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POLLENKITT IS ASSOCIATED WITH THE COLLECTABILITY OF MALVOIDEAE POLLEN FOR CORBICULATE BEES

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Abstract—Pollen grains of Malvoideae (Malvaceae) which corbiculate bees cannot collect constitute a floral filter that excludes pollen-collecting bumble bees and honey bees from exploiting pollen resources. Although large, spiny pollen grains are in fact harder to compact for collection by corbiculate bees, pollen morphology (e.g., grain diameter, spine length) is not by itself a reliable indicator of pollen collectability. In this study, we discovered that two Malvoideae species, *Anoda cristata* and *Malope trifida*, possess large, spiny pollen grains that can be groomed and collected by corbiculate bees. To gain insight into the underlying cause of collectability of Malvoideae pollen, we tested pollen adhesion to bumble bee setae and found that significantly less of the collectable pollen grains of *A. cristata* and *M. trifida* adhere to bees' setae compared to uncollectable pollen grains of *Hibiscus trionum*. As the primary mediator of pollen adhesion is pollenkitt, a viscous lipid-rich substance covering pollen of zoophilous plants, we examined the surface of uncollectable and collectable Malvoideae pollen using cryo-SEM. Fresh pollen grains were abundantly covered with pollenkitt that also coated the long spines and formed liquid bridges between the grains. Washing pollen with hexane removed all pollenkitt, whereas washing pollen with water only removed pollenkitt on the collectable pollen grains of *M. trifida*, but not the uncollectable pollen grains of *Hibiscus syriacus*. We hypothesise that pollenkitt composition differs between Malvoideae species with uncollectable and collectable pollen. Specific pollenkitt properties might elicit excessive viscidity which affects adhesion to insect visitors but prevents pollen collection by corbiculate bees.

Keywords—Apidae, floral filter, Malvaceae, pollen adhesion, pollen collection, pollenkitt

INTRODUCTION

Although bees are known to be valuable pollinators for numerous plant species, pollen harvesting by bees can negatively affect plant reproduction (reviewed by Hargreaves et al. 2009). Consequently, it may be beneficial for plants to narrow the spectrum of pollen-feeding visitors through floral filters which restrict resource exploitation to a specific group of effective pollinators (reviewed by van der Kooi et al. 2021). This can be achieved by various means, for example through bees' avoidance of flowers that are visually attractive to birds, but achromatic and thus unattractive to bees (Lunau et al. 2011). A physiological filter would be pollen that is difficult to digest or even toxic to (some) bees, as described for representatives of Asteraceae and Cucurbitaceae (Brochu et al. 2020; Vanderplanck et al. 2020). While the principles of these sensory and physiological filters are fairly well studied, the puzzle of the physicochemical filter of Malvoideae pollen has yet to be solved (Konzmann et al. 2019).

The Malvaceae are characterised by large pollen grains with long, sometimes dimorphic spines (Christensen 1986). Plants of the Malvoideae – a subfamily of the Malvaceae containing the tribes Malveae, Gossypieae (cotton), and Hibisceae – typically possess large pollen grains with long spines that are hard to collect or even not collected by corbiculate bees, e.g., honey bees and bumble bees, meaning that they cannot pack the grains into their corbiculae (pollen baskets) at all (Vansell 1944; Christensen 1986; Buchmann & Shipman 1990; Vaissière & Vinson 1994; Lunau et al. 2015; Konzmann et al. 2019). Nectar foragers can be observed frequenting the flowers and becoming densely covered with pollen, yet they hardly even attempt grooming themselves during a foraging bout, which makes them ideal pollen vectors for the plants (Vansell 1944; Konzmann et al. 2019). In this study, pollen is considered collectable or uncollectable based on the outcome of pollen collection assays with buff-tailed bumble bees. Collectability partly depends on the bees' ability to groom pollen grains and store them in the transport organs (e.g., corbiculae, combs, or branched bristles), and partly on pollen grain properties, such as stickiness, size, and ornamentation (Thorp 1979, 2000; Amador et al. 2017; Hasegawa et al. 2021). Corbiculate bees usually facilitate pollen collection by wetting their legs with regurgitated nectar, which mixes with pollenkitt and bonds the pollen grains (Michener et al. 1978; Hesse 1980; Thorp 2000; Nicolson 2011; Pokorny et al. 2014; Wappler et al. 2015). After grooming, the pollen mass is stored and compacted into a pellet in the corbicula, a slightly concave part of the hind tibia which is framed by rigid setae (Michener et al. 1978; Thorp 2000). In contrast, specialised bees such as *Tetralonia malvae* Rossi (Eucerini), *Diadasia* spp., and *Ptilothrix* spp. (Emphorini) are morphologically adapted to collect pollen grains of Malvoideae that are stored loosely in their long, sparse scopal setae (Michener 2007; Schlindwein et al. 2009; Tepedino et al. 2016). The mode of pollen transportation – loose and dry, or agglutinated with nectar – affects the ability of pollen grains to contribute to pollination when the scopa or corbicula directly contact a conspecific stigma (Parker et al. 2015). As pollen packaging with nectar prevents pollen grains from reaching a receptive stigma, it is advantageous for Malvoideae to possess pollen grains that exclude corbiculate bees from collecting pollen, which potentially reduces the plant's male fitness.

Previous studies have drawn inconsistent and partly contradictory conclusions regarding the collectability of Malvoideae pollen for corbiculate bees. Pollenkitt, pollen grain size, spine length, and the combination of pollenkitt and spines, respectively, were considered to physically impede pollen compaction in the bees' corbiculae (Vansell 1944; Buchmann & Shipman 1990; Vaissière & Vinson 1994; Lunau et al. 2015). It is known that on natural surfaces, the combined effects of surface structure and chemistry can determine adhesion or repellence of liquids (e.g., Adam 1952; Koch et al. 2008; Hensel et al. 2016), which has also been discussed for pollen (Lin et al. 2013). Recent experiments revealed that bumble bees cannot collect pollen grains of several Malvoideae species and one Dipsacoideae species that share a large diameter (≥ 100 µm) and spiny surface structures (Konzmann et al. 2019). However, neither of these morphological factors proved to be an exclusion criterion for collectability as the bees were able to collect pollen grains of *Cucurbita pepo* L. (Cucurbitaceae) which resemble Malvoideae pollen regarding size and spine length (Konzmann et al. 2019). Morphological traits alone do not render Malvoideae pollen uncollectable and thus protected from collection by corbiculate bees. Accordingly, the missing decisive factor might be pollenkitt as was already suspected by Vansell (1944) and Lunau et al. (2015). Pollenkitt is a lipid-rich and highly viscous liquid that covers pollen grains of almost all animal-pollinated plant species (Hesse 1980; Pacini & Hesse 2005). Among other functions, it connects pollen grains and enables adhesion to the inner anther wall, flower-visiting insects, and conspecific stigmas (Hesse 1980; Pacini & Hesse 2005).

In the early trials of this study, we observed honey bee workers collecting pollen of *Malope trifida* Cav. (Malvoideae) in their corbiculae. Consequently, we divided our research on the cause of pollen collectability into three parts. Firstly, we wanted to confirm the collectability of specific Malvoideae pollen for corbiculate bees. This was tested with bumble bees and three Malvoideae species in a comparative field study similar to our previous research (Konzmann et al. 2019). When visiting Malvoideae flowers, bees are often densely covered with pollen grains – demonstrating the excellent adhesion of pollen grains to the bee's body. We assume that pollen of most Malvoideae species is too sticky to be groomed and collected by corbiculate bees, i.e., that adhesive properties of pollen might be crucial for its collectability. To determine whether collectable and uncollectable pollen differs in its adhesion to bees, we applied Malvoideae pollen to bumble bee setae and evaluated its adhesion using high-speed rotation. A similar experimental setting has previously been successfully applied in pollen adhesion experiments with different *Salvia* species indicating that pollen adhesion in high-speed rotation tests depends on the duration and speed of rotation (Baumann 2006). Lastly, we assessed whether the discrepancy in pollen collectability may be caused by differences in pollenkitt. A crucial aspect of pollen collection by corbiculate bees is the wetting of pollen grains with regurgitated nectar before they are transferred to the corbiculae (Thorp 1979, 2000). As an approach to this aspect of pollen collection, we tested the effect of different solvents on pollenkitt with cryo-scanning electron microscopy. In addition to the solubility treatment, pollenkitt presence, abundance, and distribution were examined in Malvoideae species with collectable and uncollectable pollen using cryo-SEM.

MATERIALS AND METHODS

POLLEN COLLECTABILITY ASSAYS

We observed the foraging behaviour of naturally occurring Western honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.) (Hymenoptera, Apidae) on 7 species of the Malvaceae subfamily Malvoideae (*Althaea officinalis* L., *Anoda cristata* L., *Hibiscus syriacus* L., *Hibiscus trionum* L., *Lavatera trimestris* L., *Malope trifida*, and *Malva sylvestris* L.) in the Botanical Garden of the TU Dresden (51° 02' 33.3" N, 13° 45' 30.3" E) from July to August 2017 on predominantly sunny days without precipitation. The pollen collection behaviour of single buff-tailed bumble bee (*Bombus terrestris* L.) workers from a standard hive (Biobest Group NV, Westerlo, Belgium) was experimentally tested on *A. cristata*, *M. trifida*, and *H. trionum* in a flight cage (approximately 3 x 2 x 2 m steel frame covered with net fabric) in the Botanical Garden Düsseldorf (51° 11′ 13″ N, 6° 48′ 9″ O) from August to October 2018 on predominantly sunny days without precipitation. In accordance with the methods described in Konzmann et al. (2019), we recorded (a) the mass of the pollen pellet collected per corbicula, (b) the handling time (defined as the time spent on taking up nectar and collecting, grooming, and packing pollen), and (c) the number of flowers visited during one foraging bout. Twenty-six bumble bee workers were tested for *A. cristata*, 18 for *M. trifida*, and 8 for *H. trionum*.

HIGH-SPEED ROTATION TESTS

To test our hypothesis that the adhesiveness of Malvoideae pollen is an indicator of the bees' inability to groom and compact the grains into their corbiculae, pollen adhesion to bumble bee setae was tested by recording the detachment of pollen from bee bodies by centrifugal effect during high-speed rotation. This test was impossible to conduct with live bumble bees; however, we wanted to test adhesion to bee setae instead of an artificial surrogate as for example glass fibres. After preliminary testing, we glued thoraces and abdomens of recently frozen, thawed, and superficially dried *B. terrestris* workers (with the dorsal side up) into the caps of polypropylene tubes (SafeSeal reaction tube, 2 ml, SARSTEDT AG & Co. KG, Nümbrecht, Germany). Freshly collected pollen of *A. cristata*, *M. trifida*, and *H. trionum* was applied by gently manually brushing the anthers of a previously unvisited flower with recently opened anthers against the dorsal side of a thorax or abdomen (on average, 85 grains were transferred per sample). Similar to natural conditions, the pollen grains attached predominantly distally to the setae. Using a stereo microscope, we counted the number of pollen grains adhering to the bee's setae (singly and in agglomerates) before and after rotating the closed tubes at 2,600 rpm for 60 s inside a centrifuge (MIKRO 20 microliter centrifuge, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). Pre-tests showed that detachable pollen grains are released at this rotation speed. For each plant species, 5 thoraces and 5 abdomens were sampled (per plant in total $N = 10$ counts).

CRYO-SCANNING ELECTRON MICROSCOPY (CRYO-SEM)

In the Botanical Garden of the TU Dresden, recently opened flowers of *Al. officinalis*, *A. cristata*, *H. syriacus*, *H. trionum*, *L. trimestris*, *M. trifida*, and *Mv. sylvestris* were collected to visualise fresh pollen grains in their native condition. For *H. syriacus* and *M. trifida*, pollen grains from 10 anthers were rinsed three times with either 0.5 ml tap water or 0.5 ml hexane (C_6H_{14}, ≈ 95 % n-hexane for HPLC, CAS: 110-54-3. Fisher Scientific GmbH, Schwerte, Germany) for 15 s at 23 °C. The liquid was removed from the pollen samples after each rinse. The effect of different solvents on pollenkitt was observed immediately after treatment using the cryo-SEM SUPRA 40VP-31-79 (Carl Zeiss SMT

Ltd., Oberkochen, Germany) equipped with an EMITECH K250X cryo-preparation unit (Quorum Technologies Ltd., Ashford, Kent, UK). Recently opened anthers and rinsed pollen samples were mounted on metal stubs using polyvinyl alcohol (Tissue-Tek, OCT, Sakura Finetek Europe B.V., Alphen aan den Rijn, the Netherlands). Subsequently, the samples were shock-frozen in liquid nitrogen in the slushing chamber, transferred to the cryo-preparation chamber at −140 °C, sublimed for 15 min at −70 °C, sputter coated with platinum (layer thickness ca. 6 nm), transferred to the SEM, and then examined in a frozen state at 5 kV accelerating voltage and −100 °C temperature. Cryo-SEM micrographs were taken using the software Smart SEM 05.03.05 (Carl Zeiss SMT Ltd., Oberkochen, Germany).

STATISTICAL METHODS

All data were analysed using R statistical software, version 4.0.3 (R Core Team 2020). After testing normality of the data distribution with the Shapiro-Wilk test, multiple comparison sets of non-parametric data were analysed with the Kruskal-Wallis rank-sum test and *post hoc* Mann-Whitney *U* test with FDR (false discovery rate) correction, while ANOVA and *post hoc* Tukey's HSD test were used for analysis of parametric data – as indicated in the results and figure legends. Pairs of data sets were compared with Wilcoxon signed-rank test (non-parametric) or Student's *t*-test (parametric).

RESULTS

POLLEN COLLECTABILITY

During four weeks of observation of seven Malvoideae species, neither honey bees nor bumble bees collected pollen of *Al. officinalis*, *A. cristata*, *H. syriacus*, *H. trionum*, *L. trimestris*, or *Mv. sylvestris*, although both honey bees and bumble bees frequently visited the flowers to take up nectar. However, several honey bees were observed collecting pollen of *M. trifida* in their corbiculae. In the collectability experiment with three Malvoideae species, single bumble bee (*B. terrestris*) workers collected on average 3.62 ± 0.39 mg (mean ± S.E.; dry mass) pollen of *A. cristata* per corbicula and 3.06 ± 0.54 mg pollen of *M. trifida*, but none of *H. trionum* (Fig. 1A-D; Kruskal-Wallis test: $\chi^2 = 21.02$, $df = 2$, $P < 0.001$).

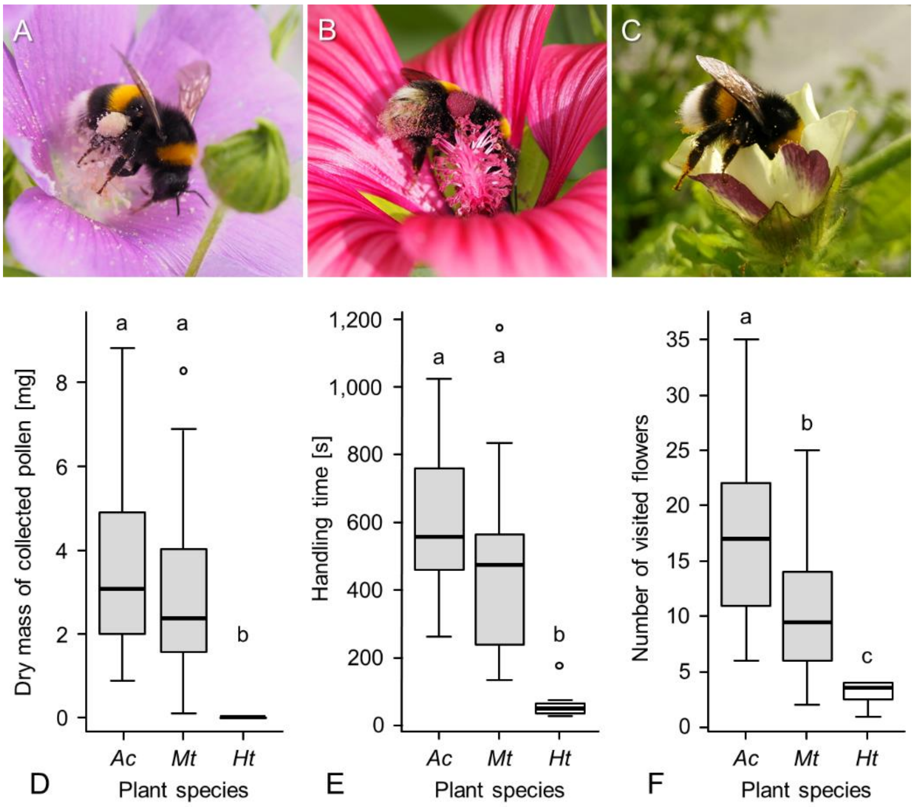

Figure 1. Pollen-foraging behaviour of single *Bombus terrestris* workers on Malvoideae. (A) *Anoda cristata* (*Ac*), (B) *Malope trifida* (*Mt*), and (C) *Hibiscus trionum* (*Ht*). (D) Dry mass of pollen collected in one corbicula, (E) handling time, and (F) number of flowers visited per foraging bout. Plant species are colour-coded for pollen collectability by corbiculate bees: grey = collectable (*Ac*, *Mt*); white = uncollectable (*Ht*). *N* = 26 bees sampled for *Ac*, *N* = 18 for *Mt*, and *N* = 8 for *Ht*. Different letters represent significant differences after Kruskal-Wallis test and Mann-Whitney *U* test with fdr correction (*P* < 0.05).

Accordingly, the handling time for the Malvoideae species with collectable pollen was also significantly higher than the handling time for uncollectable *H. trionum* pollen as the workers quickly ceased trying to pack the uncollectable pollen grains (Fig. 1E; Kruskal-Wallis test: $\chi^2 = 21.99$, $df = 2$, $P < 0.001$).

The number of flowers visited during one foraging bout differed between all three plant species (Fig. 1F; Kruskal-Wallis test: $\chi^2 = 27.14$, $df = 2$, $P < 0.001$). Pollen foraging behaviour of bumble bees on the collectable Malvoideae species *A. cristata* and *M. trifida* was comparable to pollen collection on plants of other families (Appendix I, comparison with data published in Konzmann et al. (2019)). Considering both the previously published data set and our current results, all species with collectable pollen received significantly more flower visits than species with uncollectable pollen (Appendix I; Kruskal-Wallis test: $\chi^2 = 86.06$, $df = 10$, $P < 0.001$). Whereas the dry mass of collected Malvoideae pollen was slightly lower than the fresh mass of other collectable pollen types, the handling time for Malvoideae was significantly higher compared to most other plant species with collectable pollen (Appendix I; Kruskal-Wallis test: $\chi^2 = 101.70$, $df = 10$, $P < 0.001$ and $\chi^2 = 90.81$, $df = 10$, $P < 0.001$).

POLLEN ADHESION

The high-speed rotation of pollen-covered bumble bee thoraces and abdomens revealed that pollen grains of *A. cristata* and *M. trifida*, which are commonly collected by corbiculate bees, detached in a significantly higher number from bee setae than the uncollectable pollen grains of *H. trionum* (Fig. 2A, C-H; Kruskal-Wallis test: $\chi^2 = 19.51$, $df = 2$, $P < 0.001$). Out of the initially applied pollen grains, only 2.55% ± 0.95% (mean ± S.E.) of *A. cristata* pollen and 2.31% ± 0.59% of *M. trifida* pollen, but 45.55% ± 4.37% of *H. trionum* pollen remained on the bees' bodies after the treatment (Fig. 2C-H). The number of pollen grains that were initially brushed onto the bees varied, but only differed significantly between *A. cristata* and *H. trionum* (Fig. 2B; one-way ANOVA: $F = 4.76$, $df = 2$, $P < 0.05$). Data from thoraces and abdomens were pooled for each species after tests, because comparing corresponding data sets yielded no significant differences in the presence of pollen grains between thorax and abdomen (Wilcoxon signed-rank test and Student's *t*-test: $P > 0.05$).

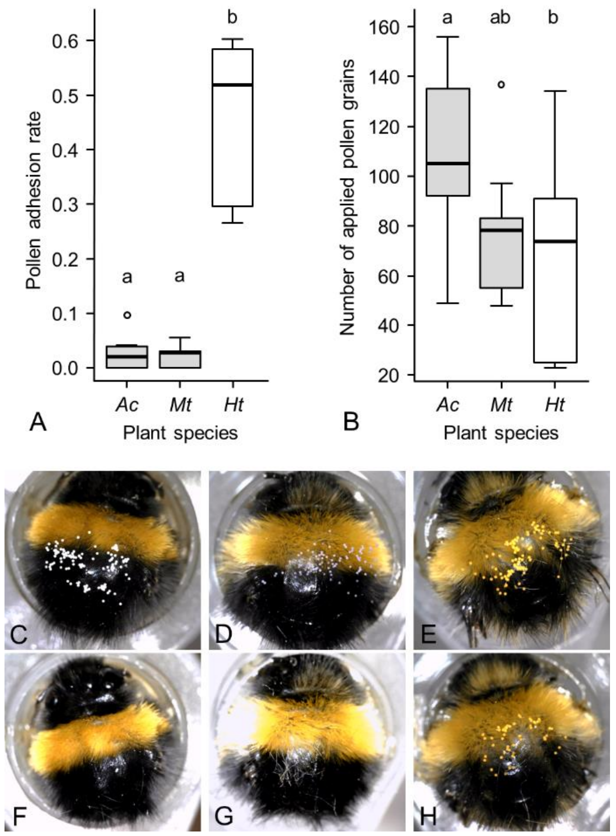

Figure 2. Adhesion of Malvoideae pollen to dorsal bumble bee setae. (A) Proportion of originally applied pollen grains adhering to the bee after 60 s of high-speed rotation at 2,600 rpm. (B) Amount of pollen grains initially applied to the bees' setae before high-speed rotation. Plant species are colour-coded for pollen collectability by corbiculate bees: grey = collectable (*Ac*, *Mt*); white = uncollectable (*Ht*). Different letters represent significant differences ($P < 0.05$) after Kruskal-Wallis test and Mann-Whitney *U* test with fdr correction (A) or ANOVA and Tukey's HSD test (B). Digital photos showing fresh pollen of (C, F) *Anoda cristata* (*Ac*), (D, G) *Malope trifida* (*Mt*), and (E, H) *Hibiscus trionum* (*Ht*) which was manually brushed on a dead, dry bumble bee (*Bombus terrestris*) worker's thorax or abdomen directly from the flower. The pollen grains were counted (C-E) before and (F-H) after high-speed rotation to calculate the pollen adhesion rate (percentage of remaining pollen grains compared to initially placed ones). Five thoraces and five abdomens were sampled for each plant species.

POLLENKITT PROPERTIES

Cryo-SEM micrographs of freshly collected pollen from recently opened flowers demonstrated that pollenkitt was abundant on pollen grains of all examined Malvoideae species (*Al. officinalis*, *A. cristata*, *H. syriacus*, *H. trionum*, *L. trimestris*, *M. trifida*, and *Mv. sylvestris*; Appendix II). The pollen spines were also covered with pollenkitt, albeit with an apparently thinner layer than the rest of the pollen grain surface. Certain differences in pollenkitt coverage could be observed between the plant species in our exemplary samples (Appendix II): uniform, even fluid film in *Al. officinalis*, irregular, even patches in *A. cristata*, even fluid film in *H. syriacus*, uniform, corrugated fluid film in *M. trifida*, uniform, even fluid film in *Mv. sylvestris*, corrugated patches with several smaller cavities in *H. trionum*, and uniform fluid film with numerous larger cavities in *L. trimestris*. A thick layer of pollenkitt was visible on non-treated pollen of both *H. syriacus* and *M. trifida*, forming liquid bridges and filling interspaces between the grains (Fig. 3C, D). After washing with water, pollenkitt of *H. syriacus* appeared swollen and was partly pulled into liquid filaments between single pollen grains, sometimes with 'beads-on-a-string' structures (Fig. 3E). In contrast, the pollenkitt is removed from *M. trifida* pollen after treatment with water (Fig. 3F) and from both *M. trifida* and *H. syriacus* pollen after rinsing with hexane (Fig. 3G, H).

DISCUSSION

This study was initiated by the question of why corbiculate bees are unable to collect Malvoideae pollen. We discovered two Malvoideae species, *Anoda cristata* and *Malope trifida*, whose pollen grains can be collected by corbiculate bees – in contrast to all previously observed and documented Malvoideae species. With a diameter of ~ 84 µm and short spines for *A. cristata* and ~ 80 µm diameter with slightly dimorphic spines for *M. trifida*, the collectable pollen grains are similar to uncollectable pollen of other Malvoideae species (Appendix II; Christensen 1986). Generally, small pollen grains (≤ 34 µm diameter) are easily collectable for corbiculate bees, irrespective of pollen exine structures (Konzmann et al. 2019). In contrast, large pollen grains (≥ 80 µm) require more effort from the bees, as evidenced by the significantly longer handling time for pollen of *A. cristata* and *M. trifida* or the smaller quantity of *Cucurbita pepo* pollen (~ 139 µm diameter) collected (Konzmann et al. 2019).

As there is no clear morphological distinction between collectable and uncollectable pollen of Malvoideae, what differentiates them? Starting from the observation that pollen of some Malvoideae seems to be too sticky for bees to groom and remove pollen properly, we tested pollen adhesion and found that attachment to bee setae differs between collectable and uncollectable pollen in this subfamily. The experimental setup was restricted as only dead bees could be tested and electrostatic charges could not be taken into account. Under natural conditions, the differential charge between the grounded flower and the bee

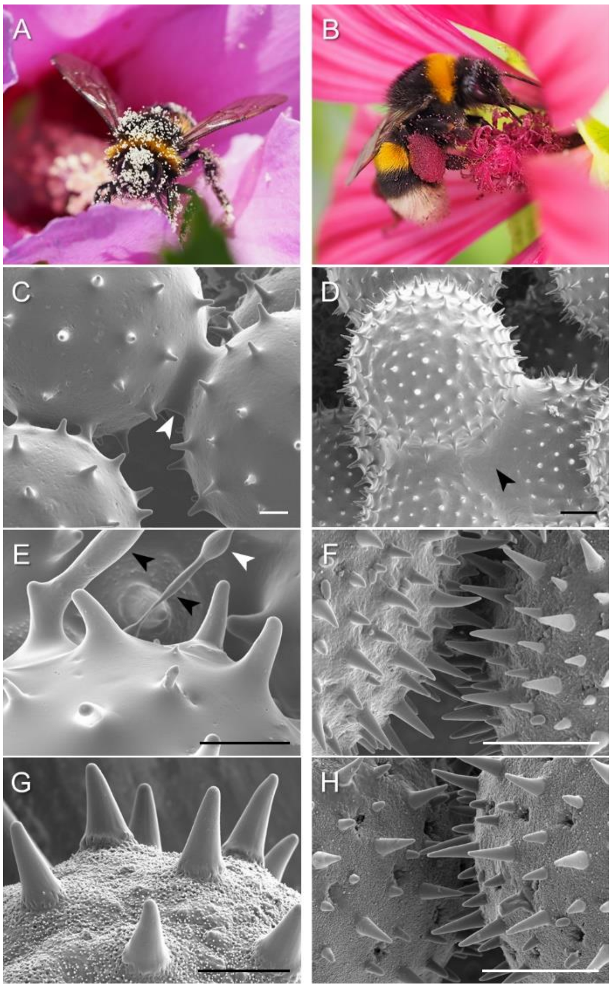

Figure 3. Cryo-SEM micrographs of pollen from two Malvoideae species. (A) *Hibiscus syriacus* pollen is not collected by corbiculate bees while (B) *Malope trifida* pollen can be collected by corbiculate bees. Freshly sampled pollen of both species (C, D) was washed with water (E, F) or hexane (G, H). A thick layer of pollenkitt is visible on fresh pollen of both species and forms wide bridges (arrow) (C) or fills interspaces (arrow) (D) between single pollen grains. After rinsing with water, pollenkitt of *H. syriacus* appears swollen and is pulled into irregular filaments between single pollen grains (black arrows), partly with 'beads-on-a-string' structures (white arrow) (E). In contrast, pollenkitt is removed from M. trifida pollen after treatment with water (F) and from both *H. syriacus* and *M. trifida* pollen after treatment with hexane (G, H). All scale bars indicate 20 µm.

that is positively charged as it flies toward the flower might play an important role in pollen adhesion to bees (Vaknin et al. 2000). The adhesion of pollen grains to flower visitors and subsequently to the floral stigma is mediated by a multitude of factors which include pollenkitt, echinate pollen surface structures (e.g., number of spines per unit area), (distance between) bee setae, electrical charge, and stigmatic papillae (Shoemaker 1911; Vansell 1944; Berger et al. 1988; Vaknin et al. 2000; Pacini & Hesse 2005; Hesse & Ulrich 2012; Lin et al. 2013; Amador et al. 2017; Ito & Gorb 2019a; Lynn et al. 2020). So far, pollen adhesion has mainly been studied regarding pollen attachment to artificial substrates, flower structures, and flower visitors but not concerning its impact on pollen collection, specifically compaction for storage in the corbiculae (Lin et al. 2013, 2015; Amador et al. 2017; Ito & Gorb 2019a,b). The Malvoideae provide a first indication that although pollen adhesion facilitates pollen uptake by bees, extremely strong adhesion may impede grooming and thus even prevent pollen collection by corbiculate bees.

Pollenkitt has long been suspected to be responsible for the lack of collectability of Malvoideae pollen (Vansell 1944; Lunau et al. 2015. Perhaps it also causes variable adhesion of pollen grains to bee setae? Pollen adhesion is influenced by a variety of factors that include the water content of pollenkitt, relative humidity, and surface properties (Lin et al. 2015; Ito & Gorb 2019b). Pollenkitt is moderately hygroscopic and generally enhances adhesion. However, it can also reduce pollen adhesion under certain conditions, e.g., on hydrophobic surfaces and upon ageing (Ito & Gorb 2019b; Prisle et al 2019). Furthermore, the volume of pollenkitt per pollen grain is plant species-specific and higher in insect-pollinated species than wind-pollinated ones (Pacini & Hesse 2005; Lin et al. 2013).

Our cryo-SEM study turned out differences in pollenkitt coverage between collectable and uncollectable Malvoideae pollen grains (Fig. 3, Appendix II). In the latter, they mostly appeared less fluid, patchy, and irregular, pulled into thin or bulky filaments between the grains, implying distinct viscosity. In comparison, uniform, even, spreading fluid films were observed on collectable pollen. Similar differences were previously observed and experimentally confirmed via pull-off force tests, e.g., for the sticky secretion released by different glandular trichomes of the carnivorous flypaper plant *Roridula gorgonias*. In contrast to the strongly adhering bulky solid secretion, the more fluid secretion adhered less (Voigt et al. 2009). Small cavities in the imaged pollenkitt (Appendix IID, E) might indicate water content evaporated during the cryo-SEM sample preparation and examination procedure by applied vacuum and high voltage (Voigt et al. 2012). After treatment with water, the hygroscopic pollenkitt of uncollectable *H. syriacus* pollen swelled and established thinning filaments with 'beads-on-a-string' structures. Such beads on a string have also been reported from pulled sticky prey-capturing water-based sugary-proteinaceous fluids, e.g., of *Drosera* plants, spider webs, or onychophoran slime (Erni et al. 2011; Mayer et al. 2015; Opell et al. 2018). The formation of 'beads on a string' and thinning filaments are a particularity of viscoelastic inertial fluids (Bhat et al. 2010). The viscous *H. syriacus* pollenkitt could be washed off with less polar hexane. In comparison, pollenkitt of collectable pollen of *M. trifida* appeared less viscous and was soluble in polar water. This fact reflects the higher polarity of less viscous pollenkitt of collectable pollen, which facilitates the miscibility with aqueous nectar that is regurgitated and used by the bees to bond the pollen mass in their corbiculae (Appendix II; Hesse 1980; Nicolson 2011). The lower viscosity and higher water solubility probably render pollenkitt of collectable pollen less adhesive and easier to handle for bees. Unfortunately, comparative data on the chemical composition of pollenkitt is scarce. In Malvaceae as well as in other plant families, neutral lipids are usually abundant in essential oils in the pollenkitt, while polar lipids are mostly found as membrane components inside the pollen grain (Dobson 1988). Our results let us speculate that the adhesive quality of differently composed pollenkitt modulates pollen collectability, which is probably influenced by distinct proportions of polar and neutral lipids.

As the primary purpose of pollenkitt is assumed to be the adherence of pollen grains to insect setae and the stigmatic surface, there is no indication that pollenkitt should facilitate grooming and pollen packing in corbiculate bees (Hesse 1980; Pacini & Hesse 2005). In fact, the interaction of pollenkitt and regurgitated nectar used by corbiculate bees might be a key factor influencing pollen grooming and compacting (Thorp 2000). In honey bees leaving the hive, the quality and quantity of nectar in the bees' crop are significantly higher in pollen foragers than in nectar foragers, which suggests that nectar quality and quantity may be decisive for pollen collection (Harano & Nakamura 2016). Furthermore, pollen-foraging bumble bees drink nectar during their foraging bout – probably not only to cover their energy requirements, but also to facilitate pollen collection (Konzmann & Lunau 2014; Konzmann et al. 2019). Nectar-collecting corbiculate bees are arguably better pollinators for many plants than pollen foragers which – by compacting pollen in their corbiculae – drastically diminish pollen viability and availability for stigmatic pollen deposition (Parker et al. 2015). In contrast, when non-corbiculate bees visit Malvoideae flowers, pollen foragers remove and deposit more pollen than nectar foragers (Tepedino et al. 2016). Thus, the floral filter of Malvoideae pollen only affects corbiculate pollen foragers and prevents pollen loss to less suitable pollinators (Gorenflo et al. 2017). However, the properties and effects of nectar-pollenkitt blends deserve further attention and might clarify the lack of collectability in Malvoideae pollen.

We detected a discrepancy in pollen forager visitation of Malvoideae species with collectable pollen. During our observations of foraging behaviour, neither bumble bees nor honey bees collected pollen on flowers of *A. cristata*, and only honey bees collected pollen of *M. trifida*. When plants for the collectability test were temporarily placed outside the flight cage, *A. cristata* received frequent visits by pollen-collecting honey bees and nectar-foraging bumble bees, but not by pollen-foraging bumble bees. These discrepancies might be explained by situation-dependent foraging decisions influenced by the abundance of a food plant in a specific patch, effort needed for resource collection, availability of alternative food plants, and (avoidance of) inter- and intra-species competition.

In conclusion, pollen of *A. cristata* and *M. trifida* differ from other studied Malvoideae pollen in three respects: collectability by corbiculate bees, adhesion rate to bumble bee setae, and water solubility of pollenkitt. Collectable pollen grains of Malvoideae adhere to bees in smaller numbers and their pollenkitt is water-soluble. These aspects do not all have to be causally linked to each other. However, we presume that they are related, i.e., that pollenkitt properties are responsible for excessive pollen adhesion which in turn prevents collection by corbiculate bees. This assumption is supported by previous findings that experimentally removing pollenkitt of uncollectable pollen renders it collectable and that bees are inherently capable of handling even large, spiny pollen grains (Lunau et al. 2015; Konzmann et al. 2019). Pollenkitt composition and consistency seem to be the key features of the floral filter of Malvoideae pollen, enhancing pollen uptake by bees while simultaneously impeding grooming and preventing the eventual compaction of pollen grains in the corbiculae. Whether pollen is collectable or uncollectable affects both the plant and its pollinators. While pollen-foraging bumble bees avoid flowers with uncollectable pollen, nectar foragers are not deterred from visiting these flowers (Konzmann et al. 2019). Flower visitors that collect only nectar might be more efficient pollinators than flower visitors that actively collect pollen or both pollen and nectar. In this respect, the lack of collectability in Malvoideae pollen – in combination with the presentation of a nectar reward – can be regarded as a floral filter against a subset of the pollinator species which, by collecting pollen, reduces the amount of pollen available for pollination.

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DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Original data of all experiments is provided in the Appendices, see below.

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I. Comparison of present and previously published data on pollen collection by single *Bombus terrestris* workers.

Appendix II. Cryo-SEM of freshly collected Malvoideae pollen and a *Malope trifida* pollen pellet in the corbicula of an *Apis mellifera* worker.

Appendix III. Original data of the pollen collectability assays (results presented in Fig. 1 and Appendix I).

Appendix VI. Original data of the high-speed rotation test (results presented in Fig. 2).

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