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PLANT-MICROBE-ANIMAL INTERACTIONS – ORIGINAL RESEARCH

Pollinator effectiveness is affected by intraindividual behavioral variation

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Abstract

Variation in pollinator quality is fundamental to the evolution of plant-pollinator mutualisms and such variation frequently results from differences in foraging behavior. Surprisingly, despite substantial intraindividual variation in pollinator foraging behavior, the consequences for pollen removal and deposition on flowers are largely unknown. We asked how two pollen foraging behaviors of a generalist pollinator (*Bombus impatiens*) affect removal and deposition of heterospecific and conspecific pollen, key aspects of pollinator quality, for multiple plant species. In addition, we examined how bee body size and pollen placement among body parts shaped pollen movement. We manipulated foraging behavior types using artificial flowers, which donated pollen that captive bees then deposited on three recipient plant species. While body size primarily affected donor pollen removal, foraging behavior primarily affected donor pollen deposition. How behavior affected donor pollen deposition depended on the plant species and the quantity of donor pollen on the bee's abdomen. Plant species with smaller stigmas received significantly less pollen and fewer bees successfully transferred pollen to them. For a single plant species, heterospecific pollen interfered with conspecific pollen deposition, such that more heterospecific pollen on the bee's abdomen resulted in less conspecific pollen deposition on the flower. Thus, intraindividual variation in foraging behavior and its interaction with the amount and placement of acquired pollen and with floral morphology can affect pollinator quality and may shape plant fitness via both conspecific and heterospecific pollen transfer.

Keywords Interspecific pollen transfer · Pollination quality · Conspecific pollen · Heterospecific pollen · Foraging behavior

Introduction

A fundamental question in the study of mutualisms is how variation in the quality of interacting partners affects the maintenance of mutualism (Frederickson 2013; Bronstein 2015; Yoder and Tiffin 2017). Plant-pollinator interactions are a model for the study of mutualism, and different species of pollinators frequently vary in their quality as vectors of pollen among conspecific flowers. For instance, in terms of both removal and deposition of conspecific pollen, pollinator taxa that are more faithful to a single plant species (e.g., specialist solitary bees) can be more effective pollinators than pollinators that visit multiple plant species (e.g., generalist fly species) (e.g., Larsson 2005). Yet while interspecific and even intraspecific differences in pollination effectiveness (e.g., by male versus female pollinators) are well documented (Minnaar et al. 2018; Moreira-Hernandez and Muchhala 2019; Smith et al. 2019), intraindividual variation in pollination effectiveness has been largely ignored. Differences in foraging behavior are thought to contribute substantially to among-species differences in pollination effectiveness (Wilson and Thomson 1991; Duffy et al. 2014; Minnaar et al. 2018; Moreira-Hernandez and Muchhala 2019). While intraindividual variation in the foraging behavior of a given pollinator species is common and is frequently proposed to affect the removal and deposition of conspecific and heterospecific pollen (e.g., Waser 1986; Chittka et al. 1999; Mitchell et al. 2009), this interaction remains poorly understood (but see Ish-am and Eisikowitch 1993; Pellmyr 1996; Young et al. 2007).

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A common way in which the behavior of an individual may vary is in how the pollinator extracts food (e.g., pollen and nectar) from flowers (e.g., Laverty 1980, 1994; Gegear and Laverty 1995; Ye et al. 2018). For instance, bees across many taxa commonly use and readily switch between two kinds of behaviors to extract pollen from flowers, bees' primary source of protein (Nicolson 2011; Russell et al. 2017a). Buzzing (estimated used by > 58% of bee species; Cardinal et al. 2018) consists of powerful thoracic vibrations that dislodge pollen from the stamens; scrabbling to collect pollen, however, uses vigorous leg movements instead (Buchmann 1985; Russell and Papaj 2016; Russell et al. 2017a). Further, bees are frequently observed using both behaviors on the same plant species, including on the same flower (documented across 29 plant families; Russell et al. 2017a). While these two behaviors are a common component of intraindividual variation in foraging, whether they affect pollen acquisition and deposition on flowers—together constituting pollination effectiveness of the individual bee—differently remains unknown.

Intraindividual variation in pollen foraging behavior could affect bees' pollination effectiveness in several non-mutually exclusive ways (Fig. 1). One, the total amount of pollen acquired from the flower might depend on foraging behavior. Greater pollen acquisition frequently translates to increased pollen deposition (e.g., Willmer et al. 1994; Thomson and Goodell 2001; Willmer and Finlayson 2015). Two, behavior may dictate the distribution of pollen acquired among body parts. For instance, pollen deposited on so-called safe sites (e.g., the venter of the thorax) is largely accessible to plant stigmas while also being protected from bee grooming (Herrera 1987; Huang et al. 2015; Koch et al. 2017; Tong and Huang 2017). Likewise, pollen stored by the bee in its pollen baskets for feeding (i.e., scopae or corbiculae) is in many bee taxa glued together with nectar, making it inaccessible for deposition or inviable for fertilization (Pankiw and Goplin 1967; Parker et al. 2015). Three, behavior could alter patterns of interference between conspecific and heterospecific pollen (Ashman and Arceo-Gómez 2013; Minnaar et al. 2018; Ashman et al. 2020). For example, behavior that results in greater acquisition of one species' pollen may bury another species' pollen on the bee body or prompt more intense grooming, thereby resulting in less conspecific pollen being transferred to the plant when multiple species are visited.

The degree to which intraindividual behavioral variation shapes pollination effectiveness may also depend on the fit between pollinator body and flower morphology (Herrera 1987; Minnaar et al. 2018; Moreira-Hernandez and Muchhala 2019; Wei et al. 2021). For example, smaller individuals may contact floral reproductive structures less frequently than larger individuals on a given flower species (e.g., Willmer and Finlayson 2015; Solís-Montero and Vallejo-Marín 2017; Konzmann et al. 2020), regardless of foraging behavior. Accordingly, differences in behavior might shape pollination effectiveness only when individuals are of good morphological fit with the flower. Likewise, the pollination effectiveness of a given behavior may depend on flower morphology (Fig. 1). For instance, a small stigma receives less total heterospecific and conspecific pollen than a larger stigma (e.g., Cruden and Miller-Ward 1981; Cruden 2000; Montgomery and Rathcke 2012). Or, for example, the orientation of flower reproductive parts may shape pollen placement on the pollinator, for instance on the pollinator's dorsum or venter (e.g., Muller 1996). While foraging behavior that alters pollen placement on the pollinator should alter subsequent pollen deposition on the flower, direct experimental manipulation of foraging behavior is very rare (see Tong et al. 2018; Wang et al. 2019).

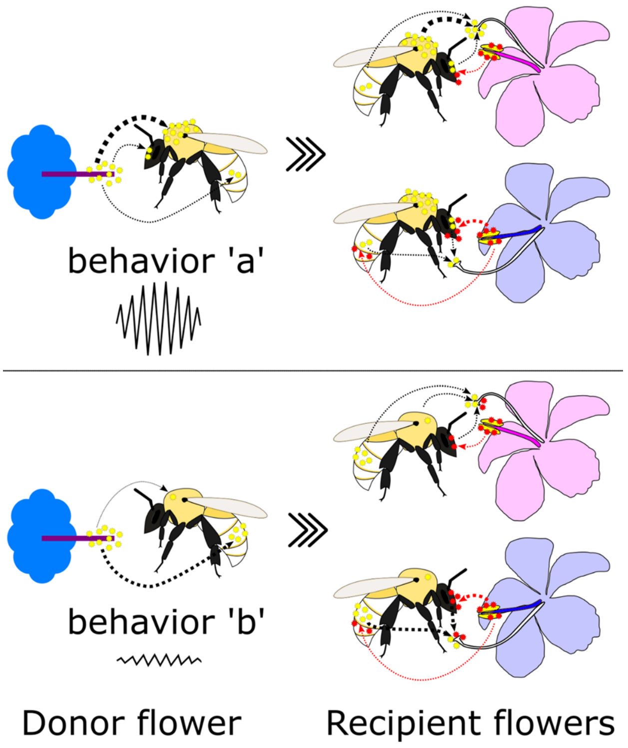

Fig. 1 Conceptual schematic showing how pollination effectiveness may depend on the interaction of pollinator behavior, pollen placement on the bee body, and flower morphology. Behavior **a** results in more total pollen acquired from the donor flower relative to behavior **b** and also alters pollen placement among bee body parts. Heterospecific (yellow) pollen transfer to the recipient flower depends on flower morphology (pink and blue flowers here differ in anther and style position) and pollen placement on the bee body; pink versus blue flowers always receive more heterospecific pollen and behavior **a** transfers more than behavior **b**. Conspecific (red) pollen is distributed differently among bee body parts depending on the recipient flower's morphology. Conspecific pollen transfer to the recipient flower depends on interference with heterospecific pollen on the bee body; behavior **a** causes more such interference than behavior **b**. Bee body size likely also affects each of these patterns (not shown)

In this laboratory study we tested whether intraindividual variation in the pollen foraging behavior and body size of the generalist bumble bee *Bombus impatiens* influenced their effectiveness as pollinators of three plant species (*Begonia odorata*, *Exacum affine*, and *Solanum houstonii*) that varied in floral morphology, most prominently in the size of the exposed stigma (Table 1; Fig. 2, S1). To control for potential consequences of pollen and floral morphology of donor flower on pollen dispersal, and to focus on behavior alone, we allowed bees to buzz or scrabble for a standardized pollen (heterospecific) from standardized artificial donor flowers. Thereafter these bees performed a single behavior (buzzing) when visiting recipient flowers of one of three plant species. We predicted that scrabbling versus buzzing behavior would affect the amount of donor pollen acquisition and deposition, but this would depend on bee body size and placement of pollen on the bee's body (Fig. 2e). Further, we predicted that the quantity and distribution of donor (heterospecific) pollen would affect the deposition of self-conspecific pollen. Additionally, we hypothesized that plant species with larger stigmas would receive more pollen overall than those with smaller stigmas, regardless of pollen foraging behavior. While we stimulated individual bumble bees to use only a single behavior under carefully controlled conditions, each bee would normally exhibit intraindividual variation by switching between scrabbling and buzzing.

Methods

Experimental subjects

To study how pollinator behavior affected pollen acquisition and deposition on flowers, we used 138 workers from

Table 1 Prominent features of the three recipient flowers

Recipient flower	Stigma	Anther	Petals	Pollen/flower (mg)	Pollen size (diameter)
Begonia odorata	Three very large bilobed stigmas	Not applicable (pistillate flower)	Five; large open flower	Not applicable (pistillate flower)	Not applicable (pistillate flower)
Exacum affine	Small stigma at the end of the single short style	Five short rigid anthers dehiscing by pores	Five; small open flower	2.22	22 μm
Solanum houstonii	Tiny stigma at the end of the single long style	Five short rigid anthers dehiscing by pores	Five fused into a corolla; large open flower	4.03	20 μm

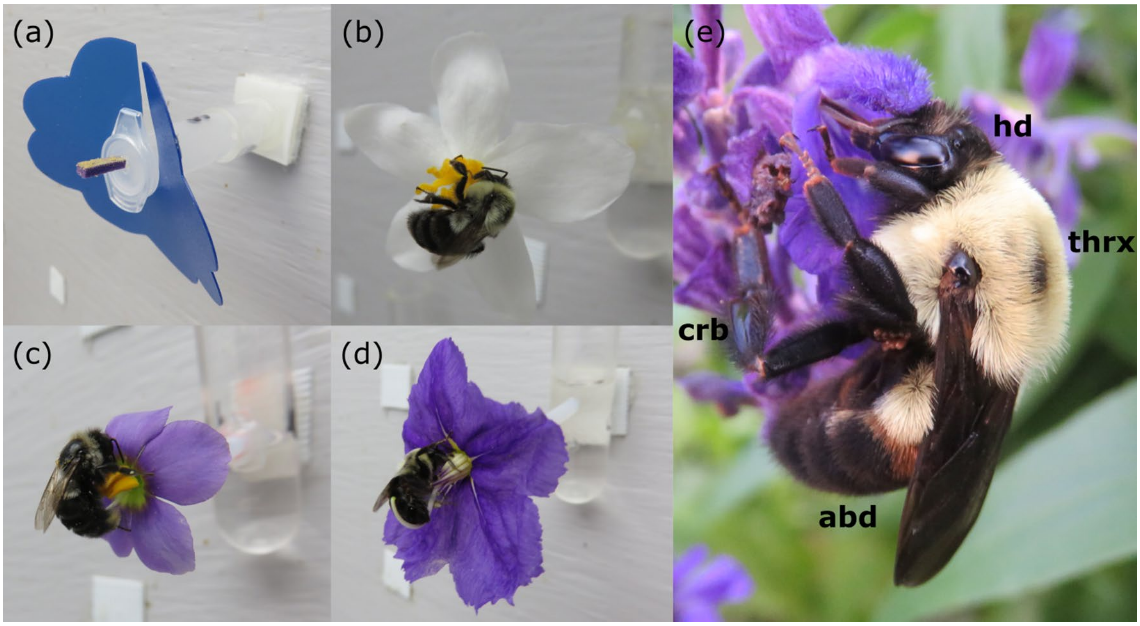

Fig. 2 Flowers used for behavioral trials and body parts examined for pollen acquisition. **a** Artificial donor flower with 1 mg of cherry pollen on the foam stamen. Bee (*B. impatiens*) visiting recipient **b** female *Begonia odorata* flower, **c** bisexual *Exacum affine* flower, and **d** bisexual *Solanum houstonii* flower. Styles of *B. odorata* are yellow; styles of *E. affine* and *S. houstonii* are white to the human eye. **e** Bee (*B. griseocollis*) with head (hd), thorax (thrx), abdomen (abd), and corbicula on the hind leg (crb) indicated

five captive commercially obtained colonies (Koppert Biological Systems, Howell, MI, U.S.A.) of the generalist and widespread bumble bee, *Bombus impatiens* (Plowright and Laverty 1984). In brief, following Russell et al. (2017a), we maintained colonies on 2 M sucrose solution and pulverized honey bee-collected pollen (Koppert Biological Systems) from artificial feeders within enclosed foraging arenas (LWH: 82 × 60 × 60 cm) set to a 14 h:10 h light: dark cycle.

We used artificial flowers as pollen donors, and we used live flowers from three plant species differing in floral morphology, including stigma surface area (Table 1; Fig. 1, 2) as recipients of heterospecific donor pollen (and donors and recipients of conspecific self-pollen). We constructed artificial flowers from blue polypropylene plastic (Index Dividers; Target, Pittsburgh, Pennsylvania, USA) fit around the mouth of 2-mL microcentrifuge tubes, with a purple foam cuboid 'stamen' (0.2 × 0.2 × 2 cm; Fibrecraft, Artist and Craftsman Supply, Pittsburgh, Pennsylvania, USA) glued to the center of the microcentrifuge cap (Fig. 2a). Live flowers were cut from 11 *Begonia odorata* (Begoniaceae), 65 *Exacum affine* (Gentianaceae), and 12 *Solanum houstonii* (Solanaceae) plants, which were grown in a greenhouse with supplemental halogen lights to extend day length to a 14:10 h light: dark cycle, and were fertilized weekly (PlantTone, NPK 5:3:3, Espoma, Millville, New Jersey, USA).

Experimental protocol

To precisely manipulate bee behavior, we loaded the stamen of each artificial donor flower with 1 mg of cherry pollen (*Prunus avium* pollen; Pollen Collection and Sales, Lemon Cove, California, USA). Cherry pollen is readily collected by bumble bees (e.g., Russell et al. 2019) and using a single type of donor pollen standardized for potential effects of pollen morphology and preference on foraging behavior. The live recipient flowers receive donor cherry pollen (heterospecific) as well as their own pollen (conspecific) during bee visits. To prevent desiccation, freshly cut live flowers were placed into custom water tubes (Russell et al. 2017a).

To initiate a behavioral trial we mounted a single donor flower on the arena wall in a cleaned test arena. From the foraging arena, a single flower-naïve female worker bee was gently captured from the nectar feeder using a 40 dram vial (Bioquip Products, Inc.) and released in the test arena. Before releasing a bee, we visually confirmed the absence of pollen on her body. During a single trial, we allowed a bee to first forage on four donor flowers across eight visits and then deposit donor pollen on a single recipient flower over four visits, as well as recipient self-pollen (conspecific pollen) (Figure S1). All flowers were presented sequentially and individually, such that only one flower was in the arena at a time. Each donor flower was replaced with the next after having received two foraging visits, using 'jumbo' forceps (BioQuip Products, Inc.) while the bee was in flight. Bees did not exhibit signs of being disturbed by our activity, such as aggressive behavior or attempts to escape from the arena. Replacement did not appear to interrupt visits to successive donor flowers, as replacement took approximately 3 s, less than the length of time between visits to the same recipient flower. The average length of a trial was 11 min and a typical flower visit lasted < 1 min. Immediately after trials, artificial stamens were discarded, bees were sacrificed and stored in a − 20 °C freezer for later body size measurements and pollen counting, and styles of recipient flowers were preserved in 70% ethanol for pollen counting. Bees stopped moving within < 1 min after being stored and containers were handled carefully to reduce the possibility of pollen being dislodged.

To test how foraging behavior on donor flowers affected pollen acquisition and deposition by bees on the flowers of different plant species, we divided flower-naïve bees into three treatments, with two sub-treatments each. Treatments differed in terms of the recipient flower that was used: either a female *B. odorata*, bisexual *E. affine*, or bisexual *S. houstonii* flower (Table 1; Fig. 2b–d), so chosen because bumble bees only buzz them and for their large differences in stigma surface area. The two sub-treatments differed in the foraging behavior we stimulated bees to use to collect pollen from donor flowers. In one sub-treatment, we stimulated bees to 'buzz' the stamens (see Russell et al. 2017a) and in the other sub-treatment we stimulated bees to scrabble with their legs for pollen ('scrabbling', see Russell and Papaj 2016). To stimulate buzzing, we soaked donor stamens in a *Solanum houstonii* stamen extract and let the solvent dry completely before adding cherry pollen (following Russell et al. 2017a; as a control, donor stamens in the 'scrabbling' treatment were soaked with the solvent minus the *S. houstonii* extract, prior to adding pollen). We systematically alternated assignment of bees to each sub-treatment to control for effects of time and day on behavior. Most bees buzzed donor flowers only in the buzzing treatment and scrabbled on donor flowers only in the scrabbling treatment. However, a minority of test bees in the scrabbling treatments only buzzed donor flowers, and we therefore re-assigned these bees to the buzzing treatment for analyses. Bees always buzzed the live recipient flowers and appeared to manipulate the recipient flower very similarly regardless of sub-treatment on recipient flowers of the same species. From analyses, we excluded sixteen bees that had failed to complete all 12 flower visits (leaving us with $N = 122$ bees, or ~ 20 bees/sub-treatment; some analyses used up to 6 fewer bees due to missing values).

To test whether pollen acquired by different body parts shaped heterospecific (donor) and conspecific (recipient self-pollen) pollen transfer to the recipient stigma, we dissected frozen bees into four parts: the head, thorax, abdomen, and hind legs (encompassing the pollen baskets; i.e., corbiculae; Fig. 2e). We washed and vortexed each body part separately and condensed each to 40uL using a centrifuge. We counted donor and conspecific pollen in a 10 uL aliquot using a hemocytometer (Hausser Scientific, Horsham, PA) at 400 × or 100 × (Leica DM 500) to arrive at an estimate for the total volume; if we counted zero grains in the first aliquot we counted grains in all four aliquots. Estimated pollen counts were rounded to the nearest whole number. To count pollen grains on flower stigmas, we acetolyzed styles from recipient flowers (following Dafni 1992) and counted pollen as above. Since patterns of pollen transfer might reflect differences in recipient stigma surface area or bee body size, we measured body size of each test bee (head width in mm) and the top–down 2D stigma surface area (in mm^2) of 10 representative recipient flowers of each plant species using a stereoscope and ImageJ (National Institutes of Health, Bethesda, MD, http://imagej.nih.gov/ij/) following Russell et al. (2017b).

Data analyses

All data were analyzed using R v.4.1.0 (R Development Core Team 2021).

Does body size and foraging behavior affect the quantity of donor pollen available for pollination?

Because bumble bees store pollen in their corbiculae to feed their colonies and this pollen is packed wet (with nectar) and thus largely unavailable for pollination, whereas pollen on the rest of the body remains viable and accessible for pollination (Parker et al. 2015), we analyzed how foraging behavior affected the proportion of donor pollen in the pollen baskets (corbiculae) versus on the rest of the body. To do so we used a generalized linear mixed effects model with a binomial distribution (GLMM) using the glmer() function in the lme4 package (Bates et al. 2015), specifying type II Wald Chi-square (χ^2) tests via the Anova() function in the car package (Fox 2015). The response variable was a binomial of pollen location (pollen in the corbiculae versus on the rest of the body) weighted by the total pollen count on the body; the explanatory variables were 'behavioral treatment' (scrabble or buzz) and 'body size' (head width). We included 'bee ID' within 'colony ID' as random factors. We checked model assumptions for all models using the DHARMa package (Hartig 2018).

To analyze how foraging behavior influenced the quantity of donor pollen on the body (but not in the corbiculae), we fit a zero-inflated negative binomial GLMM, using the glmmTMB() function in the glmmTMB package (Magnusson et al. 2018) and specifying Wald χ^2 tests as above. The response variable was 'body pollen not in the corbiculae', the explanatory variables were 'behavioral treatment' and 'body size', and we included 'bee ID' as a random factor (few GLMMs would converge with 'colony ID').

Do plant species, foraging behavior, and pollen placement on the bee shape donor pollen receipt?

We used a GLMM as above to analyze how foraging behavior, body size, and species of recipient flower influenced the amount of donor pollen transferred to recipient stigmas. The response variable was 'donor pollen on the stigma', the explanatory variables were 'behavioral treatment' and 'body size' and 'species of recipient flower', and we included 'bee ID' within 'colony ID' as a random factor.

We likewise used GLMMs to analyze how foraging behavior and donor pollen placement on the bee body influenced the amount of donor pollen transferred to recipient stigmas of *B. odorata*, *E. affine*, or *S. houstonii*. Large differences in pollen dispersal among species precluded use of a single model, even with rescaling and centering; we therefore used separate GLMMs for each plant species. To determine whether body size and bee body part(s) affected pollen transfer, we first ran maximal GLMMs with 'behavioral treatment', 'body size', and pollen on the head, thorax, abdomen, and corbiculae as explanatory variables. We treated body parts separately, because the frequency with which different body parts contact flower reproductive parts may depend on behavior and the morphological fit between plant and bee. To meet model assumptions we added 0.1 to body part pollen counts and log transformed them. We performed backward elimination using the anova() function in R to examine significance relative to the respective maximal model and found that only behavioral treatment and pollen on the abdomen had significant effects. Thus, for each of the three final GLMMs, the response variable was 'donor pollen on the stigma', the explanatory variables were 'behavioral treatment' and 'donor pollen on the abdomen', and 'bee ID' was included as a random factor.

Does foraging behavior and pollen placement on the bee influence heterospecific–conspecific pollen interference?

To analyze how foraging behavior on the donor flowers and donor (heterospecific) pollen placement on the bee body affected recipient self (conspecific) pollen transfer to recipient stigmas of *E. affine* and *S. houstonii*, we also used GLMMs. Methods and models were the same as above, only with 'conspecific pollen on the stigma' as the response variable and an additional explanatory variable, 'donor pollen on the thorax'.

Results

Body size, but not foraging behavior, affected the quantity of donor pollen available for pollination

Pooled across the three treatments, bumble bees acquired on average (± SE) 10,532 ± 660 pollen grains from artificial donor flowers, with 0.7% on the head, 1.2% on the thorax, 1.2% on the abdomen, and 96.9% collected into the corbiculae (Table 2, $N = 114$ bees; Fig. 2e). Foraging behavior had no significant effect on the proportion of donor pollen in the corbiculae versus on the rest of the body (Fig. 3a; GLMM: $\chi^2_1 = 0.95$, $P = 0.33$). This pattern was the same across bee body sizes (Fig. 3a; GLMM: effect of body size: $\chi^2_1 = 1.36$, $P = 0.24$; effect of behavior X body size: $\chi^2_1 = 0.17$, $P = 0.68$). Excluding pollen in the corbiculae, scrabbling and buzzing behaviors lead to acquisition of comparable amounts of donor pollen on bee bodies (i.e., presumably available for pollination), however, contrary to prediction, larger bees acquired significantly less pollen than smaller bees (Fig. 3b; GLMM: effect of behavior: $\chi^2_1 = 0.59$, $P = 0.44$; effect of body size: $\chi^2_1 = 6.65$, $P < 0.01$; effect of behavior X body size: $\chi^2_1 = 1.16$, $P = 0.28$).

Plant species influenced donor pollen receipt, consistent with differences in stigma surface area, as did foraging behavior

Bees transferred 16% of all acquired donor flower pollen to recipient *Begonia odorata* stigmas, 0.50% to recipient *Exacum affine* stigmas, and 0.24% to recipient *Solanum houstonii* stigmas within four visits (Table 2). Furthermore, while all bees acquired pollen from donor flowers, 100%, 81%, and 40% of bees transferred donor pollen to the stigmas of recipient *B. odorata*, *E. affine*, and *S. houstonii*, respectively. These patterns of pollen receipt followed expectations based on the surface area of recipient stigmas (mm^2 ± SE: *B. odorata*: 18.4 ± 0.54; *E. affine*: 1.6 ± 0.12; *S. houstonii*: 0.48 ± 0.03; $N = 10$ per species). Pollen receipt was significantly different among plant species and was also significantly affected by foraging behavior: bees that had scrabbled on the donor flower deposited on average 22% more donor pollen than bees that had buzzed (Fig. 4a; GLMM: effect of recipient species: $\chi^2_1 = 686.19$, $P < 0.0001$;

Table 2 Quantity of donor heterospecific pollen (HP) and conspecific pollen (CP) (mean ± SE) acquired by bee body parts and received by the recipient flower

Origin of the acquired pollen	Quantity of pollen deposited (based on counts after trials)				
	On the head	On the thorax	On the abdomen	In the corbiculae	On the recipient stigma
Donor flower	37 ± 6	101 ± 17	85 ± 21	10,113 ± 644	NA
Begonia odorata	NA	NA	NA	NA	HP 1290 ± 126 CP NA
Exacum affine	810 ± 162	4213 ± 686	2113 ± 734	53,276 ± 4325	HP 43 ± 7 CP 2371 ± 334
Solanum houstonii	292 ± 86	231 ± 113	805 ± 183	29,466 ± 3179	HP 10 ± 2 CP 216 ± 31

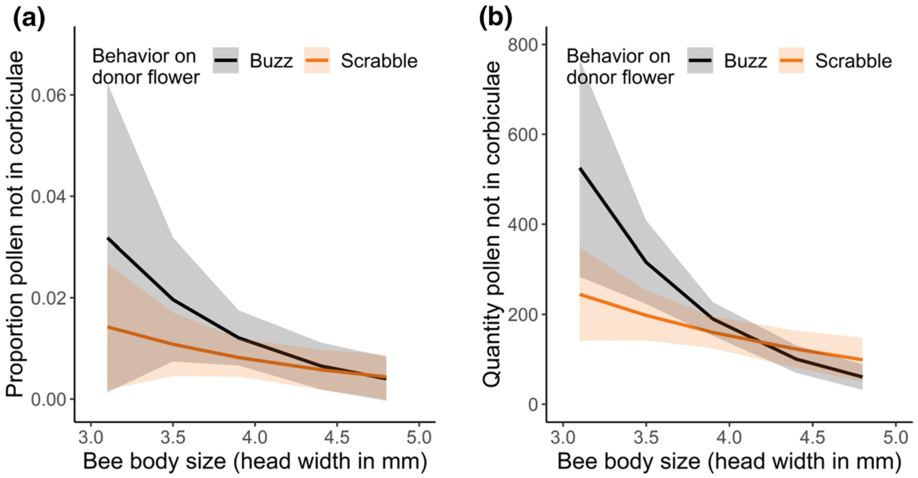

Fig. 3 Effects of donor flower foraging behavior and bee body size pooled across all treatments on **a** the proportion of donor pollen in the corbiculae versus on the rest of the body or on **b** the quantity of donor pollen on the body (excluding corbicular pollen). $N = 116$ bees. Plotted lines indicate estimated means and shaded regions indicate standard errors

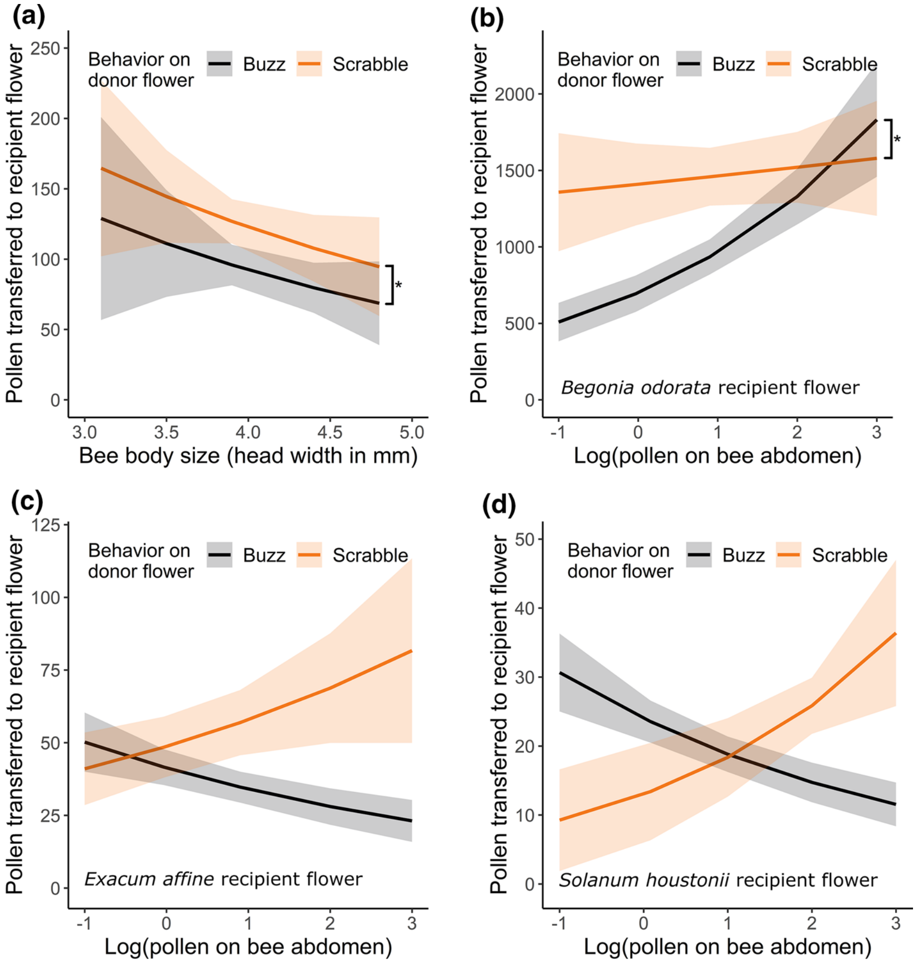

Fig. 4 The quantity of donor pollen transferred to the recipient flower's stigma as shaped by foraging behavior on donor flowers and **a** bee body size (for ease of presentation, effects of recipient flower treatment are not shown) or **b**–**d** the quantity of donor pollen on the bee's abdomen. Treatments in which the recipient flower is of **b** *Begonia odorata*, **c** *Exacum affine*, or **d** *Solanum houstonii*. The number of bees in buzz and scrabble sub-treatments, respectively, for treatments **a** $N = 20$ and 18, **b** $N = 20$ and 18, and **c** $N = 19$ and 21. Plotted lines indicate estimated means and shaded regions indicate standard errors. Asterisks indicate significant differences in the mean pollen transferred to recipient stigmas among behavior sub-treatments at $P < 0.05$

effect of behavior: $\chi^2_1 = 6.61$, $P < 0.011$; effect of behavior × recipient species: $\chi^2_2 = 0.09$, $P = 0.96$). Bee body size did not significantly affect patterns of pollen receipt (Fig. 4a; GLMM: effect of body size: $\chi^2_1 = 2.64$, $P = 0.10$; effect of body size × behavior: $\chi^2_1 = 0.04$, $P = 0.84$; effect of body size × recipient species: $\chi^2_2 = 1.16$, $P = 0.56$; effect of body size × recipient species × behavior: $\chi^2_2 = 3.30$, $P = 0.19$; the effect of body size did not differ among levels of categorical factors: $\chi^2_1 = 3.06$, $P = 0.08$).

Foraging behavior and pollen placement on the bee affected donor pollen receipt by the stigma

Foraging behavior on the donor flower and pollen placement on the bee body significantly affected the quantity of donor pollen deposited on stigmas, but effects differed with the recipient species (Fig. 4). For recipient *B. odorata*, bee behavior and quantity of pollen on the abdomen significantly affected the amount of pollen deposited on stigmas, with bees that had scrabbled on the donor flower depositing on average 19% more pollen than bees that had buzzed, and with bees with more pollen on their abdomen depositing increasingly more pollen (Fig. 4b; GLMM: effect of behavior: $\chi^2_1 = 4.17$, $P < 0.042$; effect of abdominal pollen: $\chi^2_1 = 7.70$, $P < 0.006$; behavior × abdominal pollen: $\chi^2_1 = 3.57$, $P = 0.059$). For recipient *E. affine*, the effect of foraging behavior depended on the quantity of pollen on the abdomen, such that with increasing abdominal pollen, bees that had scrabbled on donor flowers deposited more pollen on recipient stigmas than bees that had buzzed donor flowers (Fig. 4c; GLMM: effect of behavior: $\chi^2_1 = 2.29$, $P = 0.13$; effect of abdominal pollen: $\chi^2_1 = 0.59$, $P = 0.44$; effect of behavior × abdominal pollen: $\chi^2_1 = 4.26$, $P < 0.039$). For recipient *S. houstonii*, the effect of foraging behavior also depended on the quantity of pollen on the abdomen. Here also, with increasing abdominal pollen, bees that had scrabbled on donor flowers deposited more pollen on recipient stigmas than bees that had buzzed donor flowers (Fig. 4d; GLMM: effect of behavior: $\chi^2_1 = 3.68$, $P = 0.055$; effect of abdominal pollen: $\chi^2_1 = 3.83$, $P = 0.05$; effect of behavior × abdominal pollen: $\chi^2_1 = 4.61$, $P < 0.032$).

Foraging behavior and pollen placement on the bee can influence heterospecific–conspecific pollen interference

For the bisexual flowers of *S. houstonii* and *E. affine*, we could examine patterns of self-conspecific pollen transfer concurrent with donor (heterospecific) pollen transfer. Bees acquired on average 31,014 ± 3194 pollen grains from *S. houstonii*, with 1.4% on the head, 1.1% on the thorax, 4% on the abdomen, and 93.5% collected into the corbiculae (Table 2; $N = 39$ bees). Bees acquired on average 61,130 ± 4783 pollen grains from *E. affine*, with 1.6% on the head, 7.9% on the thorax, 3% on the abdomen, and 87.5% collected into the corbiculae (Table 2; $N = 39$ bees).

Prior foraging behavior on donor flowers and donor pollen placement on the bee significantly affected conspecific pollen transfer for one recipient (*S. houstonii*), but not the other (*E. affine*), (Fig. 5). Bees that had scrabbled on donor flowers deposited on average 50% less conspecific pollen on recipient stigmas than bees that had buzzed on the donor. The effect of foraging behavior depended significantly on the quantity of donor pollen on the abdomen and thorax, though in opposite ways. Bees that had buzzed versus scrabbled on donor flowers deposited more conspecific pollen on recipient *S. houstonii* stigmas as donor pollen on the abdomen decreased or donor pollen on the thorax increased. (Fig. 5a, b: GLMM: effect of behavior: $\chi^2_1 = 3.76$, $P = 0.053$; effect of abdominal pollen: $\chi^2_1 = 7.35$, $P < 0.007$; effect of thoracic pollen: $\chi^2_1 = 7.18$, $P < 0.008$; effect of behavior × abdominal pollen: $\chi^2_1 = 5.79$, $P < 0.017$; effect of behavior X thoracic pollen: $\chi^2_1 = 4.90$, $P < 0.027$; effect of abdominal pollen × thoracic pollen: $\chi^2_1 = 0.10$, $P = 0.75$; effect of behavior × abdominal pollen X thoracic pollen: $\chi^2_1 = 1.42$, $P = 0.24$).

Discussion

Our results shed light on how inter- and especially intraindividual variation affects the acquisition and deposition of pollen on flowers. Bee body size, which varied substantially (by a factor of 1.5), but not foraging behavior type (scrabbling versus buzzing, which individual bees frequently switch between; see Russell et al. 2017a), affected the total amount of pollen acquired from flowers, not accounting for losses during a trial. Conversely, foraging behavior type, but not bee body size, ultimately affected pollen deposition on flowers. Morphological fit between flower and pollinator is often considered a key factor shaping pollination effectiveness (Herrera 1987; Minnaarr et al. 2018; Moreira-Hernandez and Muchhala 2019; Wei et al. 2021). However, our results suggest that at least in some cases, intraindividual variation in behavior may be a stronger arbiter of pollination effectiveness than morphological fit between flower and bee. In addition, while field studies have reported that pollinator body size is correlated with pollen deposition on flowers (e.g.,Willmer and Finlayson 2015; Konzmann et al. 2020), differences in body size also frequently correspond to differences in foraging behavior (e.g., intraspecifically in bumble bees: Jauker et al. 2016; Stout 1999; De Luca et al. 2019). Although we did not find evidence that behavior varied with body size and affected pollen acquisition or deposition, we might have constrained any possible interaction by explicitly manipulating behavior independent of variation in bee body size. Future work is needed to determine whether body size constrains behavioral variation and how this potential interaction between behavior and body size affects pollen deposition.

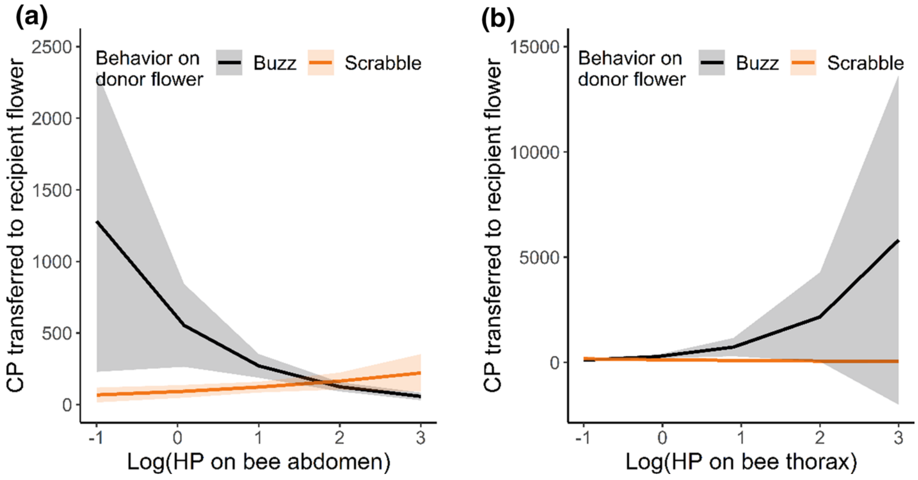

Fig. 5 The quantity of conspecific pollen (CP) transferred to the recipient *Solanum houstonii* stigma as shaped by foraging behavior on donor flowers and **a** the quantity of donor pollen (HP) on the bee's abdomen or **b** the quantity of donor pollen (HP) on the bee's thorax. Data were analyzed in a single model, but effects of bee body part are shown in separate panels for ease of presentation. $N = 19$ and 20 for bees in buzz and scrabble sub-treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate standard errors

Bee behavior affects acquisition and deposition

Just as one pollinator species or sex may be unmistakably more effective in terms of pollen deposition than another (Minnaar et al. 2018; Moreira-Hernandez and Muchhala 2019; Smith et al. 2019), across plant species, scrabbling behavior was unambiguously more effective in terms of pollen deposition on recipient stigmas than buzzing (resulting in 22% more pollen deposition on average). This is possibly due to differences in pollen placement between behaviors at a finer scale than we measured or is a result of the intense vibrations during buzzing leaving less loosely attached pollen available for pollination. Assuming our results are representative of flower-bee interactions, we might expect selection to favor floral traits that would either reduce the expression of buzzing by bees (especially as individuals readily switch between behaviors; Russell et al. 2017a) or enhance the effectiveness of buzzing relative to other behaviors in terms of pollination. In accordance with this second possibility, many plant species have evolved to be pollinated nearly exclusively by buzzing bees and possess traits presumably adapted to enhancing pollination by buzzing bees, a widespread mutualism known as buzz pollination (Buchmann 1983; De Luca and Vallejo-Marín 2013; Cardinal et al. 2018). In addition, we uncover key nuance in the relationship between foraging behavior and pollen deposition: the effect of behavior on pollen deposition varied with the amount of pollen acquired by the bee for two of the three plant species. This result suggests that the amount of pollen exported by the flower determines the pollination effectiveness of one foraging behavior relative to another.

In addition to strong effects of behavior on pollen deposition, we uncovered large differences in pollen deposition among plant species. Although we cannot disentangle whether differences in pollen deposition result from differences in stigma surface area or other differences in flower morphology, our results strongly suggest stigma surface area is important (see also Yang et al. 2002; Montgomery and Rathcke 2012; Wei et al 2021). Not only did larger stigma surface area correlate quantitatively with pollen receipt (e.g., pollen number), but the likelihood of pollen receipt also correlated with larger stigma surface area. Further, while we used a single type of donor pollen to control for effects of pollen morphology and preference on acquisition and deposition of pollen, pollen morphology can strongly affect pollen acquisition by pollinators (e.g., Roberts and Vallespir 1978; Russell et al. 2017a; Konzmann et al. 2019; Lynn et al. 2020). Yet how pollen deposition on heterospecific stigmas is affected by pollen and stigma characteristics is unknown. Future work should examine how variation in behavior, pollen and stigma characteristics might interact to drive patterns of pollen deposition.

Distribution of pollen on bees affects transport

Spatial segregation of pollen deposition on pollinator bodies is frequently proposed to dictate exact placement of pollen on flower stigmas (e.g., Muchhala and Thomson 2012; Huang et al. 2015; Tong and Huang 2017; Minnaar et al. 2018). Consistent with this, while donor pollen from artificial flowers was acquired in similar proportion by the head, thorax, and abdomen of foraging bees, only donor pollen on the abdomen significantly influenced the amount of pollen deposition. In addition, parts of pollinator bodies that are ineffectively groomed have been shown to be especially important in pollination (e.g., Buchmann et al. 1990; Koch et al. 2017; Tong and Huang 2017). These so-called safe sites have been found at least along the midline of the dorsal head and thorax and ventral and dorsal abdomen (Buchmann et al. 1990; Koch et al. 2017; Tong and Huang 2017). While we did not characterize safe sites, our results implicate safe sites on the abdomen as being particularly important to pollen receipt, at least for the plant species tested here. Moreover, it would be useful to examine whether the efficacy of grooming might be affected by pollinator body size or even prior foraging behavior. Furthermore, we confirm results of previous studies, finding that across plant species, pollen in the corbiculae (pollen baskets) contributes little to pollination, that most of the pollen (98% of the donor pollen) acquired by bees was packed into their pollen baskets, and that among plant species, between 16 and $<1\%$ of pollen acquired by the bee was transferred to the stigma (Pankiw and Goplin 1967; Hargreaves et al. 2009; Parker et al. 2015).

Consequences for mixed pollen load on plant stigmas

Heterospecific pollen receipt is common in mixed species communities in the wild (e.g., Fang and Huang 2013; King et al. 2013; Johnson and Ashman 2019), and has been proposed to be a result of plant generalization (Arceo-Gómez et al. 2016; Ashman et al. 2020; Wei et al. 2021), or pollinator traits such as generalism (Cullen et al 2021). Our results go further to demonstrate that within species behaviors on the flower can also contribute to patterns of heterospecific and conspecific pollen receipt. In particular, we found that when heterospecific pollen was acquired first, even a very small quantity on the bee could reduce conspecific pollen transfer to the stigma. Interestingly, averaging across plant species, 97% of the pollen deposited on the stigma (and 93% of the pollen available for pollination on the bee body) was conspecific. Given the small amount of heterospecific pollen on the bodies of our bees, it seems unlikely that interference was a result of limited space on the bee (see Minnaar et al. 2018). Instead, perhaps heterospecific pollen acquisition elicited increased bee grooming throughout a trial, resulting in a subsequent greater loss of conspecific pollen. Future work should also consider whether recipient flower species differ in how quickly they remove pollen from different bee body parts, which could have significant repercussions for heterospecific pollen transfer.

In addition, our results provide rare, though preliminary, evidence that spatial segregation of pollen on the pollinator body can reduce heterospecific pollen transfer (see also Muchhala and Thomson 2012; Armbruster et al. 2014a, b; Huang et al. 2015). Here, reduced heterospecific pollen on the abdomen specifically increased conspecific pollen transfer. These insights call for more integrative work examining how pollinator foraging behavior and floral morphology interact to influence community-wide patterns of heterospecific and conspecific pollen transfer. Such an agenda would add a fresh dimension to the growing body of research that suggests plant generalization is the primary driver, and shift the focus to the role of pollinator generalization instead (see Arceo-Gómez et al. 2016; Ashman et al. 2020; Wei et al. 2021; Cullen et al. 2021).

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Author contribution statement ALR, AMF, EIJ, and TLA conceived and designed the experiments. ALR, AMF, and EIJ performed the experiments. ALR analyzed the data. ALR wrote the manuscript; other authors provided editorial advice.

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Data availability The datasets supporting this article are available as electronic supplementary material.

Declarations

Conflict of interest Not applicable.

Ethical approval All bumble bee experimentation was carried out in accordance with the legal and ethical standards of the USA.

Consent to participate Not applicable.

Consent for publication Not applicable.

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