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ORIGINAL PAPER

Pollen packing affects the function of pollen on corbiculate bees but not non-corbiculate bees

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Abstract Female bees store scattered pollens grains from their bodies for transport by different modes of grooming and pollen packing. Species with corbiculae, such as honey or bumble bees, compress grains into dense pellets borne on the hind tibiae. Other species sweep grains into local concentrations of hairs (scopae), typically around the legs (in Halictidae and Andrenidae) or the ventral abdomen (Megachilidae), in which grains remain loose. Do these modes of pollen packing affect the functional value of pollen? We transferred grains from the bodies of four groups of bees—the corbiculate bees: *Bombus impatiens* and *Apis mellifera*, and the non-corbiculate bees: *Megachile rotundata* and *Halictus* spp.—onto previously unvisited stigmas of *Brassica rapa*. We wiped corbicular or scopal pollen and body pollen from each bee's body separately and measured the resulting fruit set and the number of seeds in successful fruits. The type of pollen significantly affected the number of fruits for the corbiculate bee species but not the non-corbiculate bees, and the type of pollen significantly affected the number of seeds in successful fruits for *A. mellifera* but not *B. impatiens*, *M. rotundata*, or *Halictus* spp. These results suggest that loose scopal pollen is fully functional, but corbicular pollen is sometimes impaired. In some situations, non-corbiculate bees may be more valuable pollinators than corbiculate species because their treatment of pollen leaves its capabilities intact.

Keywords Plant–pollinator interactions · Corbiculate bees · Pollen viability · Bee grooming

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Introduction

During the process of reproduction in animal-pollinated plants, plant male gametophytes (pollen grains) are dispersed and delivered to female gametophytes (ovules), where germination, pollen tube growth, and fertilization occur. Animal pollinators are responsible for dispersal and delivery, and interactions between pollinators and pollen during dispersal and delivery may affect the condition of pollen grains and ultimately impact fertilization success. The most effective and important group of pollinators, bees, also collect pollen to feed their larvae; therefore, after acquiring pollen grains at pollen-donating flowers, bees interact with those pollen grains by gathering loose pollen from their bodies ("grooming") and concentrating it into pollen transport structures ("pollen packing") (Thorp 2000). Because bees handle and manipulate pollen grains during grooming and packing, they may affect pollen function (Thorp 1979; Michener 2000). To better understand the role of bees as pollinators, it is important to understand how bees modify pollen, and how interactions between bees and pollen grains may ultimately affect plant reproductive success.

Bees vary in their grooming and pollen packing behavior during pollen dispersal and delivery (Thorp 2000). Corbiculate bees are so named for their pollen transport structure, the corbicula or "pollen basket", which

is a concave plate with long, curved hairs on the hind tibia that securely contains pollen in a nectar-moistened pellet (Michener 1979). These bees compose part of the family Apidae, including the tribes Apini (honey bees), Bombini (bumble bees), Euglossine (orchid bees), and Meliponini (stingless bees). Non-corbiculate bees—the vast majority of bee species—transport pollen using a scopa, or "brush", a dense group of elongated hairs (Thorp 1979). These non-corbiculate bees make up the remaining six families of bees and the remaining genera in the family Apidae. Here, we refer to bees within the corbiculate Apidae as "corbiculate", as they are widely known (Michener 1999). We refer to other bees not in the corbiculate Apidae as "non-corbiculate" bees. Some "non-corbiculate" bees may in fact have a small corbiculae, but the primary mode of pollen transportation is scopal hairs (Michener 1999). Although much of the available information is anecdotal and focused on one or a few bee groups, Thomson and Plowright (1980) state that non-corbiculate bees are generally "messier" and hold pollen more loosely, and Thorp (1979) generalizes that corbiculate bees perform more systematic and thorough grooming behaviors and manipulate pollen more than non-corbiculate bees. Moreover, corbiculate bees moisten pollen with nectar during grooming, while most non-corbiculate bees transport pollen dry (Thorp 1979). Added moisture clumps pollen, making it less likely to flake off during future flower visits. These differences in grooming and packing behavior may affect pollen performance.

Pollen grains on stigmas vary in their ability to germinate, grow pollen tubes to the ovary and fertilize ovules, thereby influencing seed siring and production. Pollen varies in its viability, or the capacity of the pollen grain to fertilize plant ovules when conditions are ideal. Pollen also varies in its propensity to adhere to stigmas; for example, pollination may be facilitated by electrostatic forces attracting the pollen to the stigma (Vaknin et al. 2000), or it may be deterred by nectar adhering the grains to the bee body and to one another, forming clumps (Thorp 1979). Researchers measure components of pollination and fertilization through a variety of methods, including in vitro methods, which measure pollen germination in a medium such as water, agar, or gelatin (Stanley and Linskens 1974), and in vivo methods, which look at the siring ability of pollen grains by measuring pollen tube growth or seed set.

There is evidence that contact with bee bodies and the grooming and packing of pollen grains affect pollen function. Vaissière et al. (1996) found that pollen on pollen-foraging *Apis mellifera* was less viable than pollen on nectar-foraging *A. mellifera*, indicating that some aspect of bee manipulation of pollen can be important for pollen quality. One study found pollen on *A. mellifera* bodies to be inviable after 12 h (Kraai 1962), and another found that pollen from bee bodies exhibited decreased viability in 1 year and no difference in viability in another (Kendall 1973). Corbicular pollen from *A. mellifera* is less viable both in vitro (Mesquida and Renard 1989) and in vivo (Alspach et al. 1992). However, Kraai (1962) did not control for decreasing pollen viability over time, and both of these studies did not distinguish between pollen on the body and pollen in the corbiculae.

However, there is no evidence that contact with bee bodies and the grooming and packing of pollen grains will affect the function of pollen on non-corbiculate species. Thomson et al. (2000) and Thorp (2000) state that corbicular pollen is not available for pollination, but that scopal pollen may be; however, the performance of these groups of pollen has not been empirically compared. Apple pollen from insect bodies was as viable as pollen from unvisited flowers, in 12 out of 14 species of bees and flies (Kendall 1973). Body-borne pollen declined in viability only for males of the bee *Andrena wilkella* and the syrphid fly *Rhingia campestris*. However, Kendall did not distinguish between pollen grains on the bee body and pollen grains in the corbicula or scopa.

To better understand the effect of bee grooming on pollen function, we conducted siring tests in vivo for corbicular/scopal and body pollen separately from corbiculate and non-corbiculate bee species. Based on previous literature, we predicted that pollen on corbiculate bees would differ in performance depending on where the pollen was located; we predicted reduced function of corbiculate pollen compared to body pollen. In contrast, we predicted that there would be no difference in performance between scopal and body pollen on non-corbiculate bees.

Materials and methods

We conducted this study in two parts; the first in the spring and summer of 2012 in the greenhouse at the University of Toronto using commercially reared *Bombus impatiens*, and the second in the summer of 2012 at the University of Toronto's Koffler Scientific Reserve in King City, ON (44.01.48 N, 79.32.01 W) using the locally occurring pollinators *Apis mellifera*, *Megachile rotundata*, and two species in the genera *Halictus*, *Halictus ligatus* and *Halictus confuses*; *A. mellifera* and *M. rotundata* are non-native, and these bees may have been either feral or managed on nearby farms. *B. impatiens* was studied separately because these bees were not abundant in the field and the use of commercial colonies indoors facilitated data collection. The bees *B. impatiens* and *A. mellifera* are corbiculate. The scopa of *M. rotundata* is located on the underside of the abdomen, while the scopa of the *Halictus* spp. is located on the tibia and femur of the hind legs in approximately the same location as the corbicula of *A. mellifera* and *B.*

impatiens. Both parts of the study examined pollination of the self-incompatible annual herb *Brassica rapa* L. (Brassicaceae). Naturalized populations of *B. rapa* are visited by a diverse group of insects, including but not limited to corbiculate and non-corbiculate bees, and have many ovules per flower. We grew our plants from seed harvested from populations of *B. rapa* in Quebec, Canada.

We collected foraging pollinators and wiped their bodies onto virgin stigmas of *Brassica rapa*, transferring pollen directly from the bee body onto the stigma using a method similar to an experiment by Kendall (1973). To determine the effect of pollen storage manipulations by bees on collected pollen, we wiped pollen from an individual bee's corbicula or scopa on one flower and free body pollen on another flower. We captured each bee directly into a clean vial and then placed the insect into a cooler for 10 min to slow its movement. Then, we grasped the hind leg of a bee with forceps and brought the bee into controlled contact with the stigma, ensuring that only the area of interest on the bee body made contact. We wiped the entire surface of the area of interest twice to transfer as much pollen as possible from the bee to the stigma. We returned the bee to the vial and froze it for future identification, then labeled each flower, collected the fruit when it was mature but undehisced, and counted the resulting seeds. In order to reduce our manipulation of the plants, we did not count the number of pollen grains that were transferred in each wipe, but in most cases, we were able to see that the number of pollen grains transferred greatly exceeded the number of ovules. One exception was free body pollen on *A. mellifera*, where pollen grains were sometimes not abundant enough to be visible. When corbicular or scopal pollen did not appear to transfer because of clumping, we removed a chunk of pollen from the corbicula or scopa and hand pollinated the recipient stigma. For all bee species, there were noticeably more corbicular or scopal pollen than body pollen in each wipe or hand pollination.

Greenhouse experiment

The purpose of the greenhouse experiment was to determine the functional value of corbicular and body pollen in commercially managed *B. impatiens*; this experiment was conducted in the greenhouse to increase the amount of samples that we could collect and to prevent possible pathogen spillover from our managed colonies to naturally occurring *Bombus* colonies at Koffler Scientific Reserve. In this experiment, we used three treatments: flowers wiped with corbicular pollen, body pollen, and a mix of the two. We included a mixture treatment in order to determine whether scopal/corbicular pollen would reduce the functionality of pollen that it contacts. We grew a population of *B. rapa* in the greenhouse with a 12–12 h day-night cycle; this population numbered 200–300 and was used only as a source of pollen. To use as recipient plants, we also grew plants from a line of male-sterile *B. rapa* plants previously developed through a series of crosses to integrate the autosomal loci for rapid cycling and male sterility from the Wisconsin Fast Plants® lineage into the Quebec naturalized genetic background (J. Ison and A. Weis, unpublished results); the use of male-sterile *B. rapa* plants as pollen recipients prevented contamination by self-pollen and incidental pollen transfer by contact among plants on the greenhouse bench. Recipient plants were kept in pollinator exclusion structures before and after pollinator wiping to ensure that all open flowers used for pollinations were previously unvisited. Pollinator exclusion structures were made of wood with wire mesh and measured approximately 2 m × 3 m × 2 m. In this experiment, we used captive-reared colonies of *Bombus impatiens*, a common local native, obtained from Biobest Canada Ltd. (Leamington, Ontario, Canada). We released 3–5 individuals from a colony of *B. impatiens* allowing them to forage until a mass of pollen formed in the corbicula; this occurred quickly, but each bee used in the study foraged for at least 2 min and visited at least two flowers. To ensure that enough pollen remained free on the body for the body pollen treatment, we restricted the individual's leg movement in the vial by using a smaller vial to gently "squish" the bee against the bottom. We used each bee for only one wipe.

Field experiment

The purpose of this experiment was to determine the functional value of corbicular or scopal pollen and body pollen for commonly occurring visitors of *B. rapa*. We conducted this experiment in the field because these pollinators are abundant in the field and difficult to work with in the greenhouse. In this experiment, we wiped corbicular/scopal pollen from an individual bee on one flower and body pollen from that same bee on another flower of the same plant (i.e., the data for the field experiment are paired), and we did not include a third treatment with a mix of corbicular/scopal and body pollen. We grew a population of over 1,000 plants in an open area at the Koffler Scientific Reserve. These were freely visited by the locally occurring *Apis mellifera*, *Megachile rotundata*, and *Halictus* spp. as well as other insects. When male-sterile recipient plants were unavailable for part of the experiment, we used emasculated hermaphrodites; these plants were kept in a pollinator exclusion structure with the male-sterile recipient plants. We made sure that bees had visited at least two of our population plants before we collected them for wiping.

Data analysis

All statistical analyses were done using R v3.0.1 (R Core Team 2013). We compared numbers of seeds per fruit produced by corbicular/scopal and body pollen on the

different bee taxa using generalized linear mixed models (GLMMs) using the R function *glmmADMB* (Fournier et al. 2012). We conducted separate analyses for the field experiment and the greenhouse experiment, because the experiments were conducted independently; moreover, the field experiment was paired, while the greenhouse experiment was not.

For the field experiment, we conducted two analyses that included bee type (corbiculate and non-corbiculate), bee group (genus or species: *A. mellifera*, *M. rotundata*, or *Halictus* spp.), and the type of pollen (corbiculate/scopal or loose body pollen) as well as the interaction between bee type and the type of pollen. We conducted this analysis for two response variables: the number of fruits set and the number of seeds per fruit in successful fruits. For the number of fruits set, we used a binary distribution with a logit link function. For the number of seeds per fruit in successful fruits, we used a negative binomial distribution with an ln link function. In each, we accounted for the paired design by including the bee individual as a random effect (Hall 2004; Min and Agresti 2005). To determine whether the type of recipient plant (whether it was a male-sterile or a wild-type emasculated hermaphrodite) had an effect on the results, we also included the plant type as a fixed effect. In addition, we conducted the analysis including only the male-sterile plants and obtained qualitatively similar results. Within the genus *Halictus*, we included both *Halictus ligatus* ($n = 30$) and *Halictus confusus* ($n = 5$), but the same analysis including only *Halictus ligatus* individuals resulted in qualitatively similar results.

For the greenhouse experiment, we conducted two analyses that included only the type of pollen (corbiculate/scopal or loose body pollen) because only one bee species was used. We conducted this analysis for two response variables: the number of fruits set and the number of seeds per fruit in successful fruits. For the number of fruits set, we used a binary distribution with a logit link function. For the number of seeds per fruit in successful fruits, we used a negative binomial distribution with an ln link function. Multiple comparisons were made using Tukey-corrected Wald Z tests. We included the date as a random effect because environmental factors inside the greenhouse were highly variable over time. We did not include the type of recipient plant (whether it was a male-sterile or a wild-type emasculated hermaphrodite) as a fixed effect because all plants used in this experiment were male sterile.

Results

Greenhouse experiment

Corbicular pollen on *B. impatiens* resulted in fewer *B. rapa* fruits than did body pollen on *B. impatiens* (Fig. 1a). About half (53 %) of pollinations with corbicular pollen resulted in fruits, while the majority (84 %) of pollinations with body pollen resulted in fruits. Ninety-two percentage of pollinations with mixed corbicular and body pollen resulted in fruits. There is a significant difference in fruit set between corbicular and body pollen (Fig. 1a, $df = 85$,

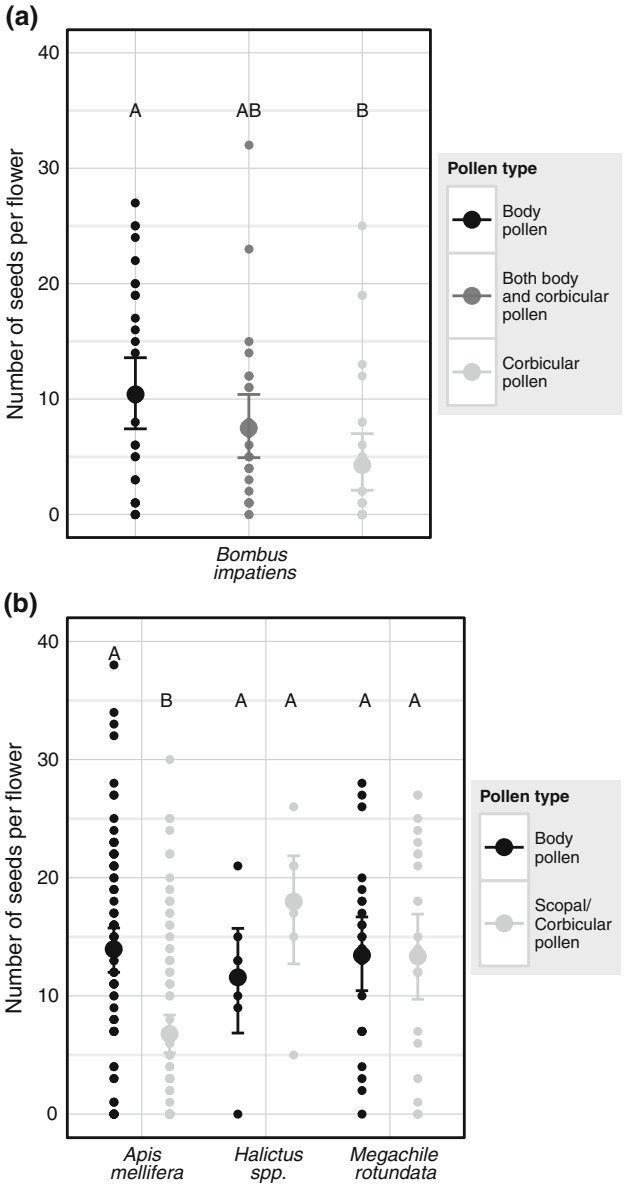

Fig. 1 The number of seeds set by a *B. rapa* flower following a wipe of pollen from bee bodies. *Points* are data points, *larger points* are means, and *error bars* are 95 % confidence intervals. Within **a** and **b**, treatments sharing a letter are not significantly different at $P < 0.05$. **a** The number of seeds set resulting from corbicular or scopal pollen ($n = 30$), a combination of corbicular and body pollen ($n = 25$), and body pollen ($n = 31$) on the corbiculate bee *B. impatiens*. **b** The number of seeds set resulting from scopal pollen and body pollen on the corbiculate bee *A. mellifera* (corbicular $n = 87$, body $n = 87$), the non-corbiculate bee *Halictus* spp. (scopal $n = 35$, body $n = 35$), and the non-corbiculate bee *M. rotundata* (scopal $n = 25$, body $n = 25$)

$Z = 2.48$, $P = 0.03$) but not body pollen and mixed pollen (Fig. 1a, $df = 85$, $Z = 0.90$, $P = 0.238$).

There were fewer seeds in successful fruits in pollinations by *B. impatiens* corbicular pollen than *B. impatiens* body pollen—with means of 8.00 and 12.42 seeds, respectively—but the difference was not significant (Fig. 1a, $df = 64$, $Z = 1.59$, $P = 0.112$).

Field experiment

In our model, there was a significant interaction between the bee type (corbiculate and non-corbiculate) and the type of pollen (corbiculate/scopal or loose body pollen) for the response variable of whether the flower set fruit (Fig. 1b, $df = 290$, $P = 0.01$). When pollinated by pollen from *A. mellifera* bodies, 87 % of flowers set fruit compared to 60 % from *A. mellifera* corbiculae. When pollinated by pollen from *M. rotundata* bodies, 96 % of flowers set fruit compared to 84 % from *M. rotundata* scopae. When pollinated by pollen from *Halictus* spp. bodies, 97 % of flowers set fruit compared to 100 % from *Halictus* spp. scopae.

There was also a significant interaction between the bee type (corbiculate and non-corbiculate) and the type of pollen (corbiculate/scopal or loose body pollen) for the response variable of the number of seeds per successful fruit (Fig. 1b, $df = 237$, $P < 0.001$). The mean number of seeds per successful fruit was 15.97 for pollinations by *A. mellifera* body pollen compared to 11.34 for pollinations by *A. mellifera* corbicular pollen. The mean number of seeds per successful fruit was 14.00 for pollinations by *M. rotundata* body pollen compared to 15.90 for pollinations by *M. rotundata* corbicular pollen. The mean number of seeds per successful fruit was 16.12 for pollinations by *Halictus* spp. body pollen compared to 17.06 for pollinations by *Halictus* spp. corbicular pollen. All analyses included both male-sterile and hermaphrodite plants; when we conducted analyses using only male-sterile plants, we obtained qualitatively similar results.

Discussion

There is a difference in fruit set between corbicular and body pollen for only corbiculate bees *A. mellifera* and *B. impatiens*, but not the non-corbiculate bees *M. rotundata* and *Halictus* spp. There is also a difference in the number of seeds per successful fruit between corbicular and body pollen for the corbiculate bee *A. mellifera*, but not *B. impatiens* or the non-corbiculate bees *M. rotundata* and *Halictus* spp. This result is evidence that differences in bee pollen packing behavior may be important in determining the functional value of pollen in pollen storage structures. The two corbiculate bees in this study moisten pollen during pollen packing, while the two non-corbiculate bees do not (along with most other non-corbiculate bees); therefore, pollen moistening is a likely mechanism for the observed difference in fruit set and potentially also the observed difference in the number of seeds per successful fruit. Moistening may cause physiological changes in the pollen grain, or it may increase pollen clumping, reducing adherence to stigmas, or both. The mixing of pollen and nectar may result in osmotic effects that reduce pollen viability (Vaissière et al. 1996); salinity has been shown to affect pollen viability in *Brassica* (Tyagi and Rangaswamy 1993). Other authors have noted that scopal pollen is generally held more loosely than corbicular pollen (Thorp 1979; Thomson et al. 2000). Tight packing may result in fewer pollen grains being transferred or fewer corbicular pollen grains adhering to stigmas.

In interpreting our results, we assumed that enough pollen grains were transferred to ensure that the number of pollen grains transferred did not affect fruit set or the number of seeds per fruit. We usually observed that enough pollen grains were transferred to greatly exceed the number of ovules of the recipient flowers. One exception was free body pollen on the *A. mellifera* body, for which we were sometimes unable to see pollen on the stigma; however, despite the low pollen load, this treatment showed high seed set (Fig. 1). When corbicular or scopal pollen on any bee species did not readily transfer, we supplemented our bee wipes by removing a chunk of pollen from the corbicula or scopa and hand pollinating the recipient stigma. We also assumed that pollen loads on the bees had enough *B. rapa* pollen to ensure adequate pollination and that the proportion of *B. rapa* pollen in corbicular/scopal and body pollen are similar in the bee species. In order to ensure that bee pollen loads included *B. rapa* pollen, we collected pollinators only from *B. rapa* flowers and only after we observed at least two visits to *B. rapa* before we caught them. However, it is possible that variation in the number of grains transferred and the amount of *B. rapa* pollen in pollen loads of the bees did affect the number of fruits and the number of seeds set; a supplemental study with more stringent controls on these factors would clarify this issue.

The use of the bee wipe technique does not simulate a pollinator visit, as it removes variability in the behavior of bees on flowers; this limits our interpretation of the results. By transferring pollen grains through bee wipes and hand pollinations, we transferred many more pollen grains than would likely be transferred in nature. Therefore, this method provides an estimate of the maximum success of pollen in each treatment. The relevancy of these results depends on the extent to which bee corbiculae or scopae contact floral stigmas during pollinator foraging. If bee corbiculae and scopae never contact stigmas during foraging, then the differences between bee groups that we

document will not be important for plant reproductive success. Very little research specifically reports contact between corbiculae or scopae and plant stigmas (but see Bosch 1992), and the extent of contact will be specific to the plants and pollinators observed. Pollen is usually not distributed uniformly on bee bodies (Beattie 1971; Bosch 1992; Vallejo-Marín et al. 2009). For example, pollen may be concentrated on "safe" areas of the bee body that are inefficiently groomed and therefore do not end up in pollen storage structures (Beattie 1971; Harder and Wilson 1998) or strategically placed on areas that are likely to contact conspecific stigmas, as demonstrated for mirror-image flowers and heteranthery (Darwin 1864; Jesson and Barrett 2005; Vallejo-Marín et al. 2009). During this study, we observed stigmatic contact of the corbiculae of *A. mellifera* and *B. impatiens*, as well as the scopae of *M. rotundata* and *Halictus* spp., but we did not quantify the frequency of such contacts. Similarly, using the bee wipe technique included pollen grains in pollination that may not ordinarily have been included; for example, pollen on the dorsal side of the bee was wiped on to stigmas, and in nature, these pollen grains may be less likely to contact the stigma than ventral grains (Vallejo-Marín et al. 2009).

How do these differences between bee groups affect these floral visitors' values as pollinators? Our results support the conclusion that scopal *B. rapa* pollen is fully intact on non-corbiculate bees, but that *B. rapa* corbicular pollen on corbiculate bees may often be impaired. While further research is needed to determine whether this effect is specific to *B. rapa* or generalizable to other systems, this difference in functional value may be important for plant reproductive success in natural and agricultural settings. Bees' interests are served when as much pollen as possible is groomed into the corbicula or scopa pollen for transportation back to the nest; for corbiculate bees, pollen packing may decrease pollination success for the plants that they visit. In addition, there may be situations in which there are not enough pollen grains free on corbiculate bee bodies to accomplish sufficient pollination. In these situations, non-corbiculate bees may accomplish a greater portion of the pollination service and be considered more valuable than corbiculate bees. The loss of function of corbicular pollen in *A. mellifera* may be a reason to maintain diversity of pollinators beyond *A. mellifera*, especially on agricultural crops (Westerkamp 1991).

By conducting tests in vivo on scopal/corbicular and free body pollen, we were able to isolate the effect of pollen packing by corbiculate and non-corbiculate bees on pollen's ability to fertilize ovules, as measured through the number of seeds produced. This is the first study to examine both corbicular and scopal pollen in this way. Our results indicate that non-corbiculate bees may be particularly valuable to plant pollination in some situations.

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