Mechanical design of apertures and the infolding of pollen grain

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When pollen grains become exposed to the environment, they rapidly desiccate. To protect themselves until rehydration, the grains undergo characteristic infolding with the help of special structures in the grain wall—apertures—where the otherwise thick exine shell is absent or reduced in thickness. Recent theoretical studies have highlighted the importance of apertures for the elastic response and the folding of the grain. Experimental observations show that different pollen grains sharing the same number and type of apertures can nonetheless fold in quite diverse fashion. Using thin-shell theory of elasticity, we show how both the absolute elastic properties of the pollen wall as well as the relative elastic differences between the exine wall and the apertures play an important role in determining pollen folding upon desiccation. Focusing primarily on colpate pollen, we delineate the regions of pollen elastic parameters where desiccation leads to a regular, complete closing of all apertures and thus to an infolding which protects the grain against water loss. Phase diagrams of pollen folding pathways indicate that an increase in the number of apertures leads to a reduction of the region of elastic parameters where the apertures close in a regular fashion. The infolding also depends on the details of the aperture shape and size, and our study explains how the features of the mechanical design of apertures influence the pollen folding patterns. Understanding the mechanical principles behind pollen folding pathways should also prove useful for the design of the elastic response of artificial inhomogeneous shells.

INTRODUCTION

Pollination is a crucial process in the life cycle of plants. For it to proceed, pollen grains must leave the anther, which exposes them to rapid desiccation as they cannot actively control their hydration status [1–3]. The grains thus require some sort of protective mechanism against desiccation in the period before they land on the stigma of a flower, where they germinate upon rehydration to complete the fertilization. The near-universal protective mechanism against desiccation in pollen during presentation and dispersal is harmomegathy: a characteristic infolding of the grain in response to a decreasing cellular volume upon dehydration [1, 4–6].

The shell of a pollen grain encapsulates the male plant genetic material it carries. It consists of two biopolymeric layers: intine, a soft cellulosic interior layer, and exine, a hard exterior layer composed of sporopollenin and impermeable to water [2, 4, 5]. The two layers are not homogeneous throughout the shell, and regions which significantly differ structurally and morphologically from the rest of the shell wall and where the exine layer is either absent or reduced in thickness are termed apertures [4–8]. Not only do apertures play a role in the harmomegathic accommodation of grain volume changes, they also function as sites for water uptake and the initiation of pollen tubes [4, 9, 10]. Despite these shared functions of apertures, their number, shape, and size vary greatly among and within pollen species [4, 11]. Most commonly, apertures occur in the form of a spherical lune (colpus or sulcus), a circular region (porus or ulcus), or a combination of the two (colporus). Monosulcate pollen with a single distal aperture is ancestral in angiosperms (flowering plants) [12, 13], and remains a distinctive feature of a large evolutionary line of monocots. In eudicots, a very large clade comprising approximately 75% of the extant angiosperm species, pollen grains are most often characterized by three apertures [13]—see Fig. 1. In general, many aperture patterns can be observed in angiosperm pollen, and some of them may not easily fall in a predetermined category. Angiosperm pollen can also be inaperturate or omniaperturate, and evolutionary trends appear to favor an increasing number of apertures [7, 9, 13]. Nonetheless, the vast majority of angiosperms produce pollen with one or three apertures [11–18].

During desiccation, apertures often fold inwards, and in many pollen species the edges of each aperture eventually touch each other and effectively close off, thus reducing the rate of water loss [20–22] [Fig. 1d]. The precise effects of the aperture number or shape on harmomegathic volume accommodation are, however, still not completely understood [6, 7]. Empirical studies have shown that harmomegathy depends on the aperture pattern, but it also appears to be influenced by other characteristics such as the size or shape of the grain [7, 23]. Some flexibility of the exine wall is also required—since even pollen without apertures is able to accommodate volume changes to a degree [10]—and exine ornamentation and its elastic properties have been suggested to be involved in the process as well [21, 22]. Overall, the entire suite of structural

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adaptations of angiosperm pollen grain seems to be tailored to favour large-scale, mostly inextensional bending of its wall, allowing the apertures to fold inward and in this way reduce the rate of desiccation [1, 22–24].

Depending on the species, pollen changes its shape during desiccation in surprisingly different ways, from a regular infolding to a seemingly random, irregular fashion—even if the type of exine ornamentation and the aperture condition appear to be almost identical [2, 4, 23]. While it is thus clear that various morphological and structural adaptations enable adjustments of pollen grain to sudden volume changes, not much is known about the precise nature of this relationship [25]. Recently, several theoretical studies have explored the role that apertures or, more generally, local soft spots play in the infolding of elastic shells. Work by Katifori et al. [22] focusing on colpate pollen has shown that apertures contribute to harmomegathy by reducing the necessity of the pollen wall to significantly stretch and bend in order to accommodate volume changes, guiding the infolding along specific pathways depending on the aperture area and shape. That local weak spots in an otherwise uniform shell can profoundly alter its buckling behaviour has also been shown by Paulose and Nelson [26] for circular soft regions. Other studies not directly focusing on pollen folding have shown that buckling behaviour of spherical shells sensitively depends on the distribution of soft spots and their shapes [27] and that placing circular soft regions in specific geometric configurations on spherical shells fundamentally alters their buckling under pressure [28]. The softness of spots can be realized by a spatial variation in either the elastic properties of the shell or its thickness, which consequently also produces elastic inhomogeneity in the shell [29–31].

A detailed knowledge of the elastic properties of the grain and their spatial variation is required in order to formulate a model of pollen infolding: a soft spot in an overall soft shell will lead to a different folding pathway than the same soft spot in an otherwise hard shell. Yet the mechanical properties of pollen remain largely unexplored, and little is known about how these properties and their variation influence folding pathways of pollen grains. Our work addresses this important question by using an elastic model which is versatile enough to investigate different modes of pollen folding based on the estimated elastic properties of pollen walls. This allows us to explore phase diagrams of folding pathways with respect to the overall elastic properties of pollen walls and the degree of weakening provided by the apertures. In this way, we are able to distinguish between regions where the apertures successfully close and the (possibly irregular) pathways where they do not close properly. We also show how the aperture size, shape, and their number all play a role in determining the folding pathways of pollen grains.

RESULTS

Elasticity of pollen grains

A significant amount of monadous pollen, shed as a single pollen grain, is approximately spherical in its hydrated form [11, 32] [Fig. 1]. For the purposes of the mechanical modelling of pollen grains, we effectively treat the entire interior of the grain as a liquid which acts only to constrain the total volume enclosed by the pollen wall. Such a model could also be formulated in terms of internal pressure imparted on the elastic spherical shell by the water inside it [22]. Using a discrete elastic model (see Materials and Methods), we assign different microscopic elastic parameters (stretching parameter $\epsilon$ and bending parameter $\rho$) to the exine region of the pollen wall and to the apertures where the exine is either absent or thinned. Parameter $f < 1$ is the ratio of the elastic parameters in the different regions and it sets the elastic difference between the pollen wall and the apertures—the smaller the value of $f$, the softer the apertural region compared to the exine wall. The overall elasticity of the pollen grain is on the other hand determined by the dimensionless Föppl-von Kármán (FvK) number $\tilde{\gamma}$, proportional both to the ratio of the stretching and bending parameters of the pollen wall and to the square of its radius $R_0^2$ (as described in Materials and Methods). It is important to stress that the FvK number $\tilde{\gamma}$ is a quantity which applies to the entire grain surface, both in the exine region and in the apertures, since the softness parameter $f$ cancels out in the ratio of the elastic parameters in the aperture.
regions. While $\tilde{\gamma}$ can be thus understood as a measure of the overall, global elasticity of the grain, $f$ indicates the extent of elastic inhomogeneity due to the presence of the apertures.

**Phase diagrams of tricolpate pollen folding**

Desiccation and the corresponding volume change lead to infolding of pollen grains. We first study folding pathways of tricolpate pollen, both because triaperturate pollen is the most common in angiosperms and because colpi usually bring about a more regular aperture closing than some other aperture shapes, such as pores [22, 33]. We model the colpi as spherical lunes extending from pole to pole, whose size is given by their azimuthal opening angle $\phi_c$. In an $n$-colpate pollen, such apertures span a total angle of $\phi_{ap} = n\phi_c$ and represent $A_{ap}/A_0 = n\phi_c/2\pi$ of the total grain area $A_0$. While it is difficult to precisely determine the total area that apertures cover in pollen grains, it can still be fairly reliably estimated from the microscopic images of pollen, where available [11, 34]. In tricolpate pollen, $A_{ap}/A_0$ typically ranges from 0.1 to 0.4, depending on the species [11], and the value which we use as representative in this section is $A_{ap}/A_0 = 1/3$.

In the next section, we address both the effect of varying the number and size of colpi on the folding pathways of pollen, and since colpi and circular pores can have different harmomegathic patterns [22, 33], we also investigate the role of the aperture shape.

Figure 2 shows the prototypical, regular infolding process of a tricolpate pollen grain with $\tilde{\gamma} = 7000$ and $f = 0.01$ as its volume decreases from the initial volume $V_0$ to about $V = 0.65V_0$ (the volume difference is denoted by $\Delta V = V_0 - V$). The overall grain shape and its changes can be characterized by the dimensions of the minimal bounding box which contain it, $(u_x, u_y, u_z)$: for a fully hydrated spherical shape, $u_x = u_y = u_z = 2R_0$. Colpi are arranged equatorially, and the poles of the pollen grain, i.e., the points where the colpi meet, are positioned on the $z$-axis. As the infolding proceeds, the equatorial dimensions of the bounding box containing the pollen grain shrink (Fig. 2a) and the distance between the poles increases (Fig. 2b). The shape of the grain thus becomes prolate, which can also be quantified by the ratio of the sizes of the bounding box in the $z$-direction and in the equatorial plane (Fig. 2c). The regular infolding of the grain and its elongation along the $z$-axis require an increase in the elastic energy of the shape (Fig. 2d). The apertures close completely once a large enough reduction in volume is reached (in this case, $\Delta V/V_0 \sim 0.35$). Past this point, the edges of the apertures touch, the shell enters a self-collision regime and further reduction of the volume requires additional deformation of the exine. At the point of the aperture closing, the ratio of the pollen long and short axes exceeds 1.6 in this case. A prolate shape is perhaps the most commonly observed feature in dry pollen grains—this is a direct consequence of a regular infolding in colpate grains, which can otherwise be perfectly spherical in their fully hydrated state, as is also the case in our calculations.

While the apertures of a tricolpate pollen grain with $\tilde{\gamma} = 7000$ and $f = 0.01$ close completely once the volume of the grain is reduced by one third, such a reduction in the initial volume is in general not necessarily enough for the apertures to close. Whether this happens at a certain $A_{ap}/A_0$ depends both on the FvK number $\tilde{\gamma}$ and on the ratio of the elastic constants of the apertures and the exine $f$. Phase diagram of infolded shapes of tricolpate pollen in the $\tilde{\gamma}$-$f$ plane, shown in Fig. 3, makes it clear that apertures close completely and in a regular fashion described in Fig. 2 only in a restricted region of the phase diagram. Complete, regular or nearly regular closure of the three colpi requires pollen grain with $\tilde{\gamma} \gtrsim 2000$ and
The relative change of volume upon desiccation is $\Delta V/V_0 = 0.35$ throughout, and the colpi (shown in red in the cross-sections) span one third of the total area of the pollen surface, $A_{ap}/A = 1/3$. Numbers next to the pollen shapes denote the elongation of the bounding box of the shapes, $2u_z/(u_x + u_y)$, indicating the prolateness of the grain. Insets show 3D shapes of desiccated pollen in different parts of the phase diagram, where the arrows indicate the viewpoint with respect to their equatorial cross-section; the colours have the same meaning as in Fig. 2 and the dashed white lines indicate the borders of the apertures. The shaded region of the phase diagram corresponds to the elastic parameters where the apertures close completely and nearly symmetrically, without asymmetric deformation of the exine.

Role of aperture shape, size, and number

Inspection of folding pathways of tricolpate pollen points to the importance of the elastic properties of the entire pollen grain and the elastic inhomogeneities provided by the apertures—both of these determine whether or not the apertures close upon desiccation and the manner in which they close. However, at a fixed FvK number of the grain $\tilde{\gamma}$ and the softness of the apertures $f$, the pollen folding pathways are further determined by the shape, size, and number of the apertures.

In the results presented thus far, the colpi extended from pole to pole: this is an idealized case (see Fig. 1). Even a small reduction in the polar span of the apertures can change the infolded shape of the desiccated pollen, which typically becomes markedly more lobate, and further reduction of the polar span partially invalidates the function of the apertures, which do not close completely anymore. In addition, large exine regions flatten upon infolding. Eventually, the apertures completely fail and the grain buckles in an irregular fashion, as large intraaperture regions become sunken so that the infolded shape resembles a cup. Such a shape is often found in dry inaperturate and porate pollen grains [11]. The progressive change of the aperture shape is addressed in Supplementary Information (SI) Methods, and these results are illustrated in Fig. S1 in SI. We can also change the area covered by the apertures, which, on the other hand, does not seem to influence much whether the apertures close completely or not. However, changing the aperture area (while retaining its shape) influences the amount of the volume reduction which can be achieved by the full closing of the apertures—the smaller the aperture area, the smaller the volume reduction upon closing (demonstrated in Fig. S2 in SI).

The area covered by the apertures can also be arranged in different ways: it can be united in a single wide colpus, or distributed among several ($n$) colpi, each one covering an area of $A_c = A_{ap}/n$. The number of colpi (and apertures in general) can vary significantly among and even within pollen species. It is thus of importance to investigate how this influences the folding pathways of pollen grains. The results are summarized in Fig. 4, which shows phase diagrams of infolded pollen shapes in the $\tilde{\gamma}$-$f$ plane for grains with $n = 1, 2, 3,$ and 4 apertures. Analysis presented earlier in Fig. 3 suggests that infolded shapes and pathways can be divided in roughly three categories: grains with apertures that infold symmetrically but do not close (light-blue regions in the diagrams in Fig. 4), grains with pronounced deformation of the exine and in which apertures do not close symmetrically and simultaneously (red regions), and grains in which all apertures completely close in a symmetrical
or nearly symmetrical fashion (dark-grey regions). This categorization of infolded shapes serves well not only for \( n = 3 \), but also for \( n = 1, 2, \) and \( 4 \) (cross-sections of the infolded shapes for \( n = 2 \) and \( n = 4 \) are shown in Figs. S3 and S4 in SI). Figure 4 makes it apparent that regular folding pathways need to be more precisely elastically regulated in grains with larger numbers of apertures, seen as the decrease in the size of the dark-grey regions in the phase diagrams as \( n \) increases. Only sufficiently soft apertures (small \( f \)) guarantee a full and symmetrical infolding of the pollen grain, and this condition becomes more stringent with larger \( n \). Interestingly, in the case of \( n = 1 \), the smallest values of \( f \) require grains with large enough FvK numbers (\( \tilde{\gamma} \gtrsim 7000 \)) in order to produce apertures which close completely.

**Differences in folding pathways**

To examine in more detail the differences in the pollen folding pathways, Fig. 5 shows three cases of tricolpate pollen grains in the three different regions of the phase diagram in Fig. 4: \( \tilde{\gamma} = 10000, f = 0.01 \) (symmetrical infolding where the apertures close; dark-grey region in Fig. 4), \( \tilde{\gamma} = 7000, f = 0.1 \) (the apertures do not close symmetrically and simultaneously; red region), and \( \tilde{\gamma} = 1000, f = 0.01 \) (symmetrical infolding but the apertures do not close; light-blue region). The changes in the bounding box dimensions of the grains in the equatorial plane and along the \( z \)-direction, brought about by the decrease in volume, are shown in Figs. 5a and 5b, respectively. The total length of the apertures, scaled by their corresponding length in the fully hydrated pollen, is shown in Fig. 5c. For the two cases with soft apertures \( (f = 0.01) \), their folding pathways are initially similar as the volume starts to decrease—the apertures gradually change their curvatures, which become inverted at about \( \Delta V/V_0 \approx 0.18 \). At this point, the folding pathways start to markedly differ and the grain with \( \tilde{\gamma} = 10000 \) almost stops shrinking in the equatorial plane as its volume is reduced further. It also stops elongating along the \( z \)-axis, after a short acceleration of elongation when \( 0.2 < \Delta V/V_0 < 0.23 \). This corresponds to a development of two “pinches” at the poles of the grain, as seen in the lower left 3D shape in Fig. 3. In the case of the grain with \( \tilde{\gamma} = 10000 \), no transitions in the change of the bounding box dimensions are observed as it continuously shrinks in the equatorial plane and elongates along the \( z \)-axis, and the apertures infold and fully close at around \( \Delta V/V_0 = 0.35 \). Even though the bounding box dimensions of the grain with \( \tilde{\gamma} = 1000 \) almost stagnate when \( \Delta V/V_0 > 0.18 \), the grain continues to reduce its volume. Figure 5c shows that the apertures of this shape start to inflate towards its interior. This process reduces the volume but requires the apertures to stretch, elongating in
the equatorial plane by 30% when \( \Delta V/V_0 \) increases from \( \approx 0.18 \) to \( \approx 0.35 \). No stretching of the apertures is observed in the case of the grain with \( \gamma = 10000 \), and this shape reduces its volume by exine bending and a complete closing of the apertures which, once fully closed, take up a much smaller interior volume when compared to the case with \( \gamma = 1000 \) (\( \Delta V/V_0 = 0.35 \)).

The irregular folding pathway pertaining to stiffer apertures (red, dashed lines in Fig. 5) is very different from the other two, as can also be observed by inspecting the cross-sections of the infolded shapes and comparing them with those in Fig. 2. The geometry of the pollen shape changes non-monotonously during infolding, and the two biggest kinks observable in the shape descriptors in Figs. 5a and 5b are indicated by arrows. At these transitional points, the symmetry of the pollen shape is reduced. The first clear transition at \( \Delta V/V_0 \approx 0.165 \) corresponds to the loss of \( C_3 \) rotational symmetry around the \( z \)-axis. The shape still retains its mirror symmetry, as can be see from its equatorial cross-section—two of the apertures continue to infold symmetrically, while the third one extends maximally. A second transition at \( \Delta V/V_0 \approx 0.215 \) corresponds to the loss of the remaining mirror symmetry, as each of the three apertures continues to infold in a different fashion afterwards.

The different folding pathways in Fig. 5 can be explained by considering the energetics of the infolding. A complete infolding of the grain apertures requires a significant bending of the exine regions as well, which adopt a smaller effective radius in the equatorial plane upon infolding. Bending deformation of the exine is also required for the elongation of the grain. The exine regions must thus deviate from their spontaneous curvature, characteristic of the fully hydrated state of the grain. While the contribution of stretching in the exine deformation is small, for sufficiently small \( \gamma \) (i.e., for grains where the ratio \( Y/\kappa \) is small) exine bending becomes prohibitively expensive energy-wise. Consequently, instead of a complete infolding of the apertures, the pollen grain reduces its volume by stretching (inflating) them towards its interior. This decreases the bending energy of the exine and lowers the total elastic energy of the grain for small \( \gamma \) and sufficiently large \( \Delta V/V_0 \). The process, however, requires very soft apertures (small values of \( f \)) and does not take place when the apertures are stiff enough—in that case, we typically observe an irregular infolding (red regions in Fig. 4), as the apertures are not soft enough to localize the deformation of the grain wall.

**DISCUSSION**

Having in mind the various mechanical influences to which pollen grains are exposed on their journey from the anther to the stigma, a robust mechanical design of the grain infolding patterns that can resist various forces and preserve the regularity of the folding pathway should provide an evolutionary advantage. According to our simulations, both the overall elastic properties of the pollen grain (as given by its FvK number \( \gamma \)) as well as the softer regions provided by the apertures (and described by the softness parameter \( f \)) play a key role in determining the folding pathway of pollen grains upon desiccation. For instance, soft apertures are required to close the apertures symmetrically—but too soft apertures will not pull in and bend the exine, and will instead tend to stretch themselves in order to reduce the volume. This will not close the grain and could result in a rupture in materials which cannot sustain large stretching deformations (\( \geq 30\% \)). On the other hand, if the apertures are too hard, they will fail to act as sites of localized deformation but will instead induce a deformation of the entire grain. If the apertures are to guide the pollen folding reliably, a delicate elastic balance is thus required which depends not only on the softness of the apertures \( f \), but also on the elasticity of the entire grain, i.e., its FvK number. Relatively small differences in the elastic properties of pollen grains are thus one possible explanation for the observed differences in folding pathways of pollen grains which otherwise appear practically the same [11, 23].

Folding pathways of pollen grains can be furthermore drastically influenced by the shape, size, and number of apertures. It is perhaps not surprising that circular pores do a much worse job at guiding pollen folding along a regular pathway compared to elongated colpi, as this has already been observed before [22, 33], while the area covered by the apertures appears to mainly influence the achievable reduction in the volume of the grain before the apertures close. The largest influence, however, on the available folding pathways of pollen grains seems to be provided by the number of apertures: the region of elastic parameters where desiccation leads to a regular, complete closing of the apertures shrinks with increasing number of apertures (Fig. 4). While there is a benefit to many apertures arising from simple geometrical considerations—the probability of a grain falling on a stigma with one of its apertures touching it increases for grains with more apertures, approaching 100\% for sufficiently large \( n \) [13], and experiments have observed a positive correlation between germination speed, number of apertures, and pollen water content at dispersal [5, 9]—the majority of angiosperm pollen has either one or three apertures, indicating that there must be factors selecting against too large a number of apertures as well. Our work shows that a possible reason favouring smaller numbers of apertures could be the requirement for a robust mechanical design of pollen grains, where the apertures close completely and in a regular fashion and thus slow the rate of water loss.

The results presented in this work emphasize the importance of a more detailed theoretical and experimental exploration of the mechanical and elastic properties of pollen grains in order to obtain a better understanding of their folding pathways. The different types of pathways we have observed should be valid for any size class of pollen, as the general range of FvK numbers we consid-
ered depends on the ratio of the pollen size and the exine thickness, and larger pollen grains tend to have thicker exines [35], thus constraining the range of FvK numbers. Given the ample variation of pollen types, however, it would not be surprising to find pollen grains which fall outside of this range, and our thin shell model should be applied with caution to the cases where the thickness of the exine becomes comparable to the size of the pollen grain. The model we presented treats the pollen wall as an infinitely thin shell and should thus also be applied with care to pollen grains with pronounced sculpting of the exine or the presence of ornamentation in the apertural region which can, for instance, lead to steric effects influencing the closure of the apertures [22].

In addition to providing a mechanical explanation for some evolved features of pollen grain structure, our work should prove useful in the design of inhomogeneous elastic shells which respond reversibly to changes in the osmotic pressure. Colloidal capsules, for example, can sustain an external osmotic pressure up to a critical point after which they buckle. This process can be strongly influenced by structural inhomogeneities in the capsule shells [30], which is consistent with our results, since apertures can be also thought of as designed inhomogeneities—these can thus dramatically change the buckling pathway of the capsule and guide it in a desired direction. Pollen grains themselves can be used as templates for containers for microencapsulation of compounds and as a drug delivery vehicles [36, 37], and it is of interest to devise strategies to manipulate pollen beyond its natural performance limits [38].

**MATERIALS AND METHODS**

**Elastic model of pollen folding and desiccation**

We assume that the fully hydrated, spherical shape of the pollen grain has no residual elastic stresses in the wall. The energy of the system $E$ is constructed by formulating the discrete bending and stretching energy contributions on a spherical mesh of triangles approximating the fully hydrated grain (more details regarding the mesh construction are discussed in SI Methods). The stretching contribution is implemented along the edges $i$ of the mesh, penalizing edge extensions and shortenings $l_i$ from their value in the fully hydrated state $l_i^0$. The bending contribution is implemented between pairs of triangles $I, J$ sharing an edge, so that the local bending energy is parametrized in terms of the deviations of the angles between the triangle normals $\theta_{I, J}$ from their value in the fully hydrated state $\theta_{I, J}^0$:

$$E = \sum_i \frac{\epsilon_i}{2} (l_i - l_i^0)^2 + \sum_{I > J} \rho_{I, J} \left[1 - \cos(\theta_{I, J} - \theta_{I, J}^0)\right].$$

(1)

The stretching and bending elastic parameters ($\epsilon_i$ and $\rho_{I, J}$, respectively) mirror the elastic inhomogeneities in the grain structure, and their values are set depending on whether the edges $i$ and faces $I, J$ are located in the aperture regions ($\epsilon_{ap}, \rho_{ap}$) or in the surrounding exine regions ($\epsilon_{ex}, \rho_{ex}$). (Cases when edge endpoints or neighboring faces are in different domains are explained in SI Methods.) There are obviously many different ways to set up the inhomogeneity of elastic properties: in order to make the problem tractable, we introduce a scaling, softness parameter $f < 1$ [22] such that $\epsilon_{ap} = f \epsilon_{ex}$ and $\rho_{ap} = f \rho_{ex}$. A smaller value of $f$ simulates a larger elastic difference between the aperture and the exine, while $f = 1$ represents the limiting point where the aperture becomes the same as its surrounding in terms of its elasticity.

Pollen desiccation is modelled by gradually decreasing the volume of the grain, treating the volume enclosed by the elastic shell $V$ as a mechanical constraint [39]. The relative change of the grain volume is given by $\Delta V/V_0$, where $V_0$ is the volume of the fully hydration pollen and $\Delta V = V_0 - V$. Dehydration in pollen varies to a great extent [32, 33, 40, 41], and the water content of pollen correlates with the volume of the grain. In *Cucurbita pepo*, for example, pollen volume has been observed to be $\sim 20\%$ higher than the hydration status of the grain [41] (meaning that the volume of the grain with, e.g., $50\%$ water content is about $70\%$ of the volume it has at $100\%$ water content: $V = 0.7 V_0$ and $\Delta V/V_0 = 0.3$ in our notation).

**Continuum thin-shell elasticity**

In the continuum theory of thin shells, their elastic behaviour is governed by a single dimensionless parameter, the FvK number $\gamma = (Y/\kappa)R_0^2$ [42], which depends on the ratio of two elastic parameters: 2D Young’s modulus $Y$ and bending rigidity $\kappa$; $R_0$ is the radius of the shell. Continuum elastic moduli $Y$ and $\kappa$ are proportional to the microscopic stretching and bending parameters $\epsilon$ and $\rho$ [42], and the constants of proportionality are on the order of one. The precise relationship between the discrete and continuum elastic constants depends, however, both on the nature of the triangulation and the geometry of the shape being triangulated, as demonstrated in Refs. [43, 44]. For our purposes, we define the quantity

$$\tilde{\gamma} = \frac{\epsilon}{\rho} R_0^2,$$

(2)

which we call the FvK number, having in mind that it may differ from the continuum value of the FvK number $\gamma$ by a multiplicative constant on the order of one. The exact value of this constant is irrelevant for our purposes, since the important effects take place in the interval spanning orders of magnitude in $\gamma$ and the elastic constants (and thus the FvK numbers) of pollen grains are largely unknown.
Elastic properties of pollen grains

While recent experiments have estimated Young’s modulus of different pollen grains (obtaining atypically high values in the process, $Y = 10–16$ GPa for pollen with $15–40$ $\mu$m in diameter [45]), elastic constants of pollen remain otherwise poorly known. To estimate the range of $\gamma$ relevant for pollen grains, we thus combine the expressions connecting 2D Young’s modulus and bending rigidity with 3D Young’s modulus and Poisson’s ratio to write the FvK number as [46, 47]:

$$\gamma = 12 \left(1 - \nu^2\right) \left(\frac{R_0}{d}\right)^2,$$

(3)

where $d$ is shell thickness and $\nu$ is 3D Poisson’s ratio. The latter can be estimated to be $\nu \approx 0.35$, a value appropriate for rigid polymers and based on the heavily cross-linked structure of sporopollenin [45]. While $\nu$ can vary from 0.2 (very rigid) to 0.5 (rubber-like), the exact number is not particularly important for our estimate of $\gamma$, since the ratio $R_0/d$ plays a more dominant role.

Even though pollen comes in sizes from less than 10 $\mu$m to more than 100 $\mu$m in diameter—depending also on the degree of hydration and the preparation method—the majority of monad, spheroidal pollen is in the range of 10–100 $\mu$m in diameter [11]. In general, pollen thickness increases with pollen size; several studies have estimated the thickness of the exine wall in different types and species of pollen to be in the range $d \in [0.5, 2.5]$ $\mu$m [21, 35, 48]. Our analysis of the available data in the online palynological database PaleDAt [11] indicates that for a large sample of spheroidal pollen with radii $R_0 \in [5, 50]$ $\mu$m an exine thickness of $d = 1–2$ $\mu$m is a very good approximation. This excludes any pronounced variations in the exine ornamentation and sculpturing in the form of, e.g., spikes or other protrusions on the scale of $R_0$. We can thus estimate that $R_0/d \approx 5–30$ (similarly to what has been reported in the literature [35, 48]), and that consequently the FvK numbers of pollen can span a large range, $\gamma \approx 10^2–10^4$. Figure 1 shows an example of pollen grain from Betonica officinalis, with an estimated $R_0/d \sim 15–20$ and $\gamma \sim 2–4 \times 10^4$.

Exine ornamentation on a scale much smaller than $R_0$ is not important for the process of harmomegathy, which takes place on the spatial scale comparable to $R_0$. Such effects can be thus included in some average sense in the elastic parameters of the exine. While the exine is very strong (either due to its thickness or its physical properties) [20], recent AFM studies estimate that aperture regions are at least an order of magnitude softer, and the smallest measured values of aperture Young’s modulus are two orders of magnitude smaller than the measured Young’s modulus of exine [17, 49]. We can thus estimate that the values of the softness parameter $f$ are in the range of $f = 0.1–0.01$, which is what we use in our analysis.

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Mechanical design of apertures and the infolding of pollen grain:
Supplementary Information

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SUPPLEMENTARY METHODS

Details of the discrete elastic model

The spherical mesh representing a fully hydrated pollen grain is constructed using the marching method for triangulation of surfaces, described in Ref. [1]. The method is modified so that the starting polygon is a pentagon, instead of a hexagon as in the original implementation—this yields more uniform triangulations with no visible patches consisting of predominantly hexagonally-coordinated vertices. Results presented in the manuscript were obtained using a mesh consisting of $V = 8597$ vertices, $E = 25785$ edges, and $F = 17190$ faces; note that $F + V - E = 2$, as guaranteed by the Euler formula for polyhedra. The mesh contains $1146$, $6314$, and $1134$ vertices with $5$, $6$, and $7$ nearest neighbours (joined by edges), respectively. The maximum and minimum edge lengths of the triangulation are $1.73\,a$ and $0.58\,a$, respectively, where $a$ is the average edge length. The radius of the spherical mesh is $R_0 = 24.34\,a$. Calculations were also performed with smaller and larger meshes, and the chosen mesh was found to be sufficiently large so that the results obtained do not depend on the nature and details of the triangulation.

Mesh vertices are assigned either to the apertures or to the exine region, depending on whether their coordinates fall within the mathematically defined borders of the apertures or not (see below). The same assignment is also made for the faces (triangles) of the triangulation based on the coordinates of their centroids. The elastic energy model is formulated for the edges of the mesh. A pair of microscopic stretching and bending constants ($\epsilon_i$, $\rho_i$) is assigned to each edge $i$, depending on the position of its endpoints and of the faces $I$ and $J$ it joins. If both endpoints are either in the aperture or in the exine region, the edge is assigned the stretching constant pertaining to the aperture, $\epsilon_{ap}$, or the exine, $\epsilon_{ex}$, respectively. If one of the endpoints belongs to the aperture and the other to the exine region, the edge is assigned a stretching rigidity of $(\epsilon_{ap} + \epsilon_{ex})/2$. If both faces joined by the edge belong either to the aperture or to the exine region, the edge is assigned the bending rigidity of $\rho_{ap}$ or $\rho_{ex}$, respectively. Similarly, if one of the triangle faces belongs to the aperture and the other to the exine region, the edge is assigned the bending rigidity of $(\rho_{ap} + \rho_{ex})/2$.

Minimization procedure

Vertex coordinates of the shapes minimizing the energy functional are calculated by a conjugate gradient minimization procedure described in Ref. [2]. The volume constraint is implemented as an energy penalty of the form $\lambda (V_{actual} - V_{target})^2$, where $V_{actual}$ and $V_{target}$ are the actual and target volumes of the system. The constraint parameter $\lambda$ is sequentially increased until the volume found is within the predefined tolerance (less than $10^{-4}$ in our calculations) [3]. Once the minimum of the energy is found, it is checked that the total contribution of the energy penalty is vanishingly small when compared with the elastic energy of the system.

Folding pathways are calculated by sequentially reducing the target volume and using the shape obtained in the previous step of the procedure as the initial guess for the conjugate gradient minimization. The procedure is started with the fully hydrated, spherical shape and its corresponding volume $V_0$. Once the minimal target volume is reached, the procedure is repeated backwards, from the maximally infolded shape towards the fully hydrated shape, by sequentially increasing the target volume. This procedure enables detection of minimal energy states which were missed in the forward minimization but can still be detected in the backward minimization, and vice versa. It also enables us to detect any hysteresis in the folding pathway, which we indeed observe in some cases, especially when the area covered by the apertures is large.

Model of the aperture shape

Apertures are arranged equatorially, and the width of each aperture along the equator is $\phi_c$. The total equatorial angular span of all $n$ apertures is $\phi_{ap} = n\phi_c$. Centres of neighbouring apertures are separated by an azimuthal angle of $\alpha = 2\pi/n$, where $n$ is the number of apertures in the pollen grain. The azimuthal angle $\varphi \in [0, 2\pi]$ is rescaled so that $\hat{\varphi} = \varphi - \text{int}(\varphi/\alpha)\alpha$, where $\text{int}(x)$ denotes the integer part of $x$. All points for which the azimuthal angle $\hat{\varphi}$ and the polar angle $\vartheta \in [0, 2\pi]$ fulfill the conditions

\[
\frac{\pi}{2} - \theta_{\Phi}(\hat{\varphi}, \phi_c) < \vartheta < \frac{\pi}{2} + \theta_{\Phi}(\hat{\varphi}, \phi_c)
\]

(1)
belong to the aperture. Note again that the apertures are arranged along the equator of the grain, where they assume
the largest azimuthal width. We have also defined \( \theta_{\Psi}(\hat{\varphi}, \phi_c) = \Psi \sqrt{\left(\frac{\phi_c}{2}\right)^2 - \left(\varphi - \frac{\phi_c}{2}\right)^2} \), where the parameter \( \Psi \) defines
the shape of the aperture. When \( \Psi = 1 \), the aperture is approximately circular (porus). As \( \Psi \to \infty \), the aperture
assumes a shape of a spherical lune (idealized colpus). The maximal polar span of the aperture \( \theta = \pi \) (pole-to-pole) is
obtained when \( \varphi = \phi_c/2 \) and \( \theta = \max[\Psi \phi_c, \pi] \). Calculations presented in the main text were performed with \( \Psi \to \infty \).
Figure S1 shows equatorial cross-sections (middle row) and 3D shapes (bottom row) of infolded triaperturate grains
for different shapes of the apertures (from an idealized colpus to porus; top row).

**MINIMUM VOLUME AND PROLaterness OF MAXIMALLY INFOLDED SHAPES**

Bounding box dimensions

Dimensions of the bounding box of a pollen grain are calculated with respect to fixed coordinate axes. This means
that the bounding box should not be considered as the minimal bounding box in some predefined sense (e.g., minimal
volume or minimal equatorial area box). While different definitions of the bounding box can produce somewhat
different ratios of the box dimensions, any difference would be small in our case, as the cross-section of the grain in
the equatorial plane is nearly isometric, i.e., it typically does not show a prominent elongation along any direction in
the equatorial plane.

Analytical approximations for the bounding box dimensions and the volume of the maximally infolded shape

The final, minimum volume of a fully infolded shape, \( V_{in} \), is a quantity which depends on the total area of the
apertures \( A_{ap} \), their number \( n \), and, in general, also on the elasticity of the grain. A rough estimate of \( V_{in} \) can be
obtained by assuming that the infolded shape does not stretch significantly and approximating it by an ellipsoid. The
cross-section in the equatorial plane can be approximated by a circle whose perimeter can be calculated by formally
excising the apertures. The longest axis of the ellipsoid can be obtained from the requirement that the exine does not
stretch significantly and approximating it by an ellipsoid. The

Analytical approximations for the bounding box dimensions and the volume of the maximally infolded shape

The final, minimum volume of a fully infolded shape, \( V_{in} \), is a quantity which depends on the total area of the
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obtained by assuming that the infolded shape does not stretch significantly and approximating it by an ellipsoid. The
cross-section in the equatorial plane can be approximated by a circle whose perimeter can be calculated by formally
excising the apertures. The longest axis of the ellipsoid can be obtained from the requirement that the exine does not
stretch when the equatorial radius of the grain shrinks. From these considerations, we can calculate the dimensions
of the bounding box of the grain at the point of complete infolding as

\[
\frac{u_x + u_y}{4R_0} = 1 - \tilde{\phi}_{ap} \\
\frac{u_x}{2R_0} = 1 + \tilde{\phi}_{ap},
\]

where \( \tilde{\phi}_{ap} = \phi_{ap}/2\pi = A_{ap}/A_0 \). For \( \tilde{\phi}_{ap} = 1/3 \), this gives \( (u_x + u_y)/(4R_0) = 2/3 \), and \( u_x/(2R_0) = 4/3 \), which
compares favourably with the values of 0.75 and 1.25 obtained in the numerical simulations (Fig. 2 in the main text).
The volume of the grain at the point of maximal infolding is given by

\[
\frac{\Delta V_{in}}{V_0} \approx \tilde{\phi}_{ap} + \tilde{\phi}_{ap}^2 - \tilde{\phi}_{ap}^3,
\]

where \( \Delta V_{in} = V_0 - V_{in} \). This rough estimate does not account for the volume subtracted from the grain by the
infolded apertures themselves. For \( \phi_{ap} = 0.25, 0.33, \) and \( 0.41 \), the equation predicts \( \Delta V_{in}/V_0 \approx 0.30, 0.41, \) and \( 0.51 \),
respectively, in reasonable agreement with the values obtained for \( n = 3 \) (0.27, 0.35, and 0.5; see Fig. S2 and Fig. 2 in
the main text). Equation 3 less accurately describes grains with \( n = 1 \) and \( n = 2 \), because the infolded apertures take up
a significant volume of the infolded grain. This effect is rather difficult to estimate analytically, and we have found
the following approximation to be useful:

\[
\frac{\Delta V_{in}}{V_0} \approx \tilde{\phi}_{ap} + \tilde{\phi}_{ap}^2 - \tilde{\phi}_{ap}^3 - \frac{\tilde{\phi}_{ap}^3}{2n} (1 + \tilde{\phi}_{ap})
\]

The last term, subtracted from the approximation in (3), accounts for the volume taken from the grain by the infolded
apertures. The correction is small when \( n > 2 \), but becomes important for \( n = 1 \) and \( 2 \). The smallest infolded volume
of a grain is obtained when the entire aperture area is lumped in a single, large aperture (\( n = 1 \)).
Figures S3 and S4 show phase diagrams, analogous to the one shown for tricolpate \((n = 3)\) pollen in Fig. 3 in the main text, for pollen grains with two and four colpi, respectively. The shapes of infolded pollen, shown in these diagrams, were analyzed in order to produce Fig. 4 in the main text. We also analysed the phase diagram for monocolpate \((n = 1)\) pollen; however, for the case of a single and sufficiently large aperture, the minimal infolded volume can no longer be thought of as a quantity independent of the elasticity of the problem. Consequently, representing the cross-sections of infolded pollen shapes in a diagram for a single volume of the infolded grain, as was done for \(n = 2, 3,\) and \(4\), can thus be somewhat misleading. In order to produce the data for \(n = 1\) required to produce Fig. 4, we must instead follow the folding pathway all the way to the point of minimum achievable volume or to the point where it becomes obvious that the apertures do not close at all. This analysis was performed for \(n = 1\) as well as for \(n = 2, 3,\) and \(4\), although the information in the latter three cases can be also reliably obtained from Figs. 3, S3, and S4.

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Figure S1. Shapes of desiccated triaperturate grains with \( \hat{\gamma} = 7000 \) and \( f = 0.01 \) at \( \Delta V/V_0 = 0.35 \) for different aperture sizes and shapes. The span of the aperture along the azimuthal angle is the same in all four cases, \( \phi_c = 0.22\pi \), while the span along the polar angle is gradually reduced from \( \theta = \pi \) (pole-to-pole colpus) to \( \theta = 0.22\pi \) (circular pore). This means that the total proportion of the aperture area \( A_{ap}/A_0 \) is gradually reduced as well—see the main text and SI Methods for more details.

Figure S2. Regular aperture closing during desiccation of tricolpate pollen with \( \hat{\gamma} = 7000 \), \( f = 0.01 \), and \( A_{ap}/A_0 = 0.25 \) (black line) and 0.41 (gray line). The elastic energy of the shapes is shown as a function of volume reduction \( \Delta V/V_0 \) (top panel). The characteristic shapes of the shells are shown in the middle and bottom rows of graphs for \( A_{ap}/A_0 = 0.25 \) and 0.41, respectively, for \( \Delta V/V_0 = 0.012, 0.13, 0.26 \) (middle row) and \( \Delta V/V_0 = 0.012, 0.13, 0.26, 0.35, 0.49 \) (bottom row). Shape colours indicate the surface curvature, with the brightest yellow and the darkest blue corresponding to largest positive and negative curvatures, respectively.
Figure S3. Phase diagram of bicolpate pollen folding in the γ-f plane, showing equatorial cross-sections of the pollen shapes. The relative change of volume upon desiccation is ∆V/V₀ = 0.42 throughout, and the colpi (shown in red in the cross-sections) span Aₚ/ₐ₀ = 1/3 of the total area of the pollen surface. Numbers next to the pollen shapes denote the elongation of the bounding box of the shapes, 2ux/(ux + uy), indicating the prolateness of the grain. The shaded region of the phase diagram corresponds to the elastic parameters where the apertures close completely and nearly symmetrically, without asymmetric deformation of the exine.

Figure S4. Phase diagram of tetracolpate pollen folding in the γ-f plane, showing equatorial cross-sections of the pollen shapes. The relative change of volume upon desiccation is ∆V/V₀ = 0.35 throughout, and the colpi (shown in red in the cross-sections) span Aₚ/ₐ₀ = 1/3 of the total area of the pollen surface. Numbers next to the pollen shapes denote the elongation of the bounding box of the shapes, 2ux/(ux + uy), indicating the prolateness of the grain. The shaded region of the phase diagram corresponds to the elastic parameters where the apertures close completely and nearly symmetrically, without asymmetric deformation of the exine.