviour.

Material and methods

Ripe sori were collected from a specimen of *Angiopteris* cf. *evecta* (Forst.) Hoffm. (Marrattiaceae, Marattiales), cultivated in the Leiden Botanical Garden under nr. 8088, a plant obtained from the National Botanic Garden of Belgium, Meise, in 1964; previous origin unknown. The genus *Angiopteris* has a wide Paleotropic distribution, and contains some of the largest ferns known, with fronds reaching 6 m in length or more. Altogether, some 200 species have been described, but the current taxonomy is “contentious” (Christenhusz & Toivonen, 2008; see also Murdock, 2008) and our identification must be regarded as tentative.

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We collected entire pinnules with ripe sori in plastic bags, where we could store them for up to several days, after which we were still able to induce dehiscence by drying. We observed the dehiscence of sporangia under room conditions both in entire pinnules and on small excised parts, without noticing any difference in behaviour of the sporangia. We isolated spores from sporangia just before dehiscence and observed their behaviour under room conditions in a free state and while immobilised with double-stick tape. For light microscopical observations (LM) we made observations in water or, to inhibit rehydration, in lactic acid, or, to induce dehydration by osmosis, in glycerol; in all cases with an Olympus BH2 (Olympus Nederland BV, Zoeterwoude) microscope equipped with a ColorView (Olympus Soft Imaging Solutions, Münster, Germany) digital camera. Standard video images were captured with an Euromex VC 3021 camera (Euromex microscopen B.V., Arnhem), high-speed video-footage was shot with a Phantom V4.2 B/W camera (Vision Research, Wayne, New Jersey) at a frame rate of 2 000 frames/second.

For scanning electron microscopy (SEM) spores were sputter-coated with gold and examined in a JEOL JSM 5300 (JEOL Europe B.V., Nieuw Venneep, The Netherlands) microscope. For transmission electron microscopy (TEM), spores were embedded in Spurr resin, sectioned with an LKB Ultratome III (LKB, Bromma, Sweden), post-stained with uranylacetate and lead citrate and examined with a JEOL-1010 microscope.

Observations

Spore movement during dehiscence

During dehiscence of the sporangium, the sporangium opens relatively slowly, the movement apparently driven by dehydration of the cells in the wall. Spore movement occurs without any apparent involvement of the sporangium wall. While the mass of spores is forced out of the sporangium with a more or less continuous movement, individual spores or small groups of spores may be propelled in an abrupt and explosive manner [see Supplementary material – Video 1], to reach distances of up to 4 mm, although most spores are propelled over much smaller distances. The movement of the spores persists for a few minutes.

When mature spores are isolated from fresh sporangia they show the same jumping movement [see supplementary material – Video 2]. High-speed video recordings of erupting spores clearly show the explosive nature of the movement [see supplementary material – Videos 3, 4].

These observations clearly show that spores of *Angiopteris* are able to propel themselves over distances of up to several millimetres without any application of external force. Taking into account their size of c. 30 μm , this would imply a maximum launch speed of c 2 m/s (Vogel, 2005; 2009, pers. comm.), which is more or less consistent with the high-speed recordings where we have observed initial speeds generally below 1 m/s.

This movement of the spores is limited to mature spores and is strictly a one-time behaviour. Although it is possible to induce dehiscence of immature sporangia by dehydration, the spores from immature sporangia do not move. Spores also do not show any movement when they are rehydrated after dehiscence and again are left to dry out.

Structure of the spores

Spores are almost perfectly spherical in shape, with diameters of (24–) 26 – 28 (–30) μm , and have a trilete laesura that is weakly marked with c. 10 μm long ridges (Figure 1A). As usual in ferns, the spore wall consists of two layers: an inner exospore and an outer perispore (Tryon & Lugardon, 1991). The exospore is composed of a very thin internal layer and a ca 0.4 μm thick outer layer which is not visibly further stratified. The perispore is as thick as or somewhat thicker (to 0.6 μm) than the exospore and is composed of a number of electron-dense layers which appear to be separated after fixation. Both exospore and perispore are weakly rugulate, with the perispore following the exospore contours (Figure 1B). Similar spores are present in other species of *Angiopteris* (Tryon & Lugardon, 1991).

Effect of dehydration on individual spores

Before dehiscence, the spores are embedded in a wet matrix inside the sporangium and are completely hydrated and spherical in shape. After dehiscence and the associated movement, spores are partially dehydrated, and nearly always show either a distinct depression or a gas bubble inside the spore wall (Figure 1C, arrows). When freshly dehiscid spores are immediately afterwards rehydrated, the gas disappears without trace within a few minutes. Gas also appears when the spores are immersed in glycerol after they are removed from nearly dehiscid sporangia. Together, this clearly indicates that it is cavitation, not airflow, that produces the gas bubble, and that accordingly the changes are highly abrupt. This is confirmed by the observation that immediately prior to the appearance of the gas bubble no deformation of the spore is visible (Figure 1D).

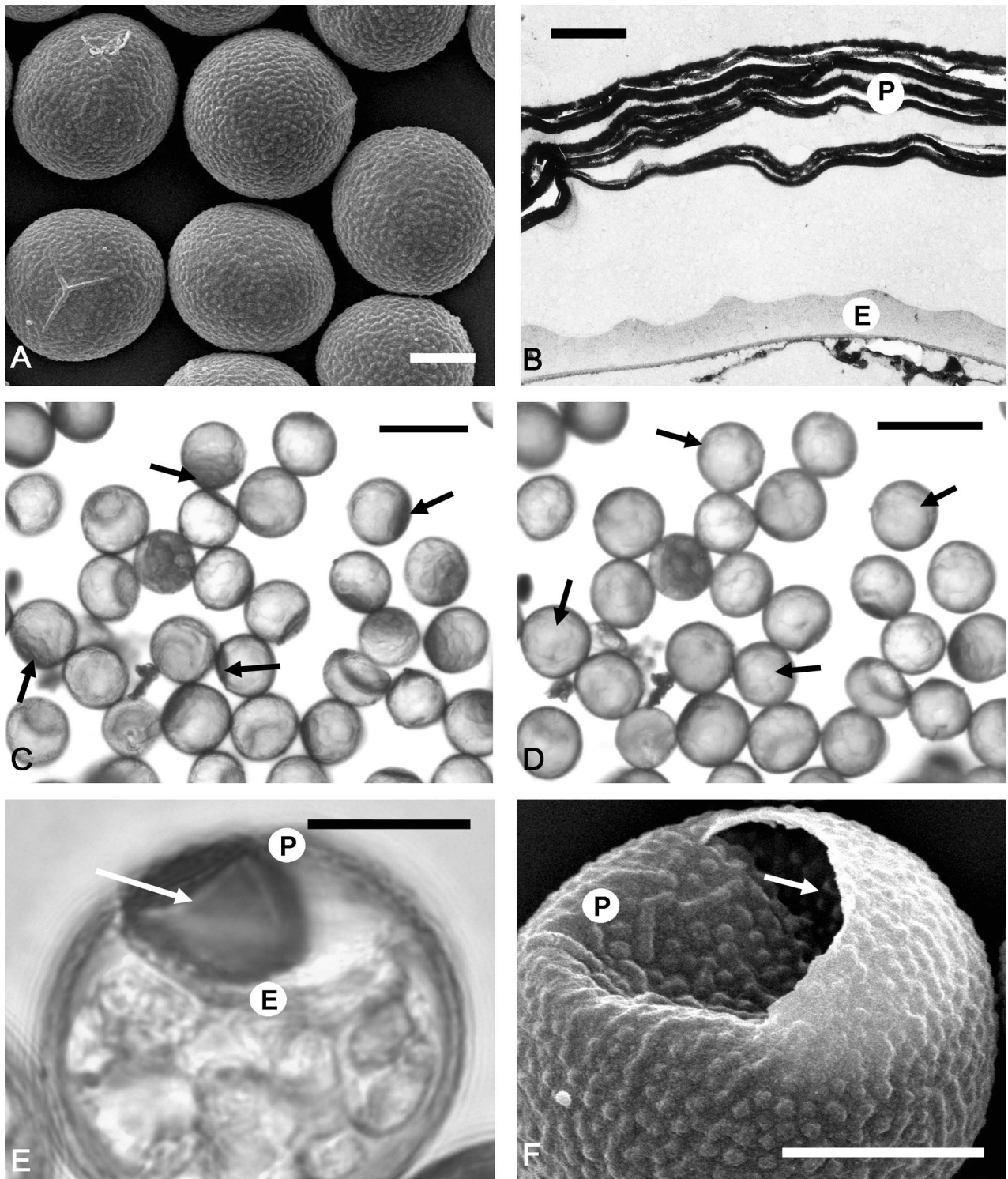


Figure 1. *Angiopteris* cf. *evecta*. **A.** Spores taken after release (SEM). **B.** Cross-section through the spore wall (TEM); note the multilayered perispore (*P*) and the homogeneous exospore (*E*). **C.** Spores isolated from sporangium before release and immobilised on double-stick tape until cavitation took place (LM); cavitation indicated (*arrows*); room conditions, immobilised on double-stick tape. **D.** As (*C.*) few seconds earlier; note the absence of visible deformation in the as yet uncavitated spores, indicated (*arrows*). **E.** Artificially damaged spore taken after release (LM), with partly enclosed gas bubble (*arrow*) between exospore (*E*) and perispore (*P*) immersed in lactic acid. **F.** Spore taken after release (SEM); the hollow space indicated where part of the perispore (*P*) has become detached from the exospore (*arrow*). Scale bars – 10 μm (*A* & *E*, *F*); 1 μm (*B*); 40 μm (*C*, *D*).

Further observation of spores in a water-free medium (to inhibit rehydration) shows that the bubble is trapped between two spore wall layers, not inside the spore wall itself. This is particularly clear when the perispore is ruptured (which can easily be effected by applying some pressure and/or movement to the cover slip), in which case the gas bubble can escape, sometimes only partially (Figure 1E). In the latter case, it is usually clearly visible that the exospore is deformed and that the bubble is captured in a cavity between the exospore and the intact perispore. Examination with SEM of spores collected after dehiscence shows a small minority of cases where the perispore is damaged and remains partially attached to the depressed exospore, showing the cavity that forms between exo- and perispore (Figure 1G).

Discussion

Both the occurrence of the movement and the absence of it after rehydration require an explanation. The observation that spore movement occurs after isolation of the spores definitely rules out the possibility that the sporangium is involved in the generation of the movement, as was suggested by Goebel (1930). Instead, the source of the movement has to be found solely in the spore wall. As the best explanation for all the observed phenomena, we suggest the following process (Figure 2):

As the spore contents gradually dehydrate, the spore shrinks. During the shrinking, tension builds up in the spore wall, due to the fact that a reduction of spore volume has to be achieved while the total

amount of spore wall cannot be reduced. Usually, in dehydrating spores or pollen grains, this tension is accommodated by harmomegathic movement of the wall (Blackmore & Barnes, 1986; Payne, 1981; Pacini, 1990), but the near-perfect spherical shape and uniform structure in this case instead results in uniform deformation of the spore wall during the shrinkage. If dehydration continues, this complete deformation of the spore wall becomes more and more difficult. At a certain critical dehydration level it becomes energetically more favourable for the spore wall to buckle inwards. When this happens, the exo- and perispore are separated, creating a cavity between the exo- and perispore. At that moment the perispore, which used to be under tension, is suddenly released and can expand to regain the shape it had in the hydrated spore. The elastic energy that becomes available can then generate a launch of the spore. It is clear that this must happen before tension is so high that it results in cavitation inside the cell, which is generally considered to be lethal for spores and pollen of vascular plants.

We suggest that the following structural properties are important factors in building up and fine-tuning the exact amount of tension:

1. The spherical shape of the spore. This shape maximises resistance to deformation under pressure.
2. The different structures of exospore and perispore. Generally, perispores are considered less elastic and more rigid than exospores, and the resistance of the perispore against buckling may in this specific case be increased by its multilayered structure.

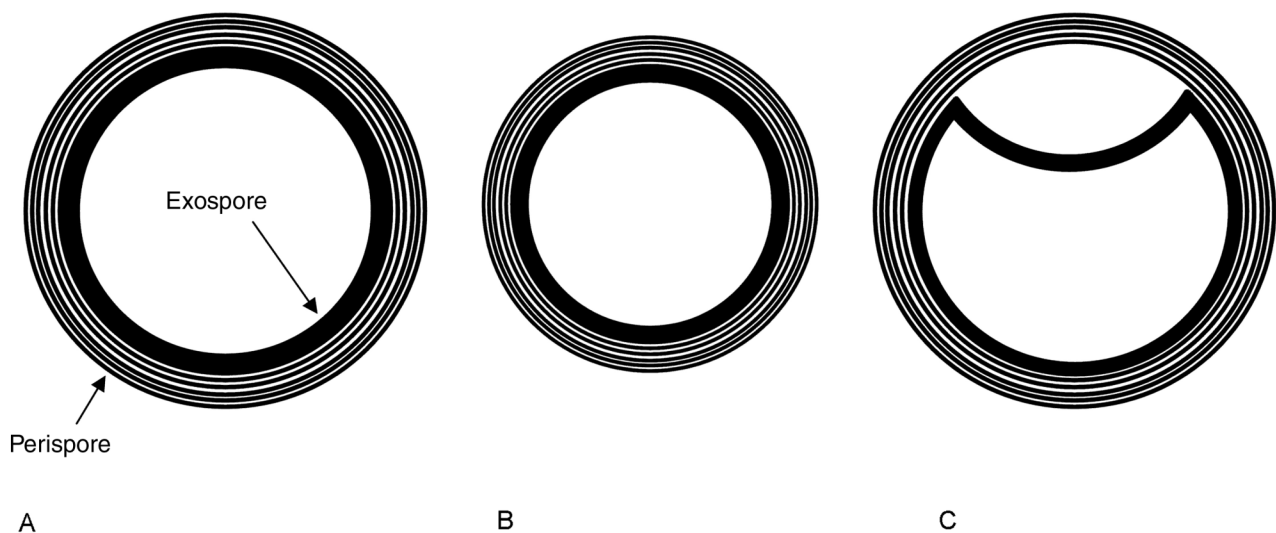


Figure 2. Schematic representation of the processes leading to spore ejection (not to scale). **A.** Fully hydrated spore. **B.** Partially dehydrated spore with reduced volume. **C.** Cavitation between cell wall layers has taken place and the spore has regained its original volume.

3. Adhesion between the exospore and the perispore. The adhesion of the exospore to the more rigid perispore initially transfers the resistance of the perispore to the exospore. Thus the level of the adhesive forces between the two layers governs the resistance of the exospore to buckling and thereby determines the degree of dehydration that can be reached before buckling takes place. (The separation between exospore and perispore and of the perispore layers seen with TEM in Figure 1B are most likely fixation artefacts). This governing role of adhesive forces also explains why rehydrated spores do not show the same movement a second time: when adhesion has been overcome once, it will not be re-established with its original force when the spore is rehydrated, and during the subsequent dehydration the exospore will more gradually dissociate itself from the perispore, and if any buckling takes place, it will generate less force.
4. The regulation of the exospore. The regulation of the exospore wall increases the contact surface between exospore and perispore and thereby the resulting adhesive force, so that the degree of regulation may be fine-tuned to the exact required force, balancing the need to maximise the amount of force released in buckling and the need to avoid cavitation inside the exospore.

In all other cases of particle ejection studied so far the source of the force resides outside the propelled particle itself. The mechanism observed here in *Angiopteris* is unique in that it is the particle itself that generates the force, relying on mechanical properties of the wall structure. The specific combination of characters that allows this movement is common in all species of *Angiopteris* but appears to be absent in other fern groups (Tryon & Lugardon, 1991), suggesting that the mechanism is typical for the genus *Angiopteris*. Species of *Angiopteris* occur mainly in moist forests and forest gullies, where low wind speeds prevail, in particular close to the lamina surface. The movement here described occurs under the dry conditions that are favourable for further dispersal. It is then able to break up the spore mass, force the bulk of the spores out of the sporangium and launch at least some spores directly into the air-stream, where they may be further dispersed.

Horsetails (with *Equisetum* as the only extant genus) are the only other group of land plants where spore movement is effectuated not by the sporangium but by the spore wall itself. There the process is different in at least two aspects: it is driven by reversible hygroscopic movement, and deformation of the inner wall does not play a role (Ingold, 1939). It may be significant, though, that both groups

where the spore wall has a function for spore movement are eusporangiates with a basal position in the phylogeny of vascular plants (Pryer et al., 2001, 2004), and also that the structures that generate the force are probably not homologous (Uehara & Kurita, 1989). In combination, this makes it likely that the two mechanisms have developed independently in response to the same need for spore dispersal. There is a clear adaptive value to any mechanism that expels spores from their container when conditions are favourable to their further dispersal, and it is therefore possible that at a time when the leptosporangium had not yet evolved, this evolutionary pressure drove the development of an elaborate multilayered spore wall. A highly elaborate and distinctively patterned outer spore wall (perispore) is currently also characteristic for many higher leptosporangiate ferns (Tryon & Lugardon, 1991), where it is likely a parallel development to the spore wall elaborations of *Angiopteris* and *Equisetum*. To date, the function of the leptosporangiate perispore remains unclear, and the identification of a possible adaptive value of an outer spore-wall layer in two other, basal, fern groups leads us to suggest that it may originally have had a similar function. An evolutionary scenario then can be envisaged in which, in the absence of other ejection mechanisms, an elaborate outer spore wall layer first developed as adaptation to the need for spore ejection, but then was released from functional constraints when a more effective ejection mechanism developed with the leptosporangium. The further development of this layer may then have been mainly the effect of various processes of self-assembly acting on the available wall material and its precursors (Van Uffelen, 1991; Gabarayeva & Hemsley, 2006).

Supplementary material – Videos

(Available with online article, and at <http://www.nationaalherbarium.nl/hovenkamp/spore%20movement.htm>)

- 1 – Real-time recording of single sporangium during dehiscence.
- 2 – Real-time recording of isolated spores taken from sporangium before release.
- 3 – High-speed recording of ejecting spores (2000 frames/second, elapsed real time for the entire video 10 ms).
- 4 – High-speed recording of ejecting spores (2000 frames/second, elapsed real time for the entire video 20 ms).

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