

Spines, microspines and electric fields: a new look at the possible significance of sculpture in pollen of basal and derived Asteraceae

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Effective adherence of pollen grains on the surface of the stigma is a crucial condition for tube germination and subsequent fertilization of ovules. We provide evidence, through computer simulations, that echinate and microechinate sculpture of pollen in Asteraceae could play an important role in that process. We show that electrostatic interaction increases in grains with few ornamental elements (echinate pollen typical of derived lineages) when compared with grains that have a large number of ornamental elements (microechinate pollen typical of basal lineages). We also show that grains of smaller size are more significantly affected by the electrostatic force. Although experimental studies are necessary to ascertain the real effect of electrostatic forces on pollen capture by the stigma in Asteraceae, our contribution suggests that these forces might have great biophysical significance in the evolution of the family.

ADDITIONAL KEYWORDS: Compositae – computer simulations – echinate and microechinate sculpture – electric fields – pollen.

INTRODUCTION

Pollination is essential in the life of flowering plants; it includes a series of complex events that culminate with the fertilization of the ovules. The physical capture of pollen, which is followed by its effective adherence on the receptive surface of the stigma, is one of the initial steps involved in pollination (Heslop-Harrison & Shivanna, 1977; Wheeler *et al.*, 2001).

Electrostatic forces had been regarded as responsible for the capture of pollen grains dispersed from

bees to the stigma (Erickson & Buchmann, 1983). The electrostatic force on a charged pollen grain is proportional to the electric field at the pollen grain location and to its electrical charge. Bowker & Crenshaw (2007a, b) studied the role of this force in wind pollination. They estimated the forces and the resulting trajectories of pollen grains approaching the plants by modelling the plant as an earthed conducting sphere and the pollen grain as a point charge. In the case of insect pollination, the process was investigated in terms of the electrostatic forces between bees and flowers. It is known that bees are positively charged during the flight, whereas flowers have a negative

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surface charge; the electrostatic interaction between them has been shown to be significant in facilitating the detachment and the flight of the pollen from the anther to the pollinator and from it to the stigma (Corbet *et al.*, 1982; Gan-Mor *et al.*, 1995; Clarke *et al.*, 2017). Chaloner (1986) suggested that the sculpture or ornamentation of pollen could play a major role in this event. According to this author, a prominent exine sculpture (e.g. echinate) could maintain the electrostatic attraction between pollen and stigma for a long period, providing more possibilities for the hydration of pollen, the germination of pollen tubes and, ultimately, for the fertilization of ovules. Taking into account that prominent sculpture is common in entomophilous pollen, Chaloner (1986) suggested that this feature might have significance in biotic pollination.

The pollen of the large family Asteraceae is particularly attractive to explore the relationship between sculpture and electrostatic forces because spines and microspines (i.e. tapering pointed elements, > 1.5 or < 1.5 μm in length, respectively) are the typical sculptural elements of the pollen exine in this family (e.g. Wodehouse, 1935; Skvarla *et al.*, 1977; Punt & Hoen, 2009). Lophate pollen (psilo- or echinolophate) is also present in some tribes of Asteraceae, but in a small proportion compared with echinate or microechinate pollen (Blackmore *et al.*, 2009). Commonly known as the sunflower or daisy family, Asteraceae has an early evolutionary history evidenced by pollen fossils from the Late Cretaceous, 66–76 Myr old (Barreda *et al.*, 2015) (Fig. 1). With ~23 000 species, it is amply distributed in both hemispheres, with the exception of Antarctica (Funk *et al.*, 2005). Biotic pollination is generalized in the family, although some species are wind pollinated (e.g. Wodehouse, 1935; Lane, 1996; Müller & Kuhlmann, 2008).

Blackmore *et al.* (2009) found broad coincidence between pollen characters and phylogeny in Asteraceae. These authors hypothesized that at a large scale, the evolution of sculpture type showed a marked change from basal to derived lineages, suggesting that the microechinate condition is basal in the family, whereas the echinate condition is clearly derived. The psilate ornamentation that these authors mapped on a molecular phylogeny is consistent with the microechinate surface considered here. According to a recent study of sculpturing and pollen size in selected Asteraceae, it was shown that both features vary in an opposite manner (Tellería, 2017). Large and elliptic pollen grains of basal taxa (i.e. Carduoideae *p.p.*, Gochnatioideae, Wunderlichioideae, Stiffitioideae, Mutisioideae and Barnadesioideae) are commonly microechinate, with countless microspines unevenly distributed, whereas smaller and spheroidal pollen grains of derived taxa (i.e. Asteroideae and Cichorioideae) are commonly echinate, with spines arranged in a clear pattern (Fig. 1). In line with Chaloner (1986), it would be reasonable to

assume that in this family the sculpture, echinate or microechinate, has a pivotal significance in promoting the capture of pollen by the stigma.

Here, we explore whether the spines or the microspines contribute to the capture of the pollen grain by the stigma, by applying an electrostatic model to calculate the attractive force between the flower and the pollen grain. We also use a numerical simulation to compute the near electric field in the vicinity of the pollen grain. Finally, in the light of the results obtained here, we discuss the possible evolutionary significance of sculpture in Asteraceae within the phylogenetic scheme proposed by Blackmore *et al.* (2009).

MATERIAL AND METHODS

SELECTION OF POLLEN TYPES

With the aim of analysing the electrostatic force between the flower and the pollen grain, we have selected the length of the sculptural elements as a characteristic parameter to distinguish spines from microspines. *Mutisia decurrens* Cav. was selected as an example of a basal microechinate grain, whereas pollen of *Cosmos sulphureus* Cav. was selected to illustrate the case of an echinate grain (Fig. 2). Although other morphological features are common in spines/microspines of Asteracean pollen (Tellería, 2017), their inclusion far exceeds the scope of the present study.

ELECTROSTATIC INTERACTION BETWEEN THE FLOWER AND THE POLLEN GRAIN

Taking into account that the capitulum, the typical inflorescence of Asteraceae, constitutes a functional unit (Lane, 1996), we call the capitulum a ‘flower’ in the context of the electrostatic model. We consider the flower as an isolated, charged, perfectly conductive grounded sphere of radius A , with its centre located at a height h above the ground, immersed in a uniform vertical electric field of intensity E_0 , which corresponds to the natural field present in fair weather electric conditions (Bowker & Crenshaw, 2007a, b; Clarke *et al.*, 2017). Under these assumptions, the absolute value of the charge Q of the flower can be calculated as (Bowker & Crenshaw, 2007b):

$$Q = 4\pi\epsilon_0 AhE_0, \quad (1)$$

where ϵ_0 is the dielectric constant of vacuum.

The electric field vector at a distance r from the centre of a sphere of charge Q is given by:

$$E(r) = \frac{Q}{4\pi\epsilon_0 r^2} \hat{r}, \quad (2)$$

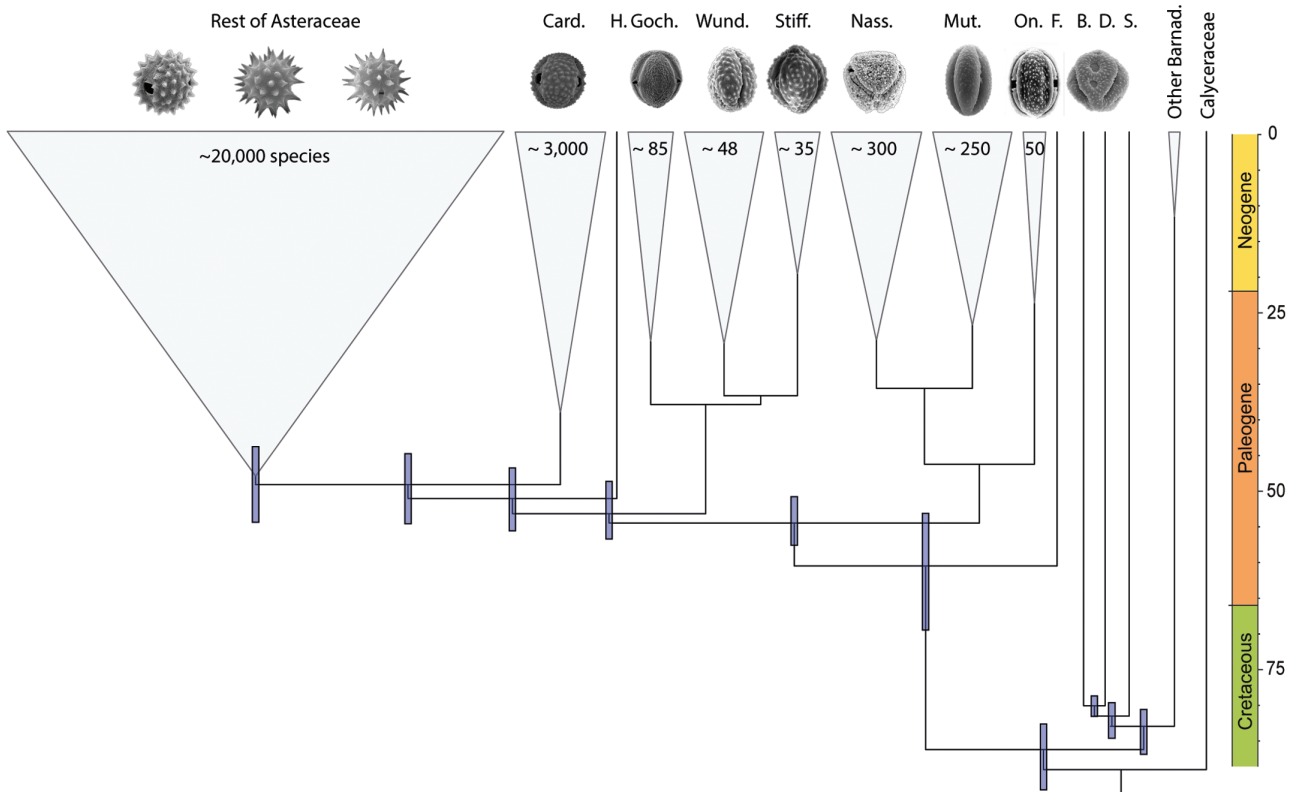


Figure 1. Chronogram (scale on the right in millions of years ago) modified from Barreda *et al.* (2015). Light blue bars at nodes represent 95% credibility intervals on estimates of divergence times. Note that most of the earliest diverging clades of Asteraceae produce microechinate pollen grains, whereas the most derived clades produce echinate pollen grains. Abbreviations: Barn., Barnadesioideae; Card., Carduoideae; D., *Dasyphyllum*; F., Famatinanthoideae (one species); Goc., Gochnatioideae; H., Hecastocleidoideae (one species); Mut., Mutisieae; Nass., Nassauvieae; On., Onoseridae; S., *Schlechtendalia*; Stiff., Stifftioideae; Wund., Wunderlichioideae. An approximated number of species was included in each clade.

where \hat{r} is a unit vector pointing outwards in a radial direction. Then, substitution of eqn. (1) in eqn. (2) yields the electric field produced by an earthed sphere immersed in the earth's field as a function of its radius A , the vertical distance between the flower and the ground h , and the ambient electric field intensity E_0 (Bowker & Crenshaw, 2007b).

In previous studies devoted to revealing the role of electrostatic forces in pollination, the pollen grain was modelled as a point charge. This assumption is valid for the investigation of many aspects of the electric interaction between the grain and the flower. However, if one is interested in investigating the effect of the ornamentalations of the pollen grain on the electrostatic attraction between the grain and the flower in their close vicinity, the assumption of the pollen grain as a point charge is no longer useful.

For an extended grain of volume V and arbitrary shape, the electrostatic force F_E exerted by the flower on the pollen grain is given by:

$$F_E = \iiint E(\mathbf{r})\rho(\mathbf{r})dV, \quad (3)$$

where $\rho(\mathbf{r})$ is the volumetric charge density of the grain and $E(\mathbf{r})$ is given by eqn. (2). For a point charge q , this expression reduces to $F_E = qE(\mathbf{r})$, which is the expression used in previous studies (Bowker & Crenshaw, 2007a, b).

On the other hand, when a charged body (pollen) approaches the earthed sphere (flower), it induces an opposite charge and is attracted towards the flower. This attractive force is called image force (F_I) and is negligible at large distances. However, it has been shown that at short distances its magnitude is much larger than that of F_E (Bowker & Crenshaw, 2007a, b). In addition, taking into account that the pollen grain is much smaller than the flower, at large distances it can always be regarded as a point charge, thus we do not expect any dependency of F_E on the particular shape and/or sculpture of the grain at large distances. Therefore, for the purpose of the present work, which

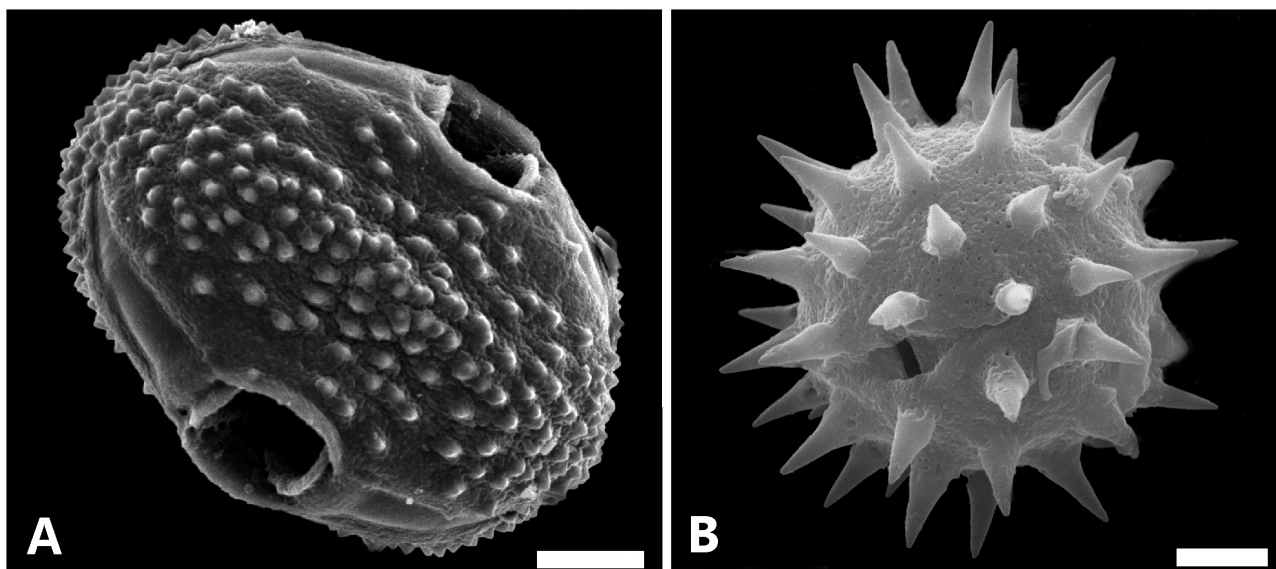


Figure 2. Pollen of *Mutisia decurrens* (A) and *Cosmos sulphureus* (B) as representatives of microechinate and echinate sculpture, respectively. Scale bars: 10 μm in A; 5 μm in B.

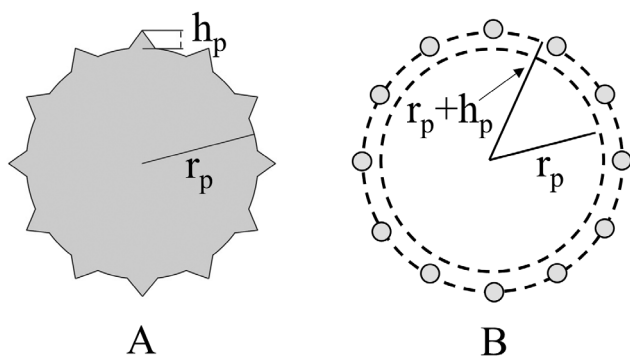


Figure 3. A, scheme of the charged pollen grain with tips. B, definition of the equivalent model of the pollen grain as a set of N point charges.

is focused on the electrostatic interaction between the flower and pollen at close proximity, we neglect \mathbf{F}_E and consider only \mathbf{F}_I , which provides us with an analytical tool to investigate the effects produced by the size and sculpture on the electrostatic interaction between the pollen and the flower.

In order to study the role of the microspines and the spines on the electrostatic attraction, we model the pollen grain as a sphere of radius r_p with an arbitrary number N of protruding tips (= spines or microspines) of length h_p (Fig. 3A).

Taking into account that in extended bodies the electric charge tends to accumulate mostly at the sharper places rather than at flat areas, we model a pollen grain with microspines as a set of N point charges q_i located on a spherical surface of radius $r_p + h_p$ (Fig. 3B).

We assume that the total charge of the grain (Q) is uniformly distributed among the tips, i.e. $q_i = Q/N$. By applying the method of images, the analytical expression of \mathbf{F}_I for a set of N point charges in front of an earthed, conducting sphere of radius A , can be written as (Jackson, 1999):

$$F_I = \sum_{j=1}^N \sum_{i=1}^N \frac{q_i q'_j (r_i - r'_j)}{4\pi\epsilon_0 |r_i - r'_j|^3}, \quad (4)$$

where \mathbf{r}_i is a vector pointing in a radial direction from the centre of the sphere to the point charge q_i (Fig. 4); q'_j and \mathbf{r}'_j denote, respectively, the value of the j -th image charge and its location, and are given by $q'_j = -q_j A/|r_j|$ and $\mathbf{r}'_j = A^2 \mathbf{r}_j / |r_j|^2$. Notice that in the case of a spherically symmetric distribution of charge, \mathbf{F}_I points in the x direction.

ELECTRIC FIELD IN THE VICINITY OF THE POLLEN GRAIN

In order to determine the electric near-field distribution generated by electrostatically charged pollen grains of different geometries, we applied a computer simulation that allows evaluation of the electric field for a two-dimensional distribution of charge. One of the features of this method is that the distribution of electrostatic charges is introduced into the simulation by means of digital images, which permits the treatment of pollen grains with arbitrary shapes. The details of the method are described by Dolinko (2008, 2009, 2018).

RESULTS

In Fig. 5 we plot the absolute value of the computed image force $|\mathbf{F}_1|$ in Newtons (N) as a function of the distance between the centers of the flower and the pollen grain (d , see Fig. 4). For a better visualization, we have considered $d - A$ as the x-axis, instead of d .

Taking as a reference the geometry of the *M. decurrens* and *C. sulphureus* pollen (Fig. 2), we considered two different cases: microechinate pollen grain with radius $r_p = 25 \mu\text{m}$, $N = 60$ and $h_p = 1.5 \mu\text{m}$ (Fig. 5A) and echinate pollen grain with radius $r_p = 10 \mu\text{m}$, $N = 12$ and $h_p = 4 \mu\text{m}$ (Fig. 5B). In both cases, the total charge Q of the grain is uniformly distributed among the N tips. In these examples, we consider $Q = 12 \cdot 10^{-14} \text{ C}$, which is a rather low value according to Bowker & Crenshaw (2007a). The rest of the parameters are $E_0 = 100 \text{ V/m}$, $A = 1 \text{ cm}$ and $h = 1 \text{ m}$. In each case, the results are compared with those of a smooth sphere of the same radius r_p , and with the same total charge. We also included a horizontal line representing the weight (gravitational force) of the pollen grain, calculated assuming that its density is equal to the density of water.

By comparing the magnitude of the attractive image force $|\mathbf{F}_1|$ for the smooth grain and for the grain with tips, no differences are observed for long distances between the flower and the pollen. However, in the close vicinity of the flower, when the distance between its surface and the grain is a few micrometres, $|\mathbf{F}_1|$ for the sculptured grain is higher than that for the smooth grain. This behaviour is observed for both the

microechinate and the echinate grain, illustrated in Figure 5A and B, respectively.

Notice that the lowest value of $d - A$ considered in Figure 5A and B is different. This is because the studied microechinate grain has $r_p = 25 \mu\text{m}$ and $h_p = 1.5 \mu\text{m}$, and therefore a value of $d - A = 26.5 \mu\text{m}$ represents the situation of maximal proximity, in which the flower and the pollen are in contact. Following the same criterion, for the echinate grain considered, the minimal distance is $d - A = 14 \mu\text{m}$. Given that the image force depends strongly on the distance between centres, the smaller grain has the possibility of getting closer to the flower and, consequently, of experiencing a greater electrostatic attraction, as observed in the inset of Figure 5B.

In addition, assuming that the mass density is independent of the type of grain, the weight of the small grain is smaller and therefore its electrostatic force will have a more decisive role in the movement of the pollen grain in the vicinity of the flower. This can be seen in Figure 5, where a horizontal line representing the weight of the grain has been included. Note that, in case A, $|\mathbf{F}_1|$ exceeds the weight for $d - A < 230 \mu\text{m}$, whereas in case B, this condition is met for $d - A < 870 \mu\text{m}$. It is to be expected that in the close proximity of the flower, the grain trajectory will be determined mainly by the electrostatic force.

As mentioned above, the comparison between the sculptured and the smooth grain, both in the echinate and in the microechinate cases, shows that the presence of tips produces an intensification of the electrostatic attraction. To investigate the influence of the number of tips on the value of the force, in

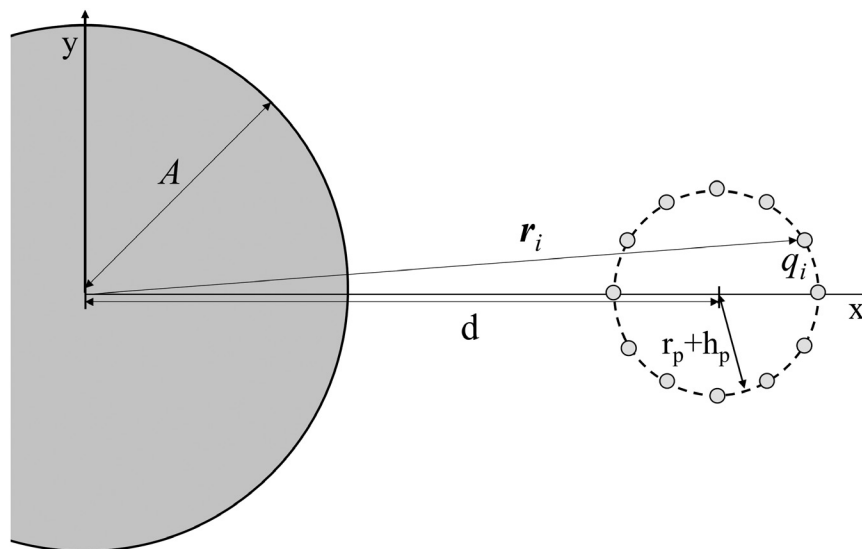


Figure 4. Scheme of the flower and the equivalent pollen grain, illustrating the geometrical parameters involved in the calculations (not to scale).

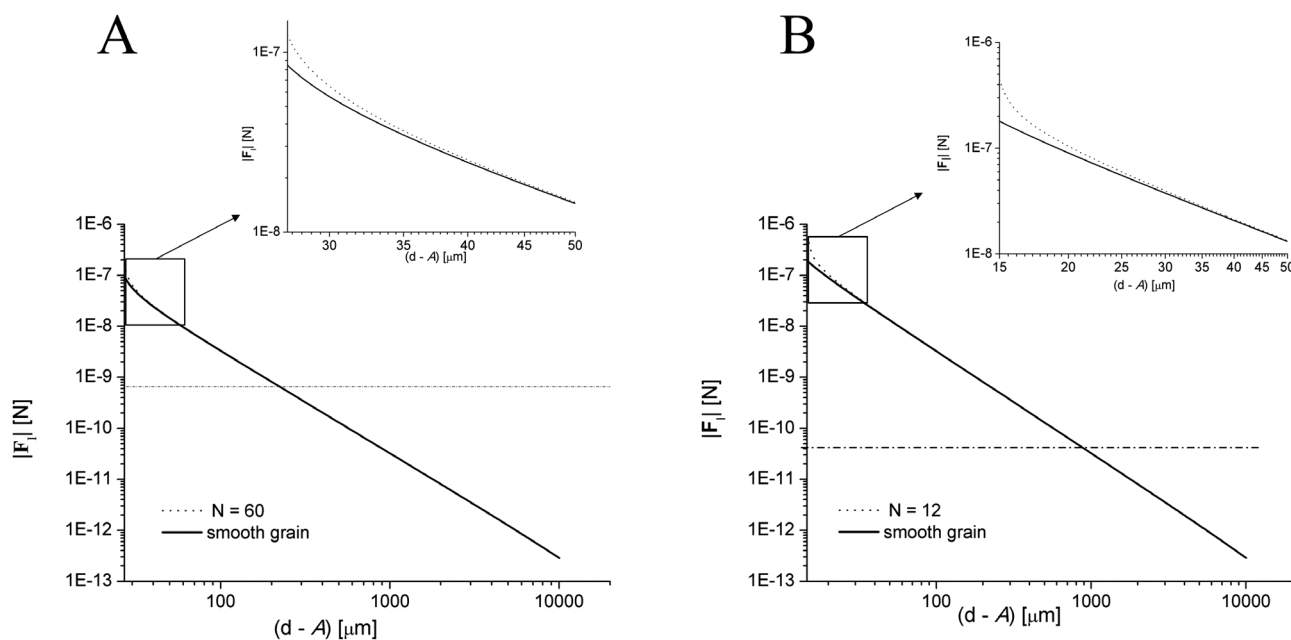


Figure 5. Absolute value of the image force $|F_1|$ as a function of $(d - A)$. A, microechinate pollen grain with $r_p = 25 \mu\text{m}$, $N = 60$ and $h_p = 1.5 \mu\text{m}$. B, echinate pollen with $r_p = 10 \mu\text{m}$, $N = 12$ and $h_p = 4 \mu\text{m}$. In both cases, $Q = 12.10^{-14} \text{C}$. The rest of the parameters are $E_0 = 100 \text{V/m}$, $A = 1 \text{cm}$ and $h = 1 \text{m}$. Results for a smooth spherical grain are also included for comparison. The inset in each panel shows a magnified zone of the figure, corresponding to the shortest pollen–flower distances. The horizontal line represents the weight of the pollen grain.

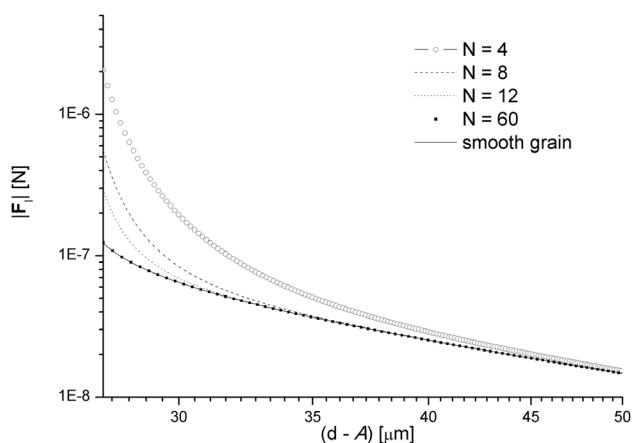


Figure 6. Absolute value of the image force $|F_1|$ as a function of $(d - A)$ for microechinate pollen grains with different N . Other parameters are $Q = 12.10^{-14} \text{C}$, $r_p = 25 \mu\text{m}$, $h_p = 1.5 \mu\text{m}$, $E_0 = 100 \text{V/m}$, $A = 1 \text{cm}$ and $h = 1 \text{m}$. Results for a smooth spherical grain are also included for comparison.

Figure 6 we show $|F_1|$ as a function of $d - A$ for a pollen grain with $r_p = 25 \mu\text{m}$, $h_p = 1.5 \mu\text{m}$ and for different values of N (4, 8, 12 and 60). We also include the curve corresponding to a smooth grain of the same radius. As observed, the number of tips

significantly affects the image force magnitude; it increases as the number of tips decreases. This result has also been verified for a different radius of the pollen grain, which suggests that this trend is independent of the pollen size. Under the assumption that the total charge of the grain is the same for all the cases considered, the amount of charge at the tips increases when the number of tips decreases. Therefore, the intensification of $|F_1|$ for low N is a consequence of the higher enhancement of the electric field in the vicinity of the tips, which is proportional to the charge of the tips.

To visualize this effect, we applied the computer simulation described before to obtain the electric field distribution generated by the pollen grain in its vicinity. The charged pollen produces an electric field and, as a result, an opposite charge is induced on the surface of the flower, and the grain is attracted to it. In Figure 7, we show contour plots of the electric field magnitude for three of the pollen types considered in Figure 5: a smooth sphere (Fig. 7A) and sculptured spheres with $N = 60$ (Fig. 7B) and $N = 12$ (Fig. 7C). The colours represent different intensity levels, and the colour scale is the same for the three plots. Notice the intensification of the electric field at the tips and that the level reached in the $N = 12$ case is higher than that of the $N = 60$ case.

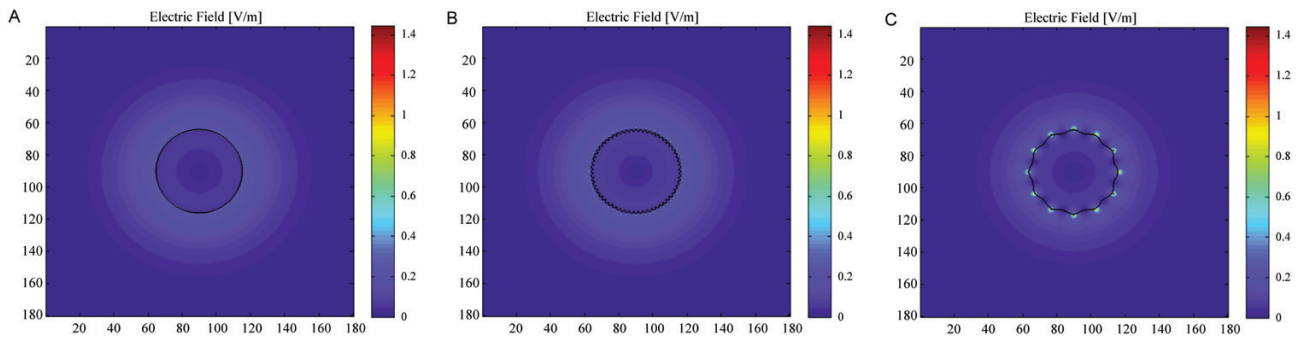


Figure 7. Contour plots of the electric field magnitude for three pollen grains considered in Figure 5. A, smooth sphere. B, sculptured sphere with $N = 60$. C, sculptured sphere with $N = 12$. The horizontal and vertical axis labels are given in micrometres.

DISCUSSION

Our study, through computer simulations, provides evidence about the significance of the electrostatic attraction between flowers and the echinate/microechinate pollen grains of Asteraceae. We demonstrated that there is an inverse relationship between the intensity of the attractive force of pollen grains (image force) and the number of sculptural elements (higher intensity/fewer sculptural elements). Therefore, those pollen grains with fewer individual sculptural elements (spines or microspines) have more chance to adhere to the stigma. These results strongly support Chaloner's hypothesis about the relevance of prominent echinate sculpture in the pollination process. Our model also showed that the size of pollen grains might play a significant role in the pollination mechanism. On the one hand, the smaller grain has an advantage over the large one, owing to the possibility of getting closer to the stigma and thus to promote pollen capture, hydration and germination. On the other hand, given that the movement of the grain is determined by the electrostatic force and by the weight, a lighter grain, i.e. a grain of smaller size, will be more significantly affected by the electrostatic force. In this family, an evolutionary trend from large to small pollen has been hypothesized to be a consequence of post-pollination processes (Torres, 2000). The present study provides evidence that the reduction of pollen size could be a favourable change for the sexual interaction within species of Asteraceae.

Our results are significant in the context of the evolution of pollen sculpture in Asteraceae suggested by Blackmore *et al.* (2009), given that these authors found broad coincidence between pollen characters and phylogeny. The evolution of sculpture type at a large scale showed a marked change from basal to derived lineages, i.e. from microechinate to echinate (Fig. 1). Interestingly, microechinate sculpture characterizes the Asteracean

pollen fossil found in Miocene sediments from Patagonia (Barreda *et al.*, 2008; Palazzesi *et al.*, 2009). In the recent evolutionary time scale of Asteraceae (Barreda *et al.* (2015), our results are even more significant. According to these authors, the onset of diversification of Asteraceae occurred during the early Cenozoic, several millions of years after the origin of the family, coinciding with a pronounced increase of insect herbivory. These derived groups, which mainly have echinate pollen (~85% of total species), represent the astonishing variety of Asteraceae that we can currently find everywhere except in Antarctica. In this context, and in the light of our results, it may be reasonable to conjecture that the shift from a microechinate to an echinate pattern and from a large to a small pollen size might have been an advantageous acquisition to the reproductive success and explosive radiation of the family. However, experimental investigations on living species of Asteraceae are required to determine the real effect of electrostatic charges in the adherence to the stigma.

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REFERENCES

- Barreda V, Palazzesi L, Tellería MC. 2008. Fossil pollen grains of Asteraceae from the Miocene of Patagonia: Nassauviinae affinity. *Review of Palaeobotany and Palynology* **151**: 51–58.

- Barreda VD, Palazzesi L, Tellería MC, Olivero E, Raine JL, Forest F. 2015.** The Cretaceous of Antarctica: witness of the early evolution of the daisy family (Asteraceae). *Proceedings of the National Academy of Sciences of the United States of America* **112**: 10989–10994.
- Blackmore S, Wortley AH, Skvarla JJ, Robinson H. 2009.** Evolution of pollen in Compositae. In: Funk VA, Susanna A, Stuessy T, Bayer R, eds. *Systematics, evolution and biogeography of the Compositae*. Vienna: IAPT, 101–130.
- Bowker GE, Crenshaw HC. 2007a.** Electrostatic forces in wind-pollination—Part 1: measurement of the electrostatic charge on pollen. *Atmospheric Environment* **41**: 1587–1595.
- Bowker GE, Crenshaw HC. 2007b.** Electrostatic forces in wind-pollination—Part 2: simulations of pollen capture. *Atmospheric Environment* **41**: 1596–1603.
- Chaloner WG. 1986.** Electrostatic forces in insect pollination and their significance in exine ornament. In: Blackmore S, Ferguson LK, eds. *Pollen and spores: form and function, Vol. 12*. London: Linnean Society Symposium Series, Academic Press, 103–108.
- Clarke D, Morley E, Robert D. 2017.** The bee, the flower, and the electric field: electric ecology and aerial electroreception. *Journal of Comparative Physiology* **203**: 737–748.
- Corbet SA, Beament J, Eisikowitch D. 1982.** Are electrostatic forces involved in pollen transfer? *Plant, Cell and Environment* **5**: 125–129.
- Dolinko AE. 2008.** Nondestructive visualization of defect borders in flawed plates inspected by thermal load. *Journal of Physics D: Applied Physics* **41**: 205503.
- Dolinko AE. 2009.** From Newton's second law to Huygens's principle: visualizing waves in a large array of masses joined by springs. *European Journal of Physics* **39**: 025201.
- Dolinko AE. 2018.** Kirchhoff and Ohm in action: solving electric currents in continuous extended media. *European Journal of Physics* **39**: 025201.
- Funk VA, Randall K, Bayer J, Keeley S, Chan R, Watson L, Gemeinholzer B, Schilling E, Panero JL, Baldwin BG, García-Jacas N, Susanna A, Jansen RK. 2005.** Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. *Biologiske Skrifter* **55**: 343–374.
- Erickson EH, Buchmann SL. 1983.** Electrostatics and pollination. In: Jones CE, Little RJ, eds. *Handbook of experimental pollination biology*. New York: Scientific and Academic Editions, 173–184.
- Gan-Mor S, Schwartz Y, Bechar A, Eisikowitch D, Manor G. 1995.** Relevance of electrostatic forces in natural and artificial pollination. *Canadian Agricultural Engineering* **37**: 189–194.
- Heslop-Harrison Y, Shivanna KR. 1977.** The receptive surface of the Angiosperm stigma. *Annals of Botany* **41**: 1233–1258.
- Jackson JD. 1999.** *Classical electrodynamics, 3rd edn*. New York: John Wiley & Sons, 407–455.
- Lane MA. 1996.** Pollination biology of Compositae. In: Caligari PDS, Hind DJN, eds. *Compositae: biology & utilisation*. Proceedings of the International Compositae Conference. Kew. 1994, **2**: 61–80.
- Müller A, Kuhlmann M. 2008.** Pollen hosts of western palearctic bees of the genus *Colletes* (Hymenoptera: Colletidae): the Asteraceae paradox. *Biological Journal of the Linnean Society* **95**: 719–733.
- Palazzesi L, Barreda V, Tellería MC. 2009.** Fossil pollen grains of Asteraceae from the Miocene of Patagonia: Barnadesioideae affinity. *Review of Palaeobotany and Palynology* **155**: 83–88.
- Punt W, Hoen PP. 2009.** The Northwest European pollen flora, 70: Asteraceae — Asteroideae. *Review of Palaeobotany and Palynology* **157**: 22–183.
- Skvarla JJ, Tomb BL, Patel A, Tomb AS. 1977.** Pollen morphology in the Compositae and in morphologically related families. In: Heywood VH, Harborne JB, Turner BL, eds. *The biology and chemistry of the Compositae*. New York: Academic Press, 141–248.
- Tellería MC. 2017.** Spines vs. microspines: an overview of the sculpture exine in selected basal and derived Asteraceae with focus on Asteroideae. *Journal of Plant Research* **130**: 1023–1033.
- Torres C. 2000.** Pollen size evolution: correlation between pollen volume and pistil length in Asteraceae. *Sexual Plant Reproduction* **12**: 365–370.
- Wheeler MJ, Franklin-Tong VE, Franklin FCH. 2001.** The molecular and genetic basis of pollen–pistil interactions. *New Phytologist* **151**: 565–584.
- Wodehouse RP. 1935.** *Pollen grains: their structure, identification and significance in science and medicine*. New York and London: McGraw-Hill.