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RESEARCH PAPER

Surprising absence of association between flower surface microstructure and pollination system

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INTRODUCTION

The variety in shape and structure of flower surfaces and their function in terms of interactions between plants and insects has intrigued scientists for decades (e.g. Kay et al. 1981; Lee 2007; Whitney et al. 2011; Papiorek et al. 2014; van der Kooi et al. 2014; Ojeda et al. 2016). There are three types of epidermal cell shapes in flowers, distinguished by their shape. Flat cells are the least common type, but are characteristic of flowers in the buttercup genera Ranunculus and Ficaria (Ranunculaceae), contributing to their glossy appearance (Kay et al. 1981; Vignolini et al. 2012; van der Kooi et al. 2017). More common types of shapes are convex and cone-shaped (Fig. 1). The epidermal cells of leaves and stems are generally flat, why then do flowers of so many species have cone-shaped epidermal cells?

At least three non-mutually exclusive hypotheses as to the functional significance of cone-shaped epidermal cells have been proposed. First, the cone shape may contribute to the flower’s visual signal by focusing or scattering incident light. Modelling studies suggested that under perpendicular illumination, conical epidermal cells could act as micro-lenses that focus incident light on the floral pigments (Gorton & Vogelmann 1996; Wilts et al. 2018), resulting in reflected light being more strongly filtered by pigments and thus generating stronger flower coloration. However, experimental studies with bees showed that presence or absence of cones does not change the flower’s salience as perceived by bees (Dyer et al. 2007).

Further, given the often strongly varying illumination in nature and varying epidermal cell shape and spacing – even between neighbouring cells (Fig. 1; van der Kooi et al. 2014; Fritz et al. 2017), it remains unclear whether focusing effects play a biologically meaningful role in natural conditions (reviewed by van der Kooi et al. 2019a).

Second, the roughness of a flower surface may play a role in the flower’s hydrophobicity and self-cleaning. Surface roughness, by means of epidermal cones or cuticle striations, reduces the contact area – and thus adhesion – of water droplets and the flower, causing water to roll off the flower (Neinhuis & Barthlott 1997; Barthlott & Neinhuis 1997; Taneda et al. 2015). Indeed, both modelling and experimental approaches showed that epidermal cell shape (in combination with a structured cuticle) determines the wettability of flowers (Taneda et al. 2015).

Third, the flower surface may provide a tactile cue and/or mediate the amount of grip an insect has upon landing on the flower. A tactile role of flower surfaces was first shown by Kevan & Lane (1985), who demonstrated that bees can discriminate flowers based on surface microstructure. Further, the minute claws and hairs at the tarsi of flower-visiting insects indeed adhere better to rough flower-like surfaces (Voigt et al. 2012; Bräuer et al. 2017). Studies using isogenic Antirrhinum majus lines and flower surface replicas that differed solely in epidermal cell shape, confirmed that bees can discriminate flowers based on touch alone and that epidermal cones may provide grip to bees that visit the flowers (Whitney et al. 2009). The importance of
microstructures in providing grip to visiting insects is further likely to increase with verticality of the floral display.

If conical epidermal cells provide grip to landing insects then, conversely, their absence could be an ‘anti-bee’ adaptation (sensu Castellanos et al. 2004). For example, (nearly) flat and smooth epidermal cells may hamper flower handling by floral antagonists such as nectar-robbing insects (Papiorek et al. 2014; Ojeda et al. 2017). The pitchers of some species of carnivorous plants (e.g. Nepenthes) feature flat epidermal cells that are indeed slippery, causing prey to slide into the pitcher and be digested (Gaume et al. 2002). By the same reasoning, there may be differences in surface structure between flower areas that are frequently touched by pollinating insects (e.g. the landing area or surfaces surrounding the nectaries and anthers) versus areas that will rarely be touched by pollinators, such as the lower (abaxial) side of the floral display.

Following the hypothesis that flower surfaces may provide grip and/or tactile cues to specific insect pollinators and having an unstructured surface can deter floral antagonists, we may expect epidermal surfaces to correlate with pollinator guild. Whereas bees and flies land on the flower to forage nectar and pollen, birds and hawkmoths do not land but hover in front of the flower while feeding. Some birds sit on a nearby branch while drinking nectar from the flower. Similarly, flowers that reproduce via uniparental reproduction (e.g. self-pollination or asexual reproduction) will also not need to provide grip or tactile cues to landing insects, so surface structure may be one of the multiple (pollinator-attracting) traits that degenerate in flowers of self-pollinating plants (e.g. Goodwillie et al. 2010; Sicard & Lenhard 2011; Dart et al. 2012).

In this study, we investigate the evolution of flower surfaces by virtue of comparing closely related species with contrasting pollination or mating systems. We include 13 genera across nine angiosperm families, and in total compare the floral epidermal cell shape for 29 congeneric sister species pairs with vertically presented flowers that differ in pollination or mating system. We test whether (i) flower surfaces that are frequently touched and/or seen by insect pollinators are more structured (i.e. have higher and/or more pointy, conical epidermal cells) than areas that are invisible/inaccessible to pollinators, and whether (ii) surfaces of bee- and fly-pollinated flowers will be more structured than species that are pollinated by hawkmoths, birds or via self-fertilisation. We found that there is no large-scale correlation of flower surface and pollinator guild or mating system, but that the adaxial surfaces of flowers are markedly more structured than abaxial surfaces, hinting at abiotic and/or visual effects as main drivers for flower surface evolution.

MATERIAL AND METHODS
Species used

Plant species pairs were chosen via a thorough literature research. We included species that were specialised in their pollinator guild inasmuch as they are exclusively bee-/fly-, moth- or bird-pollinated, not mixed. In choosing our study species, we followed the most effective pollinator principle and not the ‘pollination syndrome’ concept, because this has been proven to not hold for numerous plant groups (Ollerton et al. 2009; Funamoto & Ohashi 2017). For the same reason, we included only plant species for which actual pollinating species were reported, thus excluding cases for which only flower visitors were known. Although we realise that within each category a plant may be serviced by a wide array of different species (Waser et al. 1996), these pollinators have similar behaviour with respect to flower handling, that is, the plants are functionally specialised (sensu Ollerton et al. 2007). Species were classified as (obligate) selfer only when there was clear evidence to suggest that selfing was the main reproductive mode, because – as for the pollination syndrome concept – presence of a phenotypic trait that typically occurs in selfers need not be good
evidence that the species is actually largely selfing (e.g. Lozada-Gobilard et al. 2019). Phylogenetic relatedness was determined using recently published phylogenies (see Supplementary Information for information on pairs and references). We thus included 46 species of 13 genera in nine plant families that covered 29 species pairs pollinated by guilds with contrasting flower handling behaviour (Table 1). Flowers were taken from plants grown from seed in the University greenhouse (LD: 14 h:10 h, day:night temperature 22:20 °C) or in Botanical Gardens (see Supplementary Information for details on sources).

Investigation of flower surfaces

We first compared the adaxial and abaxial surfaces of 12 bee-/fly-pollinated species. For the comparison between pollinator guilds and mating systems, we compared the adaxial sides, for in the studied species the adaxial surface will be seen and touched by pollinators. The surface of the floral organ that constitutes the visual display (generally the petal) was examined regardless of its colour (pattern) using dental impression material (Provil Novo Light, Heraeus Kulzer, Hanau, Germany) as per van der Kooi et al. (2014). Immediately after picking the flower, it was pressed into the freshly prepared dental impression material that solidifies within minutes. The mould was subsequently filled with transparent nail polish (base coat, Hema, the Netherlands), creating a positive surface replica, i.e. a cast. The casts were examined under a microscope (Nikon Diaphot 300) with a 40× objective. The surface structure of the whole floral organ was examined and the most dominant structure (generally that on the central/distal area) was photographed with a Nikon D70 camera. Epidermal cell shape dimensions (below) were measured in Fiji (ImageJ, NIH). To avoid observer bias, the flower surfaces were observed and measured by an observer (MK) who had no prior knowledge of the pollinator, i.e. the observer was ‘blind’.

### Table 1. Summary of the taxa and pollinator mode included. Details regarding species pairs, pollinator guild/mating system and references are provided in the supplementary data file.

| family          | genus          | pollinator guild/mating system | comparison groups (number of pairs) |
|-----------------|----------------|-------------------------------|-------------------------------------|
| Acanthaceae     | *Ruella*       | Bee-bird (1)                  |                                     |
| Balsaminaceae   | *Impatiens*    | Bee-bird (4), bee-moth (2)    |                                     |
| Campanulaceae   | *Lobelia*      | Bee-bird (1)                  |                                     |
| Hippobroma      | *Silene*       | Bee-moth (1)                  |                                     |
| Caryophyllaceae | *Salvia*       | Bee-bird (2)                  |                                     |
| Oenotheraceae   | *Clarkia*      | Bee-moth (1)                  |                                     |
| Phrymaceae      | *Mimus*        | Outcrossing-selfing/sexual (1)|                                     |
| Pholemoniaceae  | *Leptosiphon*  | Outcrossing-selfing (1)       |                                     |
| Solanaceae      | *Nicotiana*    | Bee-bird (2), bee-moth (1)    |                                     |
|                 | *Petunia*      | Bee-bird (1), bee-moth (1)    |                                     |
|                 | *Solanus*      | Outcrossing-selfing (2)       |                                     |

*All species pair comparisons are within one genus, except moth-pollinated *Hippobroma longiflora*, which was compared with the closely related bee-pollinated Lobelia siphilitica.

(Holman et al. 2015; Kardish et al. 2015). Transverse sections of fresh flowers shown in Fig. 1 were obtained by photographing a transverse section of a piece of flower embedded in 6% agarose (for details, see van der Kooi & Stavenga 2019).

### Analyses of surface parameters

For cells with a round or (quasi-)hexagonal base, we measured the diameter of the cell’s base, and for cells with a rectangular base, we measured cell length and width (Fig. 2a, b). The protruding height was measured similarly for both types of epidermal cell (Fig. 2c, d). Preferably, we measured the floral surfaces of three individual plants per species, for which we succeeded in 34 out of 46 species (74%), but for nine species we could sample only two individuals and for three species only one. The approximate roughness of a surface was calculated via a ‘roughness index’, which is the ratio of the lateral, 3D surface area to the projected, geometric surface area of the cell’s base (following Aideo & Mohanta 2018). For cone-shaped cells, the lateral surface area of the cone is described by:

\[ S_l = L \times 0.5 \times d_b \times \pi \]

and the cell base size by:

\[ C_b = (0.5 \times d_b)^2 \times \pi \]

with \( L \) being the cone’s long side and \( d_b \) the cell’s base diameter (see Fig. 2e). For rectangular cells, the lateral surface area was calculated as:

\[ S_l = 2 \times l \times L \]

and the cell base size as:

\[ C_b = l \times w \]

with \( l \), \( L \) and \( w \) being the base length, the long side length and width of the cells, respectively (see Fig. 2f). The roughness index is then the ratio of the lateral surface to the ground plane:

\[ RI = S_l / C_b \]

For the exemplary cases shown in Fig. 1a–d, mean cell widths are 27, 22, 32 and 44 μm, respectively, and mean cell heights (measured as in Fig. 2c) are 34, 13, 10, 8 μm, respectively. The calculated roughness indices for these species then are 2.7, 1.6, 1.2 and 1.1, respectively, which is in line with the differences in roughness seen in Fig. 1a–d.

Statistical significance was tested via a parametric bootstrap test, using the `pbkrtest` package in R (R Foundation for Statistical Computing, Vienna, Austria). In the linear models, species was nested within genus as a random effect when testing the effect of flower side, and pair was nested within genus as random effect when testing the effect of pollination system. A likelihood ratio test (LRT) was used to see whether a model with the response variable (pollination system or flower side) fits better to the data than the same model without the response.
RESULTS

Our analysis of cell anatomy revealed significant differences between the adaxial and abaxial side of flowers (Fig. 3). The cell surface area is similar on both sides \( (LRT = 1.93, P = 0.18) \), but the adaxial cell height is on average twice that of the abaxial side \( (LRT = 48.98, P < 0.001) \). As cell height does not adequately capture overall surface curvature, we also calculated a roughness index, which is the ratio of the cell's outer surface to that of the flat, projected cell surface area (Methods, Fig. 2). Analogous to the aspect ratio, the roughness index thus summarises the cell's curvature relative to its ground plane. Indeed, the adaxial roughness index values are significantly larger than those of the abaxial surface \( (LRT = 44.3, P < 0.001) \). In ten out of the 12 studied species, the adaxial epidermal cells are higher than the abaxial cells; in one species it is approximately the same and in one there is a small decrease in height; the roughness shows a similar effect \( (Fig. 3b, c) \). In the vast majority, cone-shaped epidermal cells are thus particularly prominent at the adaxial side, and species with cone-shaped cells on both sides \( \text{e.g. Solanum citrullifolium; Fig. 1a} \) are relatively rare.

In contrast to observations between different sides of the flower, adaxial epidermal cell shape does not consistently differ between species with contrasting pollinators \( (Fig. 4; n = 29 \text{ species pairs, for all comparisons}) \). Neither cell surface area \( (LRT = 2.57, P = 0.11) \), cell height \( (LRT = 0.21, P = 0.2) \) nor roughness index \( (LRT = 0.41, P = 0.52) \) differs significantly between sister species. Also, when compared per pollinator guild, none are significantly different in epidermal cell height or roughness (Table S1).

In line with the reduction in floral display size commonly found in selfers \( \text{(Goodwillie et al. 2010; Sicard & Lenhard 2011; Dart et al. 2012)} \) is our observation that epidermal cell size is smaller in five out of six outcrossing–selfing species pairs \( \text{(see the red curves in Fig. 4a). Floral epidermal cells of selfers are approximately 35% smaller in surface area than epidermal cells in related outcrossers, a difference that is marginally significant} \ (P = 0.036, n = 6 \text{ species pairs; Table S1}) \). A similar trend is visible in epidermal cells of moth-pollinated flowers, although this is not significant \( (P = 0.072, n = 10 \text{ species pairs; Table S1}) \).

DISCUSSION

Flower surface does not correlate with pollination system

In our investigation of the microstructure of flowers with contrasting pollinators, we found no indication of large-scale correlation of epidermal cell shape and pollination system \( (Fig. 4) \). The fact that the adaxial surface, which generally faces pollinators and is close to the reproductive organs, is markedly more...
structured than the abaxial surface (Fig. 3) suggests, however, that surface roughness fulfils a biologically meaningful function. Further, there is an effect of mating system inasmuch as selfing plants have smaller epidermal cells than their outcrossing relatives (Fig. 4a; Table S1). This indicates that the reduction in floral display size commonly found in selfers (Goodwillie et al. 2010; Sicard & Lenhard 2011; Dart et al. 2012) is due, at least partly, to a reduction in cell size. Whether the number of epidermal cells, as well as the size and number of interior cells, also decrease in selfers requires additional investigation.

The fact that we find no correlation of flower epidermal cell shape and pollinator is not because sister species have not (yet) diverged sufficiently to develop phenotypic differences or because our method is inadequate to detect a biological signal. Indeed, we found up to three-fold differences in epidermal cell surface, height and roughness index between sister species, albeit in opposite directions (Fig. 4a; Table S1). This indicates that the reduction in floral display size commonly found in selfers (Goodwillie et al. 2010; Sicard & Lenhard 2011; Dart et al. 2012) is due, at least partly, to a reduction in cell size. Whether the number of epidermal cells, as well as the size and number of interior cells, also decrease in selfers requires additional investigation.

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species of Nymphaeaceae with contrasting pollinators but no difference in flower surface (Coiro & Barone Lumaga 2018), and with those in a study on surfaces of 11 (unrelated) plant species with different pollinators (Costa et al. 2017). It remains unknown how striations in the flower’s cuticle contribute to the mechanical interaction with pollinators. In the studied species, the frequent lack of cuticle structure precluded analysis of whether cuticle differences, if any, are linked to specific pollinators. Deeper species sampling would help to elucidate the importance of phylogeny on flower surface evolution. Additionally, in the systems so far considered, bird pollination was derived from bee/fly pollination and not vice versa, reflecting the asymmetry in the direction of transitions between insect and bird pollination found globally (Thomson & Wilson 2008; Barrett 2013). It remains to be investigated whether transitions from bird to bee pollination yield a systematic difference in epidermal cell shape, although we consider that unlikely.

Adaxial flower side markedly more structured than abaxial side
The marked differences between the adaxial and abaxial surfaces (Fig. 3), combined with our observation that epidermal cell shape does not correlate with pollinator guild, suggest that cone-shaped epidermal cells occur for reasons other than providing grip. Adaxial surfaces generally surround the reproductive organs, at least in the studied species, and having cone-shaped cells may increase water repellence and even self-cleaning of the flower (Neinhuis & Barthlott 1997; Barthlott & Neinhuis 1997; Watanabe-Taneda & Taneda 2019), which could benefit flower visibility and longevity, and ultimately the plant’s reproductive success. Cone-shaped epidermal cells could also modify the reflection of light by reducing the surface gloss and thus create a uniform visual signal that is well visible to pollinators approaching from different angles (van der Kooi et al. 2019a). Similar cone-like structures were found to reduce surface gloss of some animal organs, such as moth eyes and snake skin (Stavenga et al. 2006; Spinner et al. 2013). Further, a higher surface roughness means an increase in surface area, which may lead to changes in transpiration rates (Buschhaus et al. 2015), flower temperature (van der Kooi et al. 2019b) and scent emission (Effnert et al. 2005).

The asymmetry in epidermal cell shape between adaxial and abaxial flower sides may be linked with the pigmentary aspects of the flower’s visual signal. Many species that have flowers with conical epidermal cells have pigments that occur in the epidermal cells only (e.g. flavonoids and anthocyanins; Kay et al. 1981; Grotewold 2006; Lee 2007; van der Kooi et al. 2016). Anthocyanin-pigmented flowers often feature clear differences in pigmentation between abaxial and adaxial flower sides, resulting in only the pigmented side being strongly coloured, creating marked differences in coloration between the different sides (Stavenga & van der Kooi 2016; van der Kooi et al. 2016). Asymmetry in flower side coloration can be an efficient way of filtering reflected light with relatively little pigment (van der Kooi et al. 2016), and having only one coloured side can reduce conspicuousness to floral antagonists when the flower is closed (Kemp & Ellis 2019). Whether (asymmetry in) epidermal cell shape and pigmentation are indeed coupled requires further anatomical and optical investigation. Of particular interest would be to study the surfaces of species with hanging, pendant flowers where the abaxial flower side constitutes the visual signal.

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AUTHOR CONTRIBUTION
CJvdK designed the study, both authors collected the data, MK made the photographs and performed measurements, both authors performed the analyses and CJvdK wrote the manuscript. Both authors approved the final version of the manuscript.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. P-values for different comparisons. The p-values obtained for the different sublevels were Bonferroni corrected for multiple testing.

Data S1. Information on species-pairs, data file and R script.

REFERENCES
Aideo S.N., Mohanta D. (2018) Surface-wettability and structural colouration property of certain Rosaceae cultivars with off-to-dark pink appearances. Journal of Bionic Engineering, 15, 1012–1024.
Bailes E.J., Glover B.J. (2018) Intraspecific variation in species with different pollinators (Costa et al. 2017).
Bailes E.J., Glover B.J. (2018) Surface-wettability and structural colouration property of certain Rosaceae cultivars with off-to-dark pink appearances. Journal of Bionic Engineering, 15, 1012–1024.
Barthlott W., Neinhuis C. (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta, 202, 1–8.
Barthlott W., Neinhuis C. (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta, 202, 1–8.
Bräuer P., Neinhuis C., Voigt D. (2017) Attachment of honeybees and greenbottle flies to petal surfaces. Arthropod-Plant Interactions, 11, 171–192.
Buschhaus C., Hagen D., Jetter R., Feldmann K.A. (2015) Wax layers on Cosmos bipinnatus petals contribute unequally to total petal water resistance. Plant Physiology, 167, 80–88.
Castellanos M.C., Wilson P., Thomson J.D. (2004) “Anti-bee” and “pro-bird” changes during the evolution of hummingbird pollination in Penstemon flowers. Journal of Evolutionary Biology, 17, 876–885.
Coiro M., Barone Lumaga M.R. (2018) Disentangling historical signal and pollinator selection on the micromorphology of flowers: an example from the floral epidermis of the Nymphaeaceae. Plant Biology, 20, 902–915.
Costa V.B.S., Pimentel R.M.M., Chagas M.G.S., Alves G.D., Castro C.C. (2017) Petal micromorphology

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and its relationship to pollination. Plant Biology, 19, 115–122.

Dart S.R., Samis K.E., Austen E., Eckert C.G. (2012) Broad geographic covariation between floral traits and the mating system in Camissoniopsis chiaranthifolia (Onagraceae): multiple stable mixed mating systems across the species’ range. Annals of Botany, 109, 599–611.

Di Stilio V.S., Martin C., Schufur A.F., Connelly C.F. (2009) An orthology of MIXTA-like2 controls epidermal cell shape in flowers of Thalictrum. New Phytologist, 183, 718–728.

Dyer A.G., Whitney H.M., Arnold S.E.J., Glover B.J., Chittka L. (2007) Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of Antirrhinum majus flower colour. Arthropod-Plant Interactions, 1, 45–55.

Effertz U., Große J., Röse U.S.R., Ehrig F., Kägi R., Piechulla B. (2005) Volatile composition, emission pattern, and localization of floral scent emission in Mirabilis jalapa (Nyctaginaceae). American Journal of Botany, 92, 2–12.

Fritz B., Hüning R., Schmager R., Hetterich M., Lemmer U., Gomard G. (2017) Assessing the influence of structural disorder on the plant epidermal cells’ optical properties: a numerical analysis. Bioinspiration & Biomimetics, 12, 036011.

Funamoto D., Ohashi K. (2017) Hidden floral adaptation to nocturnal moths in an apparently bee-pollinated flower, Adenophora triphylla var. japonica (Campanulaceae). Plant Biology, 19, 767–774.

Gaume L., Gorb S., Rowe N. (2002) Function of epidermal surfaces in the trapping efficiency of Nepenthes alata pitchers. New Phytologist, 156, 479–489.

Glover B.J., Perez-Rodriguez M., Martin C. (1998) Development of several epidermal cell types can be specified by the same MYB-related plant transcription factor. Development, 125, 3497–3508.

Goodwillie C., Sargent R.D., Eckert C.G., Elle E., Geber M.A., Johnston M.O., Kalsi S., Moeller D.A., Rees R.R., Vallejo-Márìn M. (2010) Correlated evolution of mating system and floral display traits in flowerpitchers. New Phytologist, 187, 311–321.

Gorton H.L., Vogelmøn T.C. (1996) Effects of epidermal cell shape and pigmentation on optical properties of Antirrhinum petals at visible and ultraviolet wavelengths. Plant Physiology, 112, 879–888.

Grotewold E. (2006) The genetics and biochemistry of floral cell shape and anthocyanin synthesis influence bumblebee perception of Antirrhinum majus flower colour. Arthropod-Plant Interactions, 1, 45–55.

Kemp J.E., Ellis A.G. (2019) Cryptic petal coloration decreases floral apparency and herbivory in nocturnally closing daisies. Functional Ecology, 33, 2130–2141.

Kevern P.G., Lane M.A. (1985) Flower petal microtextures are a tactile cue for bees. Proceedings of the National Academy of Sciences of the United States of America, 82, 4750–4752.

van der Kooi C.J., Stavenaga D.G. (2019) Vividly coloured poppy flowers due to dense pigmentation and strong scattering in thin petals. Journal of Comparative Physiology. A, 205, 363–372.

van der Kooi C.J., Wils B.D., Leertouwer H.L., Staal M., Elzenga J.T.M., Stavenaga D.G. (2014) Irisescent flowers? Contribution of surface structures to optical signaling. New Phytologist, 203, 667–673.

van der Kooi C.J., Elzenga J.T.M., Staal M., Stavenaga D.G. (2016) How to colour a flower: on the optical principles of flower coloration. Proceedings of the Royal Society B: Biological Sciences, 283, 20160429.

van der Kooi C.J., Elzenga J.T.M., Dijkstra S., Stavenaga D.G. (2017) Functional optics of glossy buttercup petals. Journal of the Royal Society Interface, 17, 20160933.

van der Kooi C.J., Dyer A.G., Kevan P.G., Lanau K. (2019a) Functional significance of the optical properties of flowers for visual signalling. Annals of Botany, 123, 263–276.

van der Kooi C.J., Kevan P.G., Koski M.H. (2019b) The thermal ecology of flowers. Annals of Botany, 124, 343–353.

Lee D.W. (2007) Nature’s palette. The science of plant color. University of Chicago Press, Chicago, IL, USA.

Lozada-Gobillard S., Weigend M., Fischer E., Janssens S.B., Ackermann M., Abramczyk S. (2019) Breeding systems in Balsaminaceae in relation to pollen/ovule ratio, pollination syndromes, life history and climate zone. Plant Biology, 21, 157–166.

Nehnûs C., Barthlott W. (1997) Characterization and distribution of water-repellent, self-cleaning plant epidermal cells and structured cuticles of flower petals. Annals of Botany, 79, 567–577.

Ojeda D.I., Jaen-Molina R., Santos-Guerra A., Cau-jepe-Castells J., Cronk Q. (2017) Temporal, but not spatial, changes in expression patterns of petal identity genes are associated with loss of papillate conical cells and the shift to bird pollination in Macarone- sian Lotus (Leguminosae). Plant Biology, 19, 420– 427.

Ollerton J., Killick A., Lamborn E., Wils B., Whiston M. (2007) Multiple meanings and modes: on the many ways to be a generalist flower. Taxon, 56, 717–728.

Ollerton J., Alarcon R., Waser N.M., Price M.V., Watts S., Cranmer L., Hingston A., Peter C.J., Rotenberg J. (2009) A global test of the pollination syndrome hypothesis. Annals of Botany, 103, 1471–1480.

Papirek O., Junker R.R., Lunau K. (2014) Gloss, colour and grip: multifunctional epidermal cell shapes in bee-and bird-pollinated flowers. PLoS ONE, 9, e112013.

R.H., Vallejo-Marin M. (2010) Correlated evolution of mating system and floral display traits in flower-pitchers. New Phytologist, 187, 311–321.

No association of flower surface and pollinator