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Consequences of pollinator availability and effectiveness for pollen transfer in a gynodioecious seed crop system

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Abstract—Pollinator availability and effectiveness are important considerations in plant systems that depend on insect transfer of pollen. In mixed-flower systems (such as dioecious or gynodioecious systems), pollinators may distinguish between morphs, a behaviour which could have negative implications if it leads to reduced cross-pollination. We examined pollinator visitation, response, and effectiveness in hybrid canola seed production, a gynodioecious crop, to understand how pollinator behaviour contributes to cross-pollination between 'male' (hermaphroditic) and 'female' (male-sterile) morphs. The pollinator types found in these fields differ in visitation rate, size, and pollen load, factors that may affect pollen transfer between plants. The willingness of pollinators to move between male and female flowers can also affect pollen deposition. We used an interview bouquet to present unvisited female inflorescences to different pollinator types visiting male and female flowers to understand behavioural responses to floral morphs and the factors that contribute to better pollination effectiveness. Managed pollinators (honey and leafcutter bees) were far more abundant than wild pollinators in this system. Bees foraging on male flowers were less likely to accept a proffered female flower than those foraging on female flowers. Pollen deposition on stigmas was influenced by pollinator type (female leafcutter bees were the most effective) and increased with time spent on a flower. Pollinators transferring from a male flower to a female flower deposited more pollen than those that originated on female flowers, emphasizing the importance of transfer between male and female morphs. Surprisingly, pollinators carrying more pollen transferred less pollen to recipient stigmas.

Keywords—*Apis mellifera*, *Brassica napus*, hybrid seed canola, oilseed rape, *Megachile rotundata*, pollination effectiveness

Introduction

Plant seed production is affected not only by the resources available to the plant to produce seeds but also by the pollen supplied for fertilization (Haig & Westoby 1987; Burd 1994). Given that pollen availability can affect seed production, it is crucial to understand how pollinators deliver pollen from anthers to stigmas in animal-pollinated plants. The influence of animal pollinators on pollen transfer can be divided into two components: pollinator availability and effectiveness. Pollinator availability is a limiting factor for many plants, especially those species which are obligate outcrossers. The effectiveness of a pollinator can be defined as its ability to deposit pollen (Ne'eman et al. 2010); specifically, the number of pollen grains transferred to the female reproductive organ (the stigma) by a pollinator in a single floral visit. Effectiveness can be determined by both behavioural and morphological characteristics. To understand the influence of pollinators, therefore, we need to evaluate various attributes in the

availability and effectiveness of these animals that will impact the quantity of pollen available for a plant.

First, pollinator availability is a precondition for pollination effectiveness – even the most effective pollinator will not be important if it is uncommon. Indeed, less efficient pollinators of wild radish may be its most important pollinators due to their relatively high visitation frequency (Sahli & Conner 2007). In agricultural systems, pollinator availability depends in part on how a crop is managed, as crops that require some level of insect visitation often use managed pollinators to ensure pollination. Wild pollinators, including bees and flies, may also contribute, although their presence is more uncertain and may be affected by landscape factors (Kremen et al. 2002) or competition with managed pollinators (Lindström et al. 2016). Large fields of mass-flowering crops, which may be more typical for agriculture, can dilute the densities of wild pollinators, making adequate pollination across large fields less achievable by these groups (Holzschuh et al. 2016).

Second, pollinator effectiveness can be affected by their behavioural patterns. For example, pollinators may exhibit a preference for a specific flower or floral morph (e.g., sex) based on factors such as floral rewards, abundance, or morphological differences (Heinrich 1976; Waser 1986). Self-incompatible species, as well as species with dioecious (male and female flowers on separate plants) or gynodioecious (hermaphroditic and female flowers on separate plants) systems, require pollen transfer between different morphs. In the case of gynodioecy, a preference for one floral morph over another can result either from one sex being more attractive or rewarding than the other (Delph & Lively 1992; Ashman 2000), or a foraging preference for pollen-producing (Larsson 2005) or pollen-deficient flowers (Waller et al. 1985). In agriculture, honey bees have shown a preference for hermaphroditic plants over male-sterile ones in hybrid systems of *Brassica napus* (Mesquida & Renard 1981), carrot (Gaffney et al. 2019), and cauliflower (Gagic et al. 2021). Finally, spatial separation between patches of male and female flowers can result in pollinators individually specializing on a single morph. This has been observed in hybrid gynodioecious sunflower, a crop where the spatial separation of

morphs led to inefficient cross-pollination by insect pollinators (Martin & Farina 2016).

Other aspects of pollinator behaviour, such as the amount of time spent on a flower and flower choice, as well as the reward offered by the plant (e.g., nectar, pollen, or scent), can influence the amount of pollen deposited on a flower. Pollinators that spend more time handling flowers typically deposit more pollen (Thomson & Plowright 1980; Thomson 1986; Galen & Stanton 1989; but see Cresswell 1999). In systems where there are different types of flowers (e.g., dioecious or gynodioecious plant systems), a pollinator travelling directly from a pollen donor is likely to deposit more pollen than one travelling from a female flower. Finally, whether a pollinator is foraging for pollen or nectar can affect pollen deposition. Foragers concentrating on pollen-gathering tend to remove more pollen while depositing less (Wilson & Thomson 1991; but see Javorek et al. 2002), and visit inflorescences differently (Galen & Plowright 1985). Pollen-foraging bees may groom pollen into corbiculae or scopae, which reduces pollen carryover (Thomson 1986). This would make pollen less available for deposition (Parker et al. 2015), although 'safe sites' on pollinators may preserve pollen despite grooming efforts (Koch et al. 2017).

Third, the morphological attributes of pollinators can also influence their effectiveness in two ways: (1) the physical manner in which a pollinator contacts the stigma and (2) how much pollen is available on the pollinator's body for contact with and deposition on the stigma. In the case of easily accessible, open flowers, pollinator size influences how pollinators contact floral reproductive parts, with larger pollinators usually being more likely to come into contact with the stigma and therefore deposit pollen grains (Kandori 2002). Because larger pollinators also have more surface area onto which pollen could cling, larger pollinators may carry more pollen for deposition. In pak choi (*Brassica rapa*) pollination, visits from larger pollinator bodies with more pollen increased pollen deposition (Howlett et al. 2011). Other morphological features may cause taxonomic groups to differ in their ability to hold pollen on their bodies and to transfer it to flowers. Bees carry more pollen on their bodies than do dipterans (Lindsey 1984; Orford et al. 2015). Bees

possess dense branched hairs on their bodies that readily accumulate pollen (Thorp 1979). Pollinating flies such as hover flies are comparatively sparsely haired and may not have branched or plumose hairs (Holloway 1976), which reduces their capacity to carry pollen.

To understand how different types of flower visitors contribute to pollination in a mixed mating system, we examined pollinator visitation, pollinator response to floral morphs, and pollinator effectiveness in hybrid canola seed production fields in southern Alberta. Canola is naturally hermaphroditic and can self-fertilize, so outcrossing is controlled in the production of hybrid canola seed by inducing male sterility in the 'female' parental line, and not harvesting seed from the hermaphroditic parental line. This creates an artificially gynodioecious system of hermaphroditic and female flowers, which are grown adjacently in spatially separated bays or rows. Seed production depends on insect transfer of pollen from hermaphroditic (hereafter referred to as 'male' for brevity) to male-sterile ('female') morphs (Mesquida & Renard 1981).

Specifically, we tested the following predictions in this system: (i) that managed pollinators, including honey and leafcutter bees, would be the most common pollinators because they are placed in the field by growers, whereas wild pollinators would be fewer; (ii) that pollinators would individually specialize on floral morphs and show a low tendency to switch between floral morphs. This tendency would be especially strong in individuals foraging for pollen, for whom the female flower provides reduced reward; (iii) that larger, more hirsute pollinators would have more body pollen and deposit more pollen on stigmas, and that bees would be more effective at pollen deposition than flies; (iv) that among bee types, body size would be positively correlated with pollen deposition (for example, a bumble bee should deposit more pollen than the smaller leafcutter bee); (v) that based on diminishing pollen carryover with each subsequent visit after visiting a focal flower, pollinators travelling immediately from a male flower would deposit more pollen than those moving from a female; and (vi) that more time spent on a flower would result in higher pollen deposition.

MATERIALS AND METHODS

STUDY ORGANISMS

Two main types of canola crops are grown in Canada: (1) commodity canola, from which oil is extracted and canola meal is processed, and (2) hybrid seed canola, which produces hybrid seeds planted for commodity canola production (Westcott & Nelson 2001). Canola flowers have a central stigma, around which are spaced four nectaries and six stamens (four long and two short) (Delaplane & Mayer 2000) (Figure 1). The flowers are cruciate and both pollen and nectar are easily accessible to pollinators. Male-sterile (female) flowers, present only in hybrid production canola, have reduced, unviable stamens that present no pollen. Male flowers have larger petals and a higher rate of nectar production than do female flowers (Mesquida et al. 1991; Waytes 2017). Flowers are typically open for 3 days (Canola Council of Canada 2003; pers. obs.) and are clustered on inflorescences. The number of receptive flowers per inflorescence ranges from 1-10, with an average of three per inflorescence at a given time during bloom (Waytes 2017), although this may vary by cultivar.

The amount of pollen required for full seed set per pod is higher than the number of seeds produced for canola. For commodity canola flowers with an average of 30 ovaries, Mesquida and Renard (1984) found that 70 grains of pollen fully pollinated about half of their sample of flowers, and that flowers could benefit from receiving 100 grains or more of pollen to produce the maximum number of seeds per pod. The amount of pollen necessary for maximum seed set in commodity canola in Alberta is around 100 grains (A. Melathopoulos, pers. com.).
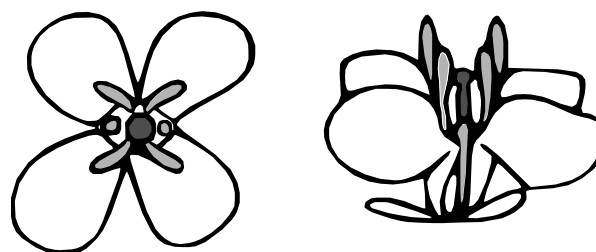
Figure 1. Illustration of a hermaphroditic canola flower in top (left) and profile (right) view. The stigma (dark grey) is surrounded by anthers (light grey), four long and two short.

Honey bees (*Apis mellifera*) and leafcutter bees (*Megachile rotundata*) are used as managed pollinators in hybrid canola seed production in Alberta. Wild pollinators, including bees and flies, contribute to commodity canola pollination (Morandin and Winston 2005), and likely contribute to hybrid seed production as well. Because they do not have the resources or habitat support that managed pollinators do and depend on nearby semi-natural habitat, wild pollinators are present at lower numbers in this system (Zink 2013).

Both bee and fly visitors are effective pollinators of canola. Yields of commodity canola increase with increasing honey bee abundance (Manning & Boland 2000; Sabbahi et al. 2005; Oz et al. 2008; Munawar et al. 2009; Durán et al. 2010). Additionally, non-*Apis* bees can deposit pollen on commodity canola flowers at a level comparable to honey bees (Rader et al. 2009; Ali et al. 2011), and hover flies can be efficient pollinators (Jauker & Wolters 2008). However, unlike the hybrid seed production fields, commodity canola fields entirely consist of hermaphroditic flowers. Studies on the pollination of commodity canola therefore do not address the potential for differential responses by pollinators to the additional presence of female flowers, as present in hybrid seed canola, which could influence pollen transfer. Additionally, seed production in commodity crops also includes pollination via self-fertilization, which is impossible for the female flowers in hybrid seed production systems. Studies of pollinators visiting gynodioecious canola in caged tents using honey bees (Mesquida & Renard 1981), alfalfa leafcutting bees (*Megachile rotundata*; hereafter 'leafcutter bees') (Soroka et al. 2001), and hover flies (family Syrphidae) (Jauker et al. 2012) suggest that these pollinators could be effective at transferring pollen (although hover flies less so than bees). However, the floral-visitation behaviours that were observed in small, screened tents might differ from behaviours in less physically constrained settings.

Study Sites

Our sites were hybrid canola production fields located in southwestern Alberta. In each field the flowers were separated into alternating bays (lines) of flowers by morph, with each bay (line) spanning the entire field. Female bays contained roughly six times the area of male bays (a width of 6 m versus 1 m). Our field sites ranged from 26 ha to 65 ha in size. We visited 21 sites in 2015 to conduct surveys on pollinator visitation, pollinator response to female inflorescences, and pollination effectiveness. All 21 sites in 2015 were of the same variety of canola. A single field was visited per day across the period of canola flowering. Due to a low sample size of wild pollinators in the 2015 season, we continued our survey efforts in 2016 to measure pollinator effectiveness with more emphasis on non-managed pollinators, visiting 14 sites in 2016. We expanded our sampling efforts to an additional variety of canola to increase the number of wild pollinators in the samples; the second variety of canola comprised three of the fourteen sites. A continued low frequency of bumble bee pollinators necessitated the collection of bumble bees in a commodity canola field. While commodity canola is effectively the same as 'male' hybrid canola, our measurements therefore represent pollination potential for bumble bees, rather than actual contributions to pollination in hybrid canola fields.

Pollinator visitation and effectiveness

To measure pollinator visitation, we established focal plots within each site composed of paired adjacent sections of male and female bay, usually with six plots per field. Each paired plot consisted of two 1 m² quadrats. Bee pollinators are central-place foragers, and distance away from their nesting source constrains their foraging range: both honey bees and leafcutter bees forage less with increasing distance from their hives or shelters (Ribbands 1951; Richards 1984; Jay 1986). To generate differences in visitation due to distance from nesting source, we placed plots within each field at distances near to and far from leafcutter bee shelters, honey bee hives, and potential sources of wild bee habitat (such as a nearby fallow field or fence line). Due to the placement of leafcutter bee shelters throughout the field and honey bee hives only at the field edges, the mean distance away from honey bee hives was 330 m, while the mean distance away from leafcutter bee shelters was 31 m.

In each field, we recorded pollinator visits to the female and male bays at each plot. During a ten-minute observation period, we recorded the identities of floral visitors and the number of visits made to inflorescences by each type of pollinator.

We separated pollinators into the following types for observations: honey bees, leafcutter bees, wild bees (Non-*Apis* bees, including bumble bees and solitary bees), hover flies (Syrphidae), calyptrate muscoid flies (Muscidae), and lepidopterans. Honey bees and leafcutter bees (*M. rotundata*), both non-native to the area, were assumed to come from managed nests. We recorded each time a pollinator visited a new inflorescence, so 'visitation' reflects visits to new inflorescences, rather than individual flowers. We estimated each plot's floral density per square meter by recording the number of inflorescences within a 0.25 m² plot in each of the male and female bays and quadrupling this number. We measured air temperature (°C) and wind speed (m/s) at each plot with a Kestrel 2000 Wind Meter®.

We measured pollinator effectiveness and response to an inflorescence of female flowers using a modified interview bouquet (Thomson 1981). The bouquet apparatus consisted of a telescoping camera stick with a GOPRO Hero 4® camera equipped with macro lens set up on the end. A micro-centrifuge tube was placed at the end of a dowel, positioned ~15 cm in front of the camera (Figure 2). The whole apparatus, from the tip of the handle to the tube at the end of the dowel, was 1 m long. During each offering we placed a virgin female inflorescence in the micro-centrifuge tube, with the camera angled towards the flower for recording, and offered the inflorescence to pollinators visiting flowers within the crop. The offered inflorescence was placed close to the pollinator, within a few centimetres.

Two methods were used to ensure female flowers were not visited before being offered to floral visitors. One involved covering inflorescences at a site with pollinator exclusion bags at least a day before collection, with all open flowers removed before covering. Another method involved covering female hybrid canola plants at a separate site permanently with a wind- and pollinator-exclusion tent to prevent pollen deposition. Virgin female inflorescences were collected from these plants at the beginning of a field day and kept in a cooler to prevent insect visitation and loss of floral function due to heat. Control stigmas from the flowers were collected daily to ensure no pollen deposition had taken place with either pollen exclusion method; measured pollen deposition on these was always zero.

Pollinators visiting flowers in both male and female bays were offered a female inflorescence. Pollinators that visited the inflorescence were collected with a Bioquip® Insect Vacuum, placed on ice until immobile, and then transferred to a micro-centrifuge tube. They were frozen until processing (see below). After the pollinator was caught, we collected and mounted the stigmas of visited flowers on slides with glycerin fuchsin jelly for later counting. For consistency, when pollinators visited multiple flowers, we collected all stigmas but only the pollen deposition on the first visited flower was counted.

We used the videos of pollinators to obtain their reactions to the proffered female inflorescences. Pollinator responses on the videos were scored as avoidance, rejection, or acceptance. Avoidance meant that the pollinator did not visit the inflorescence and implied no interest in the inflorescence before flying away. Avoidance is the broadest and least defined category, as it is unclear whether the pollinator left due to fear of the apparatus, rejection of the inflorescence, or simply did not notice the inflorescence. The second state—rejection—involved inspection of the inflorescence, possibly even contacting a non-sexual floral part (such as the petal), but ultimately leaving without visiting or contacting the stigma. The third state—acceptance—involved pollinators
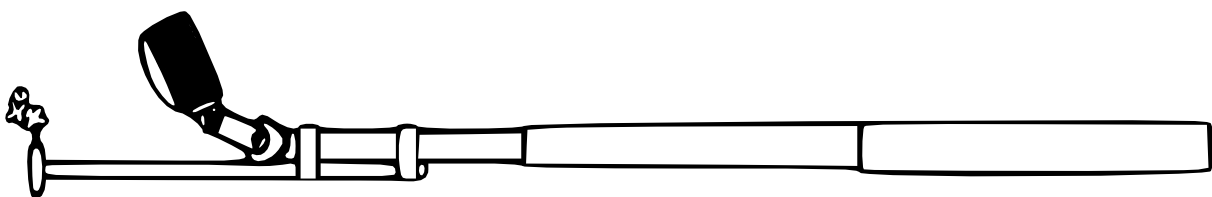

Figure 2. Stylized drawing of an interview bouquet stick (1 m long). The camera, with macro lens, is focused on a female canola inflorescence placed in a microcentrifuge tube.

contacting the reproductive parts of the flower. We processed the videos using GoPro Studio® video editing software. For each video we recorded the type of pollinator that visited the flower. If the pollinator was a honey bee, we recorded whether they were nectar or pollen foragers based on the presence of pollen in their corbiculae. While honey bees may collect both pollen and nectar on a foraging trip, most foragers collect one or the other (Free 1960).

In addition to pollinator response, we measured behaviours that could influence pollen deposition that were recorded on video, including the amount of time spent on a flower and the type of flower on which the pollinator was foraging (i.e., the 'flower of origin') before being offered a female inflorescence. In the cases where the pollinators visited (i.e., 'accepted') the flower, we measured the amount of time the pollinator was physically on the flower once it contacted the stigma. The flower of origin was assessed in the field and again checked against the video records.

We estimated the amount of pollen on each pollinator's body to understand how this affected their effectiveness of pollen transfer. We removed corbicular loads from the hind legs of honey bee and bumble bee pollen foragers prior to processing, as pollen packed in this manner is unavailable for pollination (Thorp 2000). While it is possible that pollen loads accumulated on the ventral surface of leafcutter bee abdomens may have been similarly unavailable for pollination, we decided not to remove them as they are (i) dry-packed, unlike honey bee corbicular loads, which may make them more available for pollination, (ii) located ventrally, which still may place them in range for pollination, and (iii) less well-defined than corbicular loads, which would make it difficult to tell the extent to which they should be removed.

To count the amount of pollen on a pollinator's body, we submerged pollinators in 2 mL of 70% ethanol (more ethanol was used for larger-bodied pollinators) in micro-centrifuge tubes that we then sonicated using a Branson Ultrasonic Bath Model 1800® to release the pollen from the pollinator's body in a non-destructive manner (Kearns & Inouye 1993). Pollinators were sonicated for at least one minute, until any pollen visible on the pollinator's body was suspended into the ethanol.

Ten µL of the pollen-ethanol solution was then immediately placed upon a haemocytometer and the number of pollen grains within the grid counted. We repeated haemocytometer counts ten times for each individual, each time using the same sonicated sample. We estimated the average number of pollen grains per mL on each pollinator by multiplying the average number of pollen grains for each pollinator by the degree of dilution, dividing this amount by the number of haemocytometer squares counted, and multiplying the final value by 10^4. Pollinators were not removed from the vials after sonication to minimize the loss of ethanol, although some ethanol may still have been lost via evaporation when vials were opened for the pollen-ethanol solution extraction. Because larger pollinators have more surface area onto which pollen can be held, it is possible that larger pollinators retained more pollen on their bodies compared to smaller pollinators despite sonication efforts. Although we took steps to visually scan pollinators for remaining body pollen and to minimize the amount of time vials were open to prevent ethanol loss, we acknowledge that this may have affected our dilution calculations.

Statistical analysis

All analyses were performed in R v.4.0.3 (R Development Core Team 2020). Analyses consisted of Generalized Linear Mixed Models (GLMM), which we analysed using the glmmTMB package in R (Brooks et al. 2017). Any post hoc comparisons were made using the multcomp package in R (Hothorn et al. 2008). Models were checked for collinearity of predictors, overdispersion, and outliers. Quantitative explanatory variables were standardized. Model residuals were checked for homogeneity of variance and normality. We present the means for variables ± standard error unless otherwise indicated in the text, with parameter estimates from negative binomial models in log scale and from binomial models in logit scale. In the figures we present the model-adjusted means and 95% confidence intervals.

We analysed visitation using a hurdle model to control for the presence of many zeroes in the data set (Mullahy 1986). We first analysed pollinator presence or absence in a ten-minute period using a binomial GLMM. We subsequently analysed a

zero-truncated data set to understand the number of visits to inflorescences by those pollinators who were present in plots using a GLMM with negative binomial error distribution to control for overdispersion. We tested for the significance of the effect of the variables pollinator type, temperature, flower density, and distance from nesting source on pollinator presence and visitation using likelihood-ratio tests. The variable 'pollinator type' included honey bee, leafcutter bee, fly, and native bee. The category 'native bee' included solitary bees as well as bumble bees, whose infrequent presence (bumble bees were present in 1/228 quadrat observations) prevented a more detailed assessment. The category 'fly' included hover flies and calyptrate muscoids (Calyptratae). Lepidopterans were excluded from the analysis due to their rarity (4/228 quadrats). Distance from nesting source included distance from honey bee hives for honey bees, leafcutter bee shelters for leafcutter bees, and the nearest natural habitat for native bees and flies. Because of the unmanaged nature of native bees and flies, natural habitat represents only a potential nesting source, not a definite one. Site and plot nested within site were modelled as random effects. Post hoc comparisons between pollinators were made via a Tukey Test.

We analysed pollinator response (reject versus accept) using binomial GLMMs. Responses were taken from the 2015 field season videos. Low sample sizes of non-managed pollinators necessitated that only leafcutter bees and honey bees be used to compare between responses. The ambiguity in the 'avoidance' category—which included bees that may not have seen the proffered inflorescence, or that reacted to the interview bouquet apparatus rather than the inflorescence—favoured its removal from analysis. Predictors included the type of pollinator (honey and leafcutter bees), the type of flower (male or female) on which pollinators were originally foraging, and their interaction. The significance of each predictor was compared using likelihood-ratio tests. In a separate analysis, we limited the comparison to just honey bees to understand whether nectar-foraging and pollen-foraging honey bees differed in their responses to female inflorescences. Because most pollen foragers ($N = 40$ out of 43 honey bees) were found on male flowers, forager choice of bouquet was compared between nectar and pollen

foragers only in the male bay. Julian day was included as a predictor variable for both tests because seasonal fluctuations in floral availability may have affected pollinator responses to resources. Site was a random effect.

Not all inflorescences had the same number of flowers. Inflorescences with more flowers may be more attractive to pollinators (Willson & Price 1977), and pollinators may therefore alter their responses to inflorescences depending on the number of flowers. For a subsample of video recordings ($N = 100$ total, which included $N = 25$ each of videos of honey bees and leafcutter bees rejecting or accepting bouquets) we quantified the number of flowers on all the inflorescences offered and tested to see whether this significantly affected pollinator response, controlling for other variables including pollinator type, the flower of origin, their interaction, Julian day, and site as a random effect. We found that the number of flowers per inflorescence did not significantly affect pollinator response, and as such we did not include this term in the full models.

We analysed pollen deposition (an integer, whose values frequently included zero) using a GLMM with negative binomial errors to control for overdispersion. We used log-likelihood tests to understand the significance of pollinator type (honey bee, leafcutter bee, bumble bee, and hover fly), the type of flower on which the pollinator was originally foraging (male or female), the amount of time spent on the focal flower, and the amount of pollen on a pollinator's body on the effectiveness of pollen deposition (measured as total pollen grains deposited on a stigma). The low sample size of pollen-foraging honey bees willing to visit a female flower ($N = 3$) precluded their inclusion in the model. Site was included as a random effect. Post hoc Tukey tests evaluated differences between pollinators. Small and unbalanced sample sizes necessitated our pooling of pollen deposition across variety and year. We recognize that this pooling may overlook varietal or yearly effects on pollen deposition.

RESULTS

POLLINATOR VISITATION

Pollinator types differed in their likelihood to visit a plot ($\chi^2 = 250.98$, $DF = 3$, $P < 0.0001$) (Figure

A

B
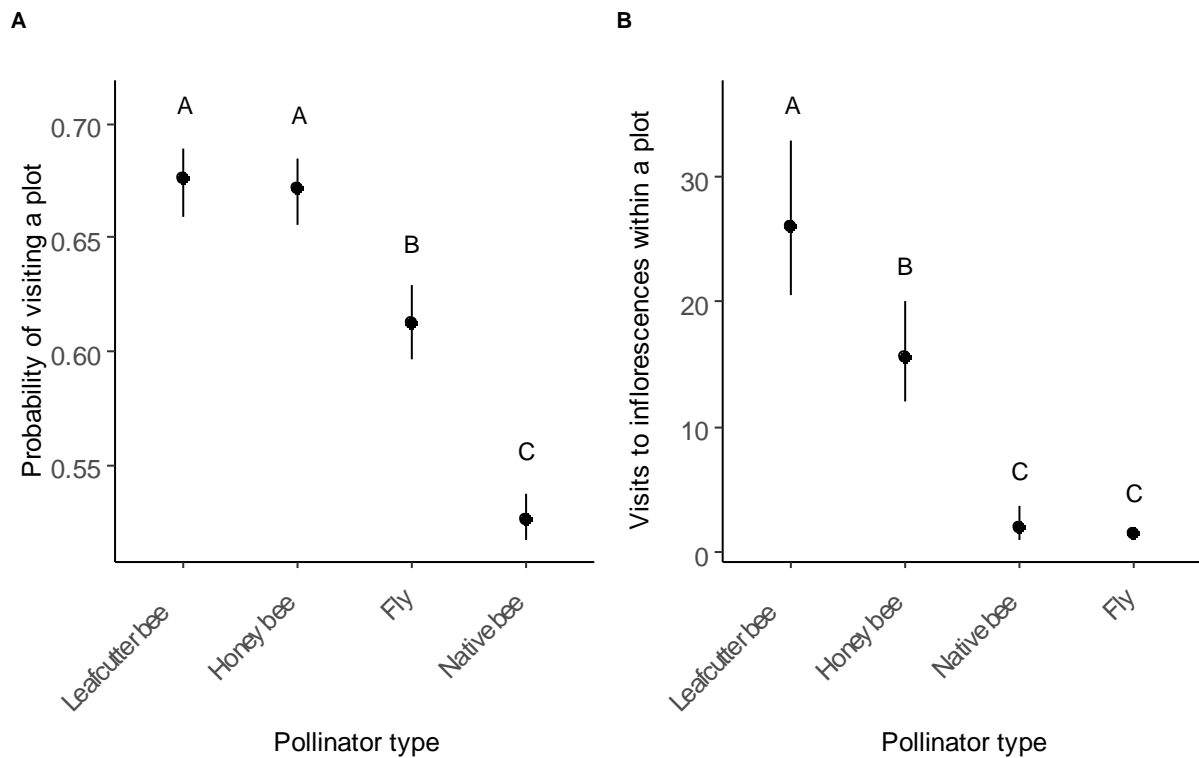
Figure 3. The effect of pollinator type on A) the probability of pollinators visiting a plot and B) the number of visits made to inflorescences within a plot by pollinators during a 10-minute observation of a 1 m² plot. The types of pollinators observed include leafcutter bees (*N* = 178), honey bees (*N* = 147), native bees (*N* = 26), and flies (*N* = 106). Points represent the model-adjusted means and lines represent the 95% CI. Letters indicate significant differences among means (*P* < 0.05); means followed by a common letter are not significantly different at the 5% level of significance.

3A). When controlling for distance from nesting source, both honey bees and leafcutter bees were equally likely to be present in a plot ($P = 0.98$) and were more common than flies or native bees ($P < 0.0001$). Flies were the next most common floral visitor, followed by native bees. We encountered more pollinators in male bays than female bays ($\chi^2 = 3.99$, $DF = 1$, $P < 0.05$). Pollinators were less common with increasing distance from their nesting source ($\chi^2 = 19.47$, $DF = 1$, $P < 0.0001$; $\beta = -0.40 \pm 0.09$), and more common at increasing temperatures ($\chi^2 = 14.65$, $DF = 1$, $P = 0.0001$; $\beta = 0.35 \pm 0.08$). Flower density had no effect on a pollinator's likelihood of visiting a plot ($\chi^2 = 1.44$, $DF = 1$, $P = 0.23$).

Of pollinators visiting inflorescences within a plot, leafcutter bees visited the greatest number of inflorescences ($\chi^2 = 173.33$, $DF = 3$, $P < 0.0001$) (Figure 3B). Leafcutter bees visited significantly more inflorescences than honey bees ($P = 0.03$) and other pollinators ($P < 0.001$), while honey bees visited more inflorescences than flies ($P < 0.001$) and native bees ($P < 0.001$). Neither native bees nor

flies differed in their visitation to inflorescences ($P = 0.84$). Pollinators visited fewer inflorescences within a plot at further distances away from their nesting source ($\chi^2 = 4.97$, $DF = 1$, $P = 0.03$; $\beta = -0.17 \pm 0.08$). Flower morph type ($\chi^2 = 0.63$, $DF = 1$, $P = 0.43$), flower density ($\chi^2 = 0.43$, $DF = 1$, $P = 0.51$), and temperature ($\chi^2 = 0.08$, $DF = 1$, $P = 0.78$) did not significantly influence the number of visits to inflorescences by pollinators.

POLLINATOR RESPONSE

Both leafcutter and honey bees were significantly less likely to 'accept' (i.e., visit) proffered female inflorescences when they were originally foraging on male inflorescences than when they were foraging on female inflorescences ($\chi^2 = 39.30$, $DF = 2$, $P < 0.001$) (Figure 4), although in general both species typically did move to the female inflorescence. Pollinator species did not affect their willingness to accept a female inflorescence ($\chi^2 = 5.37$, $DF = 2$, $P = 0.07$), and there was no significant interaction between pollinator type and flower of origin ($\chi^2 = 0.37$, $DF = 1$, $P = 0.54$). The time of season (Julian day) did not
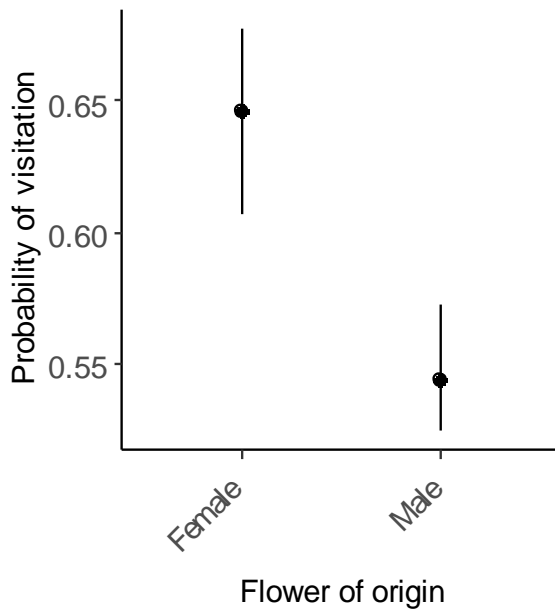
Figure 4. The effect of flower of origin on the probability of visitation to a female flower by a leafcutter bee (N = 229) or honey bee (N = 275) (P < 0.001). Points represent the model-adjusted means and lines represent the 95% CI.
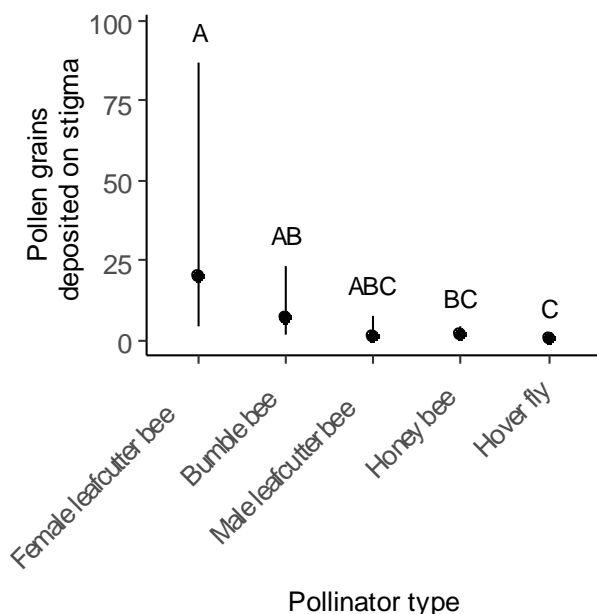

Figure 5. The effect of pollinator type on the number of pollen grains deposited on stigmas of canola flowers. The types of pollinators included female leafcutter bees (N = 20), bumble bees (N = 13), male leafcutter bees (N = 6), honey bees (N = 43), and hover flies (N = 21). Points represent the model-adjusted means and lines represent the 95% CI. Letters indicate significant differences among means (P < 0.05); means followed by a common letter are not significantly different at the 5% level of significance.

significantly influence whether pollinators accepted or rejected bouquets (χ^2 = 3.65, DF = 1, P = 0.06), although a weak trend indicated pollinators might be more likely to accept an inflorescence as the season progressed. Honey bee nectar and pollen foragers in the male bay were equally likely to accept a female flower (χ^2 = 2.18, DF = 1, P = 0.14).

POLLINATOR EFFECTIVENESS

Pollinator type (χ^2 = 27.86, DF = 4, P < 0.0001), flower of origin (χ^2 = 33.99, DF = 1, P < 0.0001), time spent on flower (χ^2 = 12.92, DF = 1, P = 0.003), and the amount of pollen on a pollinator's body (χ^2 = 4.20, DF = 1, P = 0.04) all influenced pollen deposition during a single visit to a previously unvisited canola stigma. Female leafcutter bees deposited significantly more pollen on stigmas than honey bees (P < 0.001) and hover flies (P = 0.001) (Figure 5). Pollen deposition by male and female leafcutter bees was not significantly different (P = 0.14). Bumble bees also deposited more pollen than hover flies (P = 0.009), but bumble bee pollen deposition was not significantly different than honey bee (P = 0.23) or male leafcutter bee (P = 0.57) pollen deposition. Honey bees deposited similar amounts of pollen to male leafcutter bees (P = 0.999) and hover flies (P = 0.71).

Pollinators travelling from male flowers deposited significantly more pollen on stigmas ($\bar{\chi}$ = 32.2 ± 10.1 grains) than did pollinators travelling from female flowers ($\bar{\chi}$ = 1.7 ± 1.2 grains) (Figure 6). In addition, pollinators handling flowers for longer time periods deposited more pollen (β = 0.70 ± 0.20) (Figure 7). This latter result followed the removal of an outlier of > 60 s, where a female leafcutter bee stayed motionless on a flower and did not actively interact with the flower after landing on it (discussed in more detail below). Unexpectedly, an increasing amount of pollen on a pollinator's body led to a decreased deposition (β = -0.41 ± 0.17) (Figure 8).

DISCUSSION

As we predicted, pollinator visitation in this system was highest for managed pollinators. Pollinators tended to specialize individually on floral morphs, which led to lower pollen deposition. This finding may represent a potential barrier in many zoophilous plants that require pollen transfer between floral morphs. Our
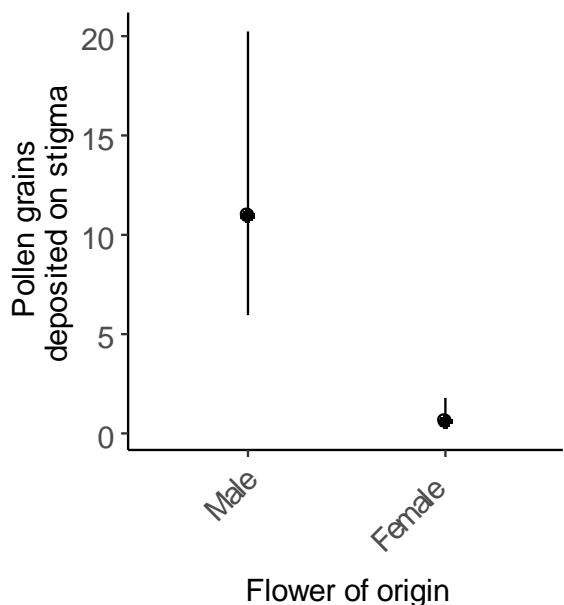
Figure 6. The influence of flower of origin, male (*N* = 48) and female (*N* = 55), on the number of pollen grains deposited on the stigma of a female flower (*P* < 0.0001). Points represent model-adjusted means and lines represent the 95% CI.
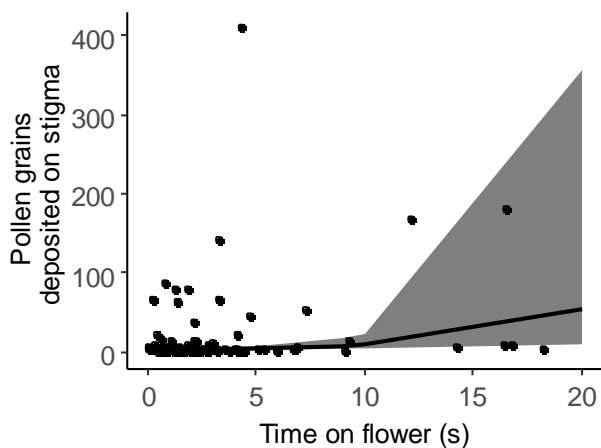

Figure 7. Relationship between the amount of time spent on a canola flower by a pollinator and the number of pollen grains deposited, with predicted trend line plotted against observed (non-adjusted) points. Shaded area represents 95% CI.

prediction that larger pollinators would more effectively deposit pollen and that bees would deposit more pollen than flies did not turn out to be entirely true. Pollen deposition increased with time spent by a pollinator on a flower, while it unexpectedly decreased with the amount of pollen on the pollinator's body. Each of these findings is discussed in more detail below.

POLLINATOR VISITATION

Managed pollinators, which included leafcutter bees and honey bees, were more common in our canola plots than flies and wild bees. This result was not unexpected; managed pollinators are actively supported and placed near or in the fields. While leafcutter bees and honey bees were equally likely to visit a plot, leafcutter bees visited more inflorescences than honey bees within a plot. Leafcutter bees have a restricted foraging range (Gathmann & Tscharntke 2002), which could explain why they visited more inflorescences within a plot than honey bees, as they are more dependent on local resources. Honey bees forage at greater distances from their colony than leafcutter bees (Greenleaf et al. 2007), but leafcutter bee shelters were also dispersed regularly throughout the field compared to the location of the honey bee colonies on the field edges. It was more difficult to sample 'far' from leafcutter bee shelters (maximum of 160 m, versus 850 m from honey bees). Hence, declines in leafcutter bee density with distance from their shelters were reduced due to their higher in-field density, which would explain why they were equally likely to visit a plot as honey bees despite potential differences in foraging ranges. Our measure of visitation reflected the number of new inflorescences visited in a 10-minute watch, and as such was not necessarily a direct representation of pollinator density on canola. More inflorescence visitation by leafcutter bees relative to honey bees could result from either a higher visitation rate or a higher bee density.

Flies were the next most abundant pollinators after managed bees. As larvae, some species of hover flies prey on crop pests such as aphids (Sarwar 2013), and so may be present in canola at multiple life stages. Canals and ditches near canola fields could be breeding places for flies with an aquatic larval stage, such as hover flies in the genus *Eristalis* (Buckton 1895). Cattle feedlots located near some of the fields may have also provided resources for the larvae of muscoid flies.

Native bees were the least frequently observed floral visitors in fields of hybrid seed canola, with a mean probability of encounter of only about 11%, and a comparatively low number of visits to inflorescences within a plot compared to managed pollinators. Mass-flowering crops such as hybrid
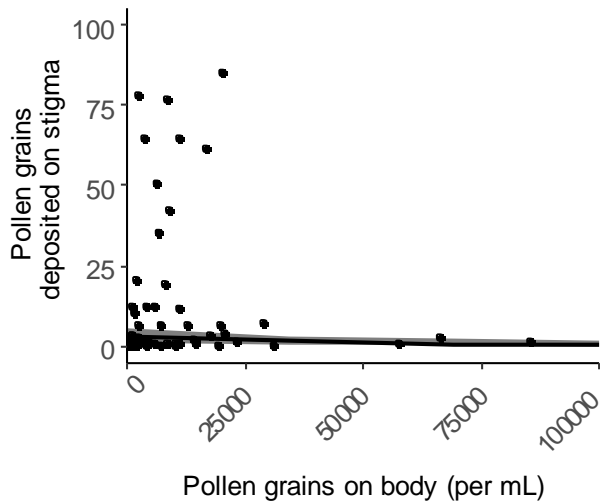
Figure 8. Effect of the number of grains on the body of a pollinator (as present per mL of fluid) on the number of pollen grains deposited on the stigma of a canola flower, with predicted trend line plotted against observed (non-adjusted) points. Shaded area represents 95% CI. The plot was capped at 100 pollen grains deposited on the stigma so that the trendline would be visible, which resulted in the removal of four points which exceeded that amount.

seed canola can increase local densities of bees (Westphal et al. 2003), and non-*Apis* wild bees have been found in some localities to be common visitors to commodity canola (O'Brien & Arathi 2018). Unlike managed pollinators, which are provided with nesting resources (such as hives for honey bees) and whose food can be supplemented by their keepers, wild pollinators depend wholly on the vagaries of nesting and floral resources in the environment. If requirements for floral and nesting resources by native bees are not locally met, large fields of flower crops may simply serve to dilute flower visitation of already low existing populations of wild pollinators (Holzschuh et al. 2016). The presence (and contributions) of native pollinators may therefore be higher in smaller agricultural fields, with more semi-natural habitats. Isaacs and Kirk (2010) found that native (bumble) bees contributed most to pollination in small-sized blueberry fields, but that honey bees dominated pollination in large fields. Native bees might also be less abundant due to be competitive displacement by managed bees (Lindström et al. 2016). The high numbers of managed pollinators in seed fields of hybrid canola could deter wild bee visitation, especially if native bees can obtain floral resources in nearby semi-natural habitat (Zink 2013).

Pollinators were more likely to be found in male bays compared to female bays. While the size ratio of male to female bays was 1:6, male flowers are more rewarding in that they offer pollen and have a higher rate of nectar production than female flowers (Mesquida et al. 1991; Waytes 2017).

POLLINATOR RESPONSE

Both honey and leafcutter bees were more likely to reject female inflorescences when they were originally foraging on male flowers than when they were foraging on female flowers. This suggests that managed pollinators in hybrid seed canola exhibit individual selectivity to floral morph. Optimal diet theory suggests that pollinators are more likely to specialize with increased differences in food types (e.g., higher rewards) or greater food density (MacArthur & Pianka 1966). This apparent tendency of pollinators to choose flowers based on abundance or rewards was described by Waser (1986) as a 'labile preference'. Male flowers in this system offer higher rewards than females, both in the amount of nectar produced as well as pollen (which female flowers lack). While they are less abundant than female flowers (in that the width of female to male bays is 6:1), the extensive size of the canola fields may make this difference in availability less limiting than what might occur in a more natural setting. The spatial separation of bays used in the production of hybrid canola seed also means that pollinators do not bypass easily accessible and similarly rewarding flowers. While pollinator directionality was not a focus of our study, pollinators may differ in their directionality of movement during foraging trips (Brunet et al. 2019), which may also affect their tendency to transfer between floral morphs in this system.

The tendency for bees to exhibit this selective behaviour has implications for flower breeding systems that require pollinator movement between floral morphs. Bees are expected not to specialize when alternative flower types are similarly rewarding and accessible, but this is not always observed (Marden & Waddington 1981). This study is not the first to show that pollinators can distinguish between morphs in gynodioecious crops and selectively visit one morph; honey bees preferred male-fertile oilseed rape (Mesquida & Renard 1981) and sunflower (Martin & Farina 2016).

Honey bees foraging for pollen in hybrid seed canola are limited to male flowers, while nectar foragers have no such restrictions. Honey bees tend to forage for either pollen or nectar, and are less likely to collect both in one foraging trip (Free 1960). Despite this, in a subset of our bees for which we had information about resource collected, we found that nectar- and pollen-foraging honey bees foraging in the male bay were similarly likely to reject to female inflorescences. One possible explanation is that the honey bees reacted to the interview bouquet apparatus and not the female inflorescence; because we did not offer male inflorescences as well, we cannot compare between reactions to inflorescences based on flower morph. Another possibility is that pollinators selectively responded to the morph of the flower. The rejection of female flowers may therefore be universally high from all honey bees foraging on the male bay. The presence of a small number of pollen foragers in the female bays suggests that not all bees classified as 'pollen foragers' collect only pollen. Hence floral morph may determine foraging behaviour more strongly than floral resource type.

POLLINATOR EFFECTIVENESS

Not surprisingly, pollinators moving from male flowers deposited more pollen on virgin stigmas than did those moving from female flowers. Female flowers lack pollen and cannot directly contribute to pollen distribution. For a foraging pollinator not picking up any new pollen, pollen deposition will decline with subsequent floral visits (Levin & Berube 1972). For hybrid canola, most pollen is likely lost in 3 to 4 successive flower visits, and virtually all of it gone after 20 successive flower visits (Cresswell et al. 1995). Even if a pollinator had switched from the male to female bay before visiting the female inflorescence, the rapidity of pollen loss would mean that amount of pollen available for deposition would likely still be less than that coming from a pollinator moving directly from a male flower. The willingness of a pollinator to switch between flower morphs, in both directions, is therefore essential for continuing pollen deposition from an individual forager.

Pollinators that spent more time on flowers generally deposited more pollen. This result agrees with previous studies that suggest that more time

on a flower leads to higher pollen deposition (Thomson & Plowright 1980; Thomson 1986; Galen & Stanton 1989). It is important to note that we focused on pollinators that were actively handling flowers. We removed one outlier which involved a female leafcutter bee spending over 60 seconds motionless on a flower, therefore not participating in the act of pollen transfer. A lack of movement by pollinators while foraging, such as in the case of *Bombus lapidarius* visitors staying fairly immobile while probing nectaries in a study by Cresswell (1999), may result in low pollination despite the amount of time the visitors spend on flowers.

We expected that the amount of pollen available on the bodies of the pollinators (based on hairiness and size) would positively influence their pollination effectiveness. Certainly flies, which tend to be more sparsely haired than bees, did not deposit large numbers of pollen grains. A previous research study indicated that flies must be present at much higher densities than bees to achieve similar levels of pollination (Jauker et al. 2012). However, in our study, per-fly pollen deposition was not significantly different from that of honey bees or male leafcutter bees, and the differences in pollination potential among these pollinator types would be due to visitation frequency rather than visitation quality.

Female leafcutter bees were the most effective at pollen deposition, depositing more per visit than either honey bees or hover flies. Leafcutter bees are smaller and less hairy than both honey and bumble bees, but canola flowers are also relatively small, so there is no morphological mismatch in terms of size. It is possible that the abdominal scopae of female leafcutter bees pick up pollen grains better than the comparatively sparser ventral abdominal hair on bumble bees and especially honey bees. The positioning of pollen on the underside of the body may make it more likely that it contacts the stigma than pollen located laterally or dorsally, despite the female leafcutter bee propensity to elevate her abdomen while visiting flowers.

Bumble bees and honey bees were similarly effective to each other at depositing pollen. The variation in bumble bee deposition could reflect differences in effectiveness among species (which, due to the limited sample size, were grouped at the level of genus), or the type of resources for which

the pollinator was foraging (pollen or nectar). The small sample size prevented comparing pollen deposition between forager type (for example, $N = 3$ for honey bee pollen foragers). The lower pollen deposition by honey bees, as compared to female leafcutter bees, may be due to how the pollinators groom and carry their pollen, rather than their size or amount of hair.

Previous studies such as Howlett et al. (2011) suggest that higher amounts of pollen on a pollinator's body should result in higher pollen deposition. Surprisingly, in this study the amount of pollen on a pollinator's body negatively affected the amount of pollen deposited on a stigma. The amount of pollen a pollinator carries may not accurately represent the pollen available for deposition. Behaviours such as grooming and foraging may make it inaccessible for pollination (Thomson 1986). Differences in how a pollinator carries its pollen may also affect pollen availability and function; corbiculate bees such as honey bees and bumble bees may treat the pollen as they pack it, which has been found to negatively affect seed set (Parker et al. 2015). For this study, we attempted to address the issue of unavailable pollen by removing corbicular loads from honey and bumble bees. However, pollen residue unavailable for stigma contact could have been left on their legs. In future, we recommend that hind legs be removed entirely prior to measuring the amount of pollen present on the bodies of bees. While leafcutter bees do not treat the pollen packed into their scopae in the same manner that corbiculate bees do, it is possible that the pollen packed on leafcutter bee scopae is unavailable for pollination depending on how the bees position their abdomens while foraging. This, too, may have contributed to our findings on the negative effect of body pollen on pollen deposition. Finally, it is possible that the method of calculating the amount of pollen on the bodies of pollinators was influenced by factors such as ethanol evaporation during extraction and pollinator body size, which we did not measure in this study.

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