

# Hold tight or loosen up? Functional consequences of a shift in anther architecture depend substantially on bee body size

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## Research Article

**Keywords:** anther cone, buzz pollination, bees, interspecific pollen transfer, pollen movement, pollinator morphology

**Posted Date:** May 31st, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1652058/v1>

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# Abstract

A fundamental question in pollination ecology is how pollinators affect the evolution of different floral forms. Yet functional effects of shifts in floral form for plant and pollinator are frequently unclear. For instance, flowers that conceal pollen within tube-like anthers that are spread apart and move freely (free architecture) or are tightly joined together (joined architecture) have evolved independently across diverse plant families and are geographically widespread. Surprisingly, how their bee pollinators affect the function of both architectures remains unknown. We hypothesized that bee body size would affect foraging success and pollination differently for free and joined anther architectures. We therefore modified the anther architecture of a single plant species (*Solanum elaeagnifolium*) and used a single species of generalist bumble bee (*Bombus impatiens*), which varies greatly in body size. We found that on free anther architecture, larger bees were better pollinators. More pollen on their bodies was available for pollination and they deposited more pollen on stigmas. Conversely, on joined anther architecture, smaller bees were better pollinators. They collected less pollen into their pollen baskets, had more pollen on their bodies available for pollination, and deposited more pollen on stigmas. While we also found modest evidence that plants benefit more from joined versus free anther architecture, further investigation will likely reveal this also depends on pollinator traits. We discuss potential mechanisms by which pollinator size and anther architecture interact and implications for floral evolution.

## Introduction

Pollinators are a primary driver of floral trait evolution and traits including flower colours, scents, and morphologies frequently reflect selection from pollinator behaviour and morphology (Darwin 1877; Barrett 2010; Johnson and Anderson 2010; Schiestl and Johnson 2013). For instance, the flowers of many plant species have evolved ultraviolet-absorbing centres that contrast strongly with the rest of the flower and are thought to reflect selection by pollinators with strong preferences for these colour patterns (Silberglied 1979; van der Kooi et al. 2019). Likewise, a variety of flowering plant taxa have evolved specialized flowers with long nectar spurs, reflecting selection by insect pollinators with long proboscises (Hodges et al. 2004; Vlašánková et al. 2017). Accordingly, floral traits evolving via pollinator-mediated selection affect pollination and foraging success. In particular, pollinator-mediated selection on flowering plants favours the evolution of floral traits that enhance the dispersal of pollen to pollinators and maximize the transfer of pollen to conspecific stigmas, while simultaneously minimizing pollen wastage (e.g., pollen lost or consumed as food by the pollinator) (Harder and Wilson 1994; De Kock et al. 2018). Yet while the functional effects of floral traits should depend on how plant and pollinator interact (e.g., Fukuda et al. 2014; Hopkins et al. 2014; Hazlehurst and Karubian 2016), consequences for pollination and foraging success are poorly understood (but see Betts et al. 2015; De Kock et al. 2018; Lichtenberg et al. 2018).

Modifications of anther architecture (i.e., sizes, degree of fusion, and spatial/functional connections) are common and thought to significantly affect flower function (Endress 2012; Nevard et al. 2021). For example, anthers frequently vary in the degree to which they are joined in the flowers of buzz pollinated plants, yet the functional significance of this variation is imperfectly understood (Faegri 1986; Vogel

1978; Glover et al. 2004; Vallejo-Marín et al. 2022). Such plants are pollinated by bees capable of generating powerful vibrations ('floral buzzing'; performed by an estimated 58% of bee species; Cardinal et al. 2018), which expel pollen from the terminal pores of the tube-like poricidal anthers (Macior 1968; Vallejo-Marín 2019; Brito et al. 2020). Many of these buzz pollinated plant species possess flowers whose anthers are spread apart and capable of relatively independent movement ('free anther architecture') (Glover et al. 2004; Vallejo-Marín et al. 2022). Yet many other buzz pollinated species across taxonomically diverse families have independently evolved a joined anther architecture, in which poricidal anthers are arranged and joined in the centre of the flower, resembling a cone (Fig. 1; found in at least 21 plant families, see supplementary; Vogel 1978; Faegri 1986; Glover et al. 2004; De Luca and Vallejo-Marín 2013; Russell et al. 2016). Both joined and free anther architectures are widely distributed among buzz pollinated species, and floral buzzing is expected to affect pollen expulsion differently depending on the degree to which the anthers are joined (Glover et al. 2004; Nevard et al. 2021; Vallejo-Marín et al. 2022). Despite this, the functional significance of both anther architectures for flower and bee remain unclear (see Vallejo-Marín et al. 2022).

The degree to which anthers are joined could affect pollination and foraging success in complementary or opposing ways. For instance, joined anther architecture could simultaneously enhance pollination (benefitting the plant) and pollen collection (benefitting the bee). Given that simulated bee vibrations are propagated more effectively and pollen release is increased when anthers are joined (Nevard et al. 2021; Vallejo-Marín et al. 2022), a bee might be able to collect more of the released pollen. Increased pollen release might also translate to more pollen deposited on the stigma. Alternatively, joined or free anther architecture could enhance either pollination or pollen collection, but not both. For instance, increased pollen release from joined anther architecture might enhance collection by the bee, without resulting in more pollen transferred to conspecific stigmas (e.g., Russell et al. 2021). This would be expected, for instance, if the anther cone more consistently deposited pollen in a readily groomed location on the bee. In contrast, loosely held, sprawling anthers of free anther architectures may more readily distribute pollen to so-called safe-sites on the bee, which are accessible to plant stigmas, but protected from bee grooming (Herrera 1987; Harder and Barclay 1994; Huang et al. 2015; Koch et al. 2017; Tong and Huang 2017). Similarly, if free anther architecture releases less pollen (Vallejo-Marín et al. 2022), more pollen may remain in the anthers for subsequent pollinators, resulting in more opportunities for pollination. Reduced pollen release might even entice a given pollinator into spending more time on the flower, thereby enhancing pollen transfer to the stigma.

How anther architecture affects pollination and foraging success also likely depends on pollinator characteristics. Pollination effectiveness is considered to be generally influenced by the physical fit between flower morphology and pollinator body, which can, for instance affect the removal of pollen and contact with floral reproductive structures (Herrera 1987; Minnaar et al. 2018; Moreira-Hernandez and Muchhala 2019; Russell et al. 2021). Given that body size frequently varies both within and among bee species (Cariveau et al. 2016; Cullen et al. 2021), physical fit may strongly influence how a given anther architecture affects pollination and foraging success. For instance, relative to larger bees, smaller bees might be less effective pollinators on flowers with free anther morphology, because their smaller bodies

would be less likely to contact the stigma as they move among anthers (Li et al. 2015; Solis-Montero and Vallejo-Marin 2017; Mesquita-Neto et al. 2021). Likewise, given that joined anthers vibrate together (Nevard et al. 2021), relatively larger bees might be less effective pollinators on flowers with joined anther morphology, because their more powerful vibrations (De Luca et al. 2013; 2019; Switzer et al. 2019) could enable them to collect more pollen or deplete anthers more completely, leaving less pollen to be transferred to conspecific stigmas by subsequent visitors.

In this laboratory study, we assessed how pollination and foraging success in a plant-pollinator mutualism were influenced by anther architecture and pollinator body size. To control for differences among species, we modified the anther architecture of a single plant species (*Solanum elaeagnifolium*) and used a single species of bumble bee (*Bombus impatiens*), which varies substantially in body size, even among individuals within a given colony (e.g., by a factor of 3.2 in size and 10-fold in body mass; Harder 1985; Couvillon et al. 2010; Kelemen et al. 2022). We hypothesized that pollination and foraging success would differ between anther architectures, with foraging bees leaving less pollen within joined anther architecture (the experimentally modified condition), and pollen being deposited more precisely on the bee, resulting in more pollen collection by the bee, and in less pollen ultimately transferred to stigmas. We also hypothesized that bee body size would substantially affect these patterns, with smaller bees leaving more pollen, collecting less pollen, and spending more time on joined anther architecture, and transferring less pollen to stigmas of flowers with free anther architecture (the natural condition).

## Methods

### Experimental subjects

To study how anther architecture affected pollen acquisition by bees and deposition on flowers, we used 67 workers from three captive commercially obtained colonies (Koppert Biological Systems, Howell, MI, USA) of the common eastern bumble bee, *Bombus impatiens*. Briefly, following Russell et al. 2017a, each colony was maintained on 2 M solution of sucrose and pulverized honeybee-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.

To donate and receive pollen, we used *Solanum elaeagnifolium* flowers (hereafter, '*Solanum*'), which across their natural range are commonly pollinated by bumble bees (Knapp et al. 2018). Flowers used in trials were freshly cut from eight plants grown in a greenhouse (seeds originally collected from plants 2.5 Km NNE of Portal, Arizona) 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

We modified the naturally free-moving anthers of *Solanum* flowers into two configurations for trials: free and joined (Fig. 2) (Vallejo-Marín et al. 2022). In the joined configuration, a small amount of PVA glue was applied to the lateral edges of anthers, which were held together tightly with the aid of a plastic straw

while the glue dried over 5 minutes. Flower styles, which were approximately centred among the free moving anthers (Fig. 2a), were also positioned at the centre of anther cones (Fig. 2b), mimicking the typical condition in plant species with naturally joined anthers (Fig. 1). In the free configuration we constructed sham flowers by applying glue to the lateral edge of anthers without pressing them together, to control for potential effects of glue on bee behaviour and pollen removal (Fig. 2a). To prevent desiccation, all freshly cut live flowers were placed into custom water tubes (Russell et al. 2017a).

To test how anther architecture affected pollen acquisition and deposition by bees, we divided flower-naïve bees into two treatments that differed in whether only free or only joined flowers were provided (Fig. 2). We systematically alternated assignment of bees to each treatment to control for effects of time and day on behaviour. To initiate a behavioural trial, we mounted four flowers in a 2 × 2 grid 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 forage twice on each of the four donor flowers for a total of eight visits. Each donor flower was removed from the arena after having received the 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 behaviour or attempts to escape from the arena. Removing a flower did not appear to interrupt visits to the other flowers, as removal took approximately 3 seconds, less than the length of time between visits to a given flower. Twelve bees made fewer or more than 8 visits, divided equally among joined and free treatments: we included these bees in analyses. Because time spent visiting flowers could have influenced pollen acquisition, collection, and deposition, trials were video recorded to measure the total flower handling time per trial; from three legs on the flower until physical contact with the flower ended (via Avidemux 2.7.6). The average time spent on all flowers in a trial was 2.7 minutes and a typical flower visit lasted 22 seconds.

Immediately after a trial, the anthers of the four flowers were pooled, as were the styles, and preserved in 70% ethanol for pollen counting, and the bee was sacrificed and stored in a -20°C freezer for later body size measurements and pollen counting. Bees stopped moving within < 1 minute of being stored and containers were handled carefully to limit the possibility of pollen being dislodged. Each bee was only used in a single trial. From analyses, we excluded 7 bees that failed to collect pollen into their pollen baskets, leaving  $N = 28$  and 32 bees within free and joined treatments, respectively.

## **Pollen counting and body size measurements**

To test whether pollen acquired by different body parts affected pollen transfer to flowers, we dissected frozen bees into four parts: the head, thorax, abdomen, and hind legs (encompassing the pollen baskets, i.e., corbiculae). We washed and vortexed each body part separately and condensed head, thorax, and abdomen samples to 100 uL each and hind leg samples to 1000 uL each using a centrifuge. We counted pollen in three 10 uL aliquots using a haemocytometer (Hausser Scientific, Horsham, PA) at 400× or 100× (Leica DM 500) to arrive at an estimate for the total volume. Estimated pollen counts were rounded to the nearest whole number. To count pollen grains on flower stigmas, we acetolyzed styles from flowers

(following Dafni 1992) pooled by trial, condensed samples to 40 uL, and counted pollen in two aliquots; if we counted zero grains, we counted grains in all four aliquots. To test whether anther architecture affected pollen removal by bees, anthers from flowers pooled by trial were acetolyzed and pollen counted in 1000 uL samples as above. To estimate the average amount of pollen removed from flowers, we also counted pollen from the anthers of 20 unvisited flowers (pooled in groups of 4 flowers) as a baseline.

Since patterns of pollen removal, acquisition, collection, and deposition might reflect differences in bee body size, we also measured body size of each test bee (head width at the widest position in mm) 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 analysed using R v.4.1.0 (R Development Core Team 2021). We checked for overdispersion, zero inflation, and uniformity for all models using the DHARMA package (Hartig 2018) and log transformed all pollen counts to meet model assumptions.

## Does anther architecture, flower handling time, and body size affect pollen remaining within the anthers?

To analyse how the amount of pollen bees left within the anthers was influenced by anther architecture, bee body size, and the amount of time bees handled flowers, we used a generalized linear mixed effects model (GLMM) with a Gaussian distribution using the `glmmTMB()` function in the `glmmTMB` package (Magnusson et al. 2018), specifying type II Wald chi-square ( $\chi^2$ ) tests via the `Anova()` function in the `car` package (Fox 2015). The response variable was 'pollen in anthers' (number of grains), and the explanatory variables were 'anther architecture treatment' (free vs joined), 'flower handling time' (total time spent handling flowers), and 'body size' (head width). We included 'colony ID' as a random factor.

### Does anther architecture, flower handling time, and body size affect pollen collection and acquisition by the bee?

Because bumble bees collect pollen into their corbiculae to feed their colonies and this pollen is packed wet (with nectar) and thus largely unavailable for pollination ('collection'), while pollen acquired on the rest of the body remains viable and accessible for pollination ('acquisition') (Parker et al. 2015), we considered both reservoirs of pollen. To analyse how the quantity of pollen accessible for pollination or in the pollen baskets was influenced by anther architecture, bee body size, and the amount of time bees handled flowers, we used two GLMMs with explanatory variables and random factor specified as above. The response variables differed for each GLMM and were 'pollen in the pollen baskets' (number of grains collected in the corbiculae) or 'pollen available for pollination' (number of grains acquired on the body, excluding the corbiculae).

Additionally, we analysed how the proportion of donor pollen in the pollen baskets versus on the rest of the body was influenced by the same factors, via a GLMM with a binomial distribution. The response variable was a binomial of pollen type (pollen in the corbiculae versus on the rest of the body) weighted by the total pollen count on the body; explanatory variables were as above. For this model only, we included 'bee ID' within 'colony ID' as random factors.

### **Does anther architecture, flower handling time, body size, and pollen placement on the bee affect pollen receipt by the stigma?**

To analyse how the amount of pollen bees deposited on stigmas was influenced by anther architecture, bee body size, bee body part, and handling time, we first ran a maximal GLMM, with 'anther architecture treatment', 'flower handling time', 'body size', and pollen on the head, thorax, and abdomen as explanatory variables. We treated body parts separately, because the frequency with which different body parts contact flower reproductive parts may depend on the physical fit between plant and bee, which could have been altered by anther architecture treatment. We performed backward elimination using the `anova()` function in R to examine significance relative to the respective maximal model, finding that all body parts contributed similarly. Thus, for the final GLMM, the response variable was 'pollen on the stigma' and the explanatory variables were 'anther architecture treatment', 'flower handling time', 'body size', and 'pollen available for pollination' (number of grains on the body, but not in the corbiculae). We included 'colony ID' as a random factor.

## **Results**

### **Anther architecture alone affected pollen remaining within the anthers after bee visitation**

Anther architecture significantly affected how much pollen bumble bees removed from anthers, such that 52% more pollen was left in joined versus free anthers (Fig. 3a; Table 1,S1; GLMM: effect of architecture:  $\chi^2_1 = 12.13$ ,  $P < 0.0005$ ). Relative to unvisited flowers, on average, bees left an estimated 63% of the pollen in joined anthers versus 41% in free anthers (Table 1). Neither bee body size nor flower handling time significantly affected how much pollen was left in anthers (Fig. 3a,b; Table 1,S1; Table S1; GLMM: effect of body size:  $\chi^2_1 = 0.55$ ,  $P = 0.46$ ; effect of handling time:  $\chi^2_1 = 0.021$ ,  $P = 0.89$ ; effect of architecture X body size:  $\chi^2_1 = 0.33$ ,  $P = 0.57$ ; effect of architecture X handling time:  $\chi^2_1 = 1.98$ ,  $P = 0.16$ ; effect of body size X handling time:  $\chi^2_1 = 1.78$ ,  $P = 0.18$ ; effect of architecture X body size X handling time:  $\chi^2_1 = 0.0001$ ,  $P = 0.99$ ).

Table 1

Quantity of pollen (mean  $\pm$  SE) acquired by bee body parts, remaining in anthers, and transferred to stigmas among anther architecture treatments

Treatment	On the head	On the thorax	On the abdomen	In the corbiculae	In anthers (est. initial: 612450 $\pm$ 19017)	On stigmas
Free	1341 $\pm$ 237	23921 $\pm$ 3256	8683 $\pm$ 1578	211697 $\pm$ 20452	252900 $\pm$ 24095	3933 $\pm$ 1055
Joined	868 $\pm$ 155	15254 $\pm$ 1615	4846 $\pm$ 522	163949 $\pm$ 16120	383540 $\pm$ 34421	3220 $\pm$ 691

## Anther architecture and body size affected pollen collection and pollen available for pollination

Pooled across treatments and counted after trials, bumble bees acquired on average ( $\pm$  SE) 216,808  $\pm$  14,317 pollen grains from flowers, with 0.5% on the head, 9% on the thorax, 3% on the abdomen, and 87% collected into the pollen baskets (corbiculae) (Table 1). The quantity of pollen bees collected into their corbiculae was significantly affected by anther architecture, with bees foraging on free anthers collecting on average 29% more pollen into their corbiculae (Fig. 4a; Table 1,S2; GLMM: effect of architecture:  $\chi^2_1 = 4.11$ ,  $P < 0.043$ ). Additionally, this effect depended significantly on bee size, such that smaller bees collected less on joined anthers versus on free anthers (Fig. 4a; Table S2; GLMM: effect of architecture X body size:  $\chi^2_1 = 4.72$ ,  $P < 0.03$ ; effect of body size:  $\chi^2_1 = 7.43$ ,  $P < 0.007$ ). Flower handling time did not significantly affect pollen collection (Fig. 4b; Table S2; GLMM: effect of architecture X handling time:  $\chi^2_1 = 0.48$ ,  $P = 0.49$ ; effect of body size X handling time:  $\chi^2_1 = 0.015$ ,  $P = 0.90$ ; effect of architecture X body size X handling time:  $\chi^2_1 = 2.28$ ,  $P = 0.13$ ).

Anther architecture also significantly affected the quantity of pollen available for pollination (i.e., pollen not in the corbiculae), such that on average bees foraging on free anthers had 62% more pollen on their bodies available for pollination (Fig. 4c; Table 1,S3; GLMM: effect of architecture:  $\chi^2_1 = 5.61$ ,  $P < 0.018$ ). Among body parts, the average bee foraging on free anthers had 54%, 57%, and 79% more pollen on its head, thorax, and abdomen (Table 1). This effect became significantly more pronounced as bee body size increased (Fig. 4c; Table S3; GLMM: effect of architecture X body size:  $\chi^2_1 = 4.15$ ,  $P < 0.042$ ; effect of body size:  $\chi^2_1 = 0.45$ ,  $P = 0.50$ ). Flower handling time did not affect how much pollen was available for pollination (Fig. 4d; Table S3; GLMM: effect of handling time:  $\chi^2_1 = 4.15$ ,  $P = 0.068$ ; effect of architecture X handling time:  $\chi^2_1 = 0.28$ ,  $P = 0.60$ ; effect of body size X handling time:  $\chi^2_1 = 1.61$ ,  $P = 0.20$ ; effect of architecture X body size X handling time:  $\chi^2_1 = 0.55$ ,  $P = 0.46$ ).

The proportion of pollen on the body of the bee available for pollination (on average, 17% of the pollen) was significantly influenced by anther architecture, but effects differed with body size (Fig. 5a; Table 1,S4;



GLMM: effect of architecture X body size:  $\chi^2_1 = 13.11$ ,  $P < 0.0003$ ; effect of architecture:  $\chi^2_1 = 0.00$ ,  $P = 0.99$ ; GLMM: effect of body size:  $\chi^2_1 = 3.95$ ,  $P < 0.047$ ). For bees on joined anthers, the proportion of pollen available for pollination declined with increasing body size, while on free anthers the proportion available for pollination increased. Flower handling time did not significantly affect the proportion of pollen available for pollination (Fig. 5b; Table S4; GLMM: effect of handling time:  $\chi^2_1 = 1.33$ ,  $P = 0.25$ ; effect of architecture X handling time:  $\chi^2_1 = 0.05$ ,  $P = 0.83$ ; effect of body size X handling time:  $\chi^2_1 = 0.79$ ,  $P = 0.37$ ; effect of architecture X body size X handling time:  $\chi^2_1 = 0.65$ ,  $P = 0.42$ ).

## **Anther architecture and body size affected pollen receipt by the stigma**

Pollen deposition on the stigma by bees was significantly affected by anther architecture, but the effect depended on the quantity of pollen on the body available for pollination (Fig. 6a; Table 1, S5; GLMM: effect of architecture X body pollen:  $\chi^2_1 = 5.30$ ,  $P < 0.021$ ). When bees carried relatively less pollen on their bodies, receipt was less for flowers with joined versus free anthers; the opposite pattern was observed when bees carried relatively more pollen. Overall, bees foraging on free anthers deposited 22% more pollen on stigmas than bees on joined anthers (Fig. 6a, Table 1). Regardless of anther architecture, pollen receipt by the stigma depended significantly on bee body size, with pollen receipt decreasing as body size increased (Fig. 6a,b; Table 1, S5; GLMM: effect of body size:  $\chi^2_1 = 4.83$ ,  $P < 0.028$ ). There was no effect of flower handling time on pollen receipt by the stigma (Fig. 6c; Table S5; GLMM: effect of handling time:  $\chi^2_1 = 1.93$ ,  $P = 0.165$ ).

## **Discussion**

Our study elucidates how the functional significance of a common and widespread shift in anther architecture (Fig. 1) can be profoundly affected by pollinator body size. This is not unexpected, because pollination effectiveness is often thought to depend substantially on the physical fit between flower and pollinator (Herrera 1987; Minnaar et al. 2018; Moreira-Hernandez and Muchhala 2019). We found that even a modest difference in bee body size (varying by a factor of 1.3) greatly affected benefits to the plant in terms of pollen transfer to the stigma and pollen on the bee body available for pollination (i.e., not held within the pollen baskets), as well as benefits to the bee in terms of pollen collected into the pollen baskets. Consistent with our initial hypothesis, relatively smaller bees were better pollinators for flowers with joined anther architecture, while relatively larger bees were better pollinators for flowers with free anther architecture. Specifically, smaller bees deposited more pollen on stigmas overall, and when foraging on flowers with joined anther architecture, absolutely and proportionally (relative to pollen in the pollen baskets) more pollen was available for pollination on smaller bee bodies. Conversely, larger bees foraging from free anther architecture acquired absolutely and proportionally more pollen available for pollination than smaller bees. Finally, smaller bees collected less pollen into their pollen baskets when foraging from joined, but not free anther architecture. Altogether, our results suggest that to understand

the functional effects of floral trait evolution, pollinator characteristics should be taken into consideration.

Multiple mechanisms could account for how bee body size affects the movement of pollen. One possibility is that pollen removal from the anthers by bees, and thus the quantity of pollen that can be transferred to conspecific stigmas or collected by the bee, is a function of bee body size. Indeed, body size can be associated with differences in foraging behaviour (e.g., Jauker et al. 2016; Stout 1999; Russell et al. 2021), including characteristics of floral buzzing predicted to affect pollen removal (e.g., Corbet et al. 2014; De Luca et al. 2013, 2014, 2019; Switzer et al. 2019). Yet bee body size did not affect pollen removal from the anthers in our study. Instead, the quantity of pollen deposited on the bee was affected by body size, with bigger bees overall carrying and collecting more pollen. This result suggests that body size likely affects how much pollen can be intercepted by the bee (affecting pollen wastage; see Vallejo-Marín et al. 2022). However, despite carrying more pollen, larger bees transferred less pollen to flower stigmas, indicating that smaller bees in our study may have been more size matched to flower reproductive anatomy (see Solis-Montero and Vallejo-Marín 2017). Future studies that exploit greater variation in bee body size – and in particular, smaller bees – will be required to determine whether size matching between bee and flower can explain patterns of pollen transfer better than bee body size alone (see also Willmer and Finlayson 2014; Konzmann et al. 2020).

Transfer of pollen to conspecific stigmas is thought to frequently depend on spatial segregation of pollen on the pollinator (e.g., Muchhala and Thomson 2012; Tong and Huang 2017; Minnaar et al. 2018; Russell et al. 2021). While joined anther architecture has been proposed to result in more precise pollen placement on the bee relative to free anther architecture (e.g., Harder and Barclay 1994; Vallejo-Marín et al. 2022), our results were not consistent with these expectations. Pollen was spatially segregated on bee bodies, with more than twice as much pollen available for pollination present on the thorax, versus on the head and abdomen combined (Table 1). However, the proportion of pollen acquired among body parts did not differ among bees foraging on one versus the other anther architecture. Additionally, no particular bee body part contributed disproportionately to pollen transfer to conspecific stigmas for either anther architecture. One possible explanation for this latter pattern is that bees had insufficient time between flower visits to groom pollen more completely. Poorly groomed parts of pollinator bodies (safe sites; e.g., the midline of the dorsal head and thorax and ventral and dorsal abdomen) are often key to pollination (Herrera 1987; Buchmann et al. 1990; Huang et al. 2015; Koch et al. 2017; Tong and Huang 2017), but excess and easily groomed pollen might reduce the influence of spatially segregated safe sites on patterns of pollen movement. Perhaps consistent with this, in this study the quantity of pollen on the bee body available for pollination relative to the quantity of pollen transferred to stigmas was large (nearly 9 times greater). Modification of anther architecture could also have had other effects on pollen transfer that we were unable to quantify. For example, joined anther architecture strongly reduces the spatial separation between the anthers and the stigma (herkogamy), and reductions in herkogamy are predicted to increase self-pollination (Webb and Lloyd 1986; Opedal 2018). Future research could therefore examine whether shifts in anther architecture alter the likelihood of outcrossing.

Prior studies have predicted that joined anther architecture evolved to more effectively release pollen and reward bees, and experimental work with simulated bee buzzes has found that joined anther architecture increases pollen release (Glover et al. 2004; Vallejo-Marín et al. 2022). In contrast, we find that joined anther architecture overall decreases both pollen release from the anthers (by 34%) and pollen collection by the bee into its pollen baskets (by 23%). Bee behaviour likely at least partially accounts for the discrepancy with earlier work: bees buzzed and released pollen from multiple anthers on visits to flowers with free anther architecture, whereas simulated buzzes were applied to only a single anther. Other possible explanations include that joined anther architecture caused bees to buzz with reduced effectiveness or in suboptimal locations on the anther cone (see De Luca and Vallejo-Marín 2013; Jankauski et al. 2022). Additionally, although pollen transfer to the stigma is also decreased (by 18%) for flowers with joined vs free anther architecture, we tentatively suggest that the proportionally greater retention of pollen by joined anthers more than offsets the reduction in pollen transfer, by resulting in more pollen being available for subsequent pollinators to transfer to conspecific stigmas. However, this prediction will require experimental validation and, as above, the relative benefits of joined anther architecture to the plant likely depend on the relative size of its bee pollinators.

In conclusion, assuming our results are broadly representative, even modest differences in bee body size have the potential to drive selection for different anther architectures. Given that interspecific differences in bee body size are often substantial within a given pollinator community (e.g., Solis-Montero and Vallejo-Marín 2017; Mesquita-Neto et al. 2021; Cullen et al. 2021), selection for different anther architectures may be common. Accordingly, patterns in the distribution and frequency of plant species with joined and free anther architecture, which offer pollen as a reward, likely reflect the degree to which physical characteristics of the bee community vary, such as occurs in the context of floral traits associated with nectar rewards (e.g., Harder 1985; Kaiser-Bunbury et al. 2014; Klumpers et al. 2019; Sponsler et al. 2021). Furthermore, given that the degree to which anthers may be joined can be less discrete than in our study (see Faegri 1986; Russell et al. 2016; Vallejo-Marín et al. 2022), effects on pollination and foraging success may be variable. Finally, while the present study focuses on how pollinator body size influences the movement of pollen, behaviour and especially learning is key to affecting how pollinators interact with different flower morphologies (e.g., Laverty 1994; Papaj et al. 2017; Russell et al. 2021). A more complete understanding of the functional effects of shifts in anther architecture will therefore require addressing the influence of both pollinator morphology and behaviour simultaneously.

## Declarations

### ACKNOWLEDGEMENTS

We are grateful to Koppert Biologicals for bee colonies, Steve Buchmann for seeds, Abilene Mosher for greenhouse care, Jenny Burrow for assistance with pollen counting, and Russell lab members for discussion. We acknowledge this work was performed on unceded traditional territory of the Kiikaapoi, Sioux, and Osage.

## FUNDING

Not applicable

## CONFLICTS OF INTEREST

Not applicable

## ETHICS 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

## DATA AND CODE ACCESSIBILITY

The code and datasets supporting this article are available as electronic supplementary material.

## References

1. Barrett SCH (2010) Darwin's legacy: the forms, function and sexual diversity of flowers. *Philos Trans R Soc B* 365:351–368. doi: 10.1098/rstb.2009.0212
2. Betts MG, Hadley AS, Kress WJ (2015) Pollinator recognition by a keystone tropical plant. *PNAS* 112:3433–3438. doi: 10.1073/pnas.1419522112
3. Brito VLG, Nunes CEP, Resende CR, Montealegre-Zapata F, Vallejo-Marín M (2020) Biomechanical properties of a buzz-pollinated flower. *Royal Soc Open Sci* 7:1–9. doi: 10.1098/rsos.201010
4. Buchmann SL, Shipman CW, Hansen HW (1990) Pollen residing in safe sites on honey bee foragers. *Am Bee J* 130:798–799
5. Cardinal S, Buchmann SL, Russell AL (2018) The evolution of floral sonication, a pollen foraging behavior used by bees (Anthophila). *Evolution* 1–11. doi: 10.1111/evo.13446
6. Cariveau DP, Nayak GK, Bartomeus I, Zientek J, Ascher JS, Gibbs J, Winfree R (2016) The allometry of bee proboscis length and its uses in ecology. *PLoS ONE* 11:e0151482. doi: 10.1371/journal.pone.0151482
7. Corbet SA, Huang S-H (2014) Buzz pollination in eight bumblebee-pollinated *Pedicularis* species: does it involve vibration-induced triboelectric charging of pollen grains? *Ann Bot*. doi: 10.1093/aob/mcu195

8. Couvillon MJ, Jandt JM, Duong N, Dornhaus A (2010) Ontogeny of worker body size distribution in bumble bee (*Bombus impatiens*) colonies. *Ecol Entomol* 35:424–435. doi: 10.1111/j.1365-2311.2010.01198.x
9. Cullen N, Xia J, Wei N, Kaczorowski R, Arceo-Gómez G, O'Neill E, Hayes R, Ashman T-L (2021) Diversity and composition of pollen loads carried by pollinators are primarily driven by insect traits, not floral community characteristics. *Oecologia*. doi: 10.1007/s00442-021-04911-0
10. Dafni A (1992) *Pollination ecology: a practical approach*. Oxford University Press, Oxford
11. Darwin C (1877) *The various contrivances by which orchids are fertilised by insects*, 2nd ed., revised. New York, USA: D. Appleton and Company
12. De Luca PA, Vallejo-Marín M (2013) What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. *Curr Opin Plant Biol* 16:1–7. doi: 10.1016/j.pbi.2013.05.002
13. De Luca PA, Bussière LF, Souto-Vilaros D, Goulson D, Mason AC, Vallejo-Marín M (2013) Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. *Oecologia* 172:805–816. doi: 10.1007/s00442-012-2535-1
14. De Luca PA, Cox DA, Vallejo-Marín M (2014) Comparison of pollination and defensive buzzes in bumblebees indicates species-specific and context-dependent vibrations. *Naturwissenschaften* 101:331–338
15. De Luca PA, Buchmann S, Galen C, Mason AC, Vallejo-Marín (2019) Does body size predict the buzz-pollination frequencies used by bees? *Ecol and Evol* 1–13. doi: 10.1002/ece3.5092
16. Endress PK (2012) The immense diversity of floral monosymmetry and asymmetry across angiosperms. *Bot Rev* 78:345–397. doi: 10.1007/s12229-012-9106-3
17. Faegri K (1986) The solanoid flower. *Trans. & Proc. Bot. Soc. Edinburgh* 45:51–59 doi: 10.1080/03746608608684993
18. Fox J (2015) *Applied regression analysis and generalized linear models*, 3rd edn. Sage Publications Inc., London
19. Fukuda Y, Suzuki K, Murata J (2001) The function of each sepal in pollinator behavior and effective pollination in *Aconitum japonicum* var. *montanum* *Plant Species Biol* 16:151–157
20. Glover BJ, Bunnewell S, Martin C (2004) Convergent evolution within the genus *Solanum*: the specialised anther cone develops through alternative pathways. *Gene* 331:1–7. doi: 10.1016/j.gene.2004.01.027
21. Harder LD, Barclay RMR (1994) The functional significance of poricidal anthers and buzz pollination: controlled pollen removal from *Dodecatheon*. *Funct Ecol* 8:509–517
22. Harder LD, Wilson WG (1994) Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants. *Evol Ecol* 8:542–559
23. Harder LD (1985) Morphology as a predictor of flower choice by bumble bees. *Ecology* 66:198–210
24. Hartig F (2018) *DHARMA: Residual diagnostics for hierarchical (multilevel/mixed) regression models*. R package version 0.1.6. Available from: [https:// cran.r- proje ct. org/ web/ packa ges/](https://cran.r-project.org/web/packages/)

25. Hazlehurst JA, Karubian JO (2016) Nectar robbing impacts pollinator behavior but not plant reproduction. *Oikos* 000:1–9. doi: 10.1111/oik.03195
26. Herrera CM (1987) Components of pollinator “Quality”: comparative analysis of a diverse insect assemblage. *Oikos* 50:79–90
27. Hodges SA, Fulton M, Yang JY, Whittall JB (2003) Verne Grant and evolutionary studies of. *Aquilegia* *New Phyt* 161:113–120. doi: 10.1046/j.1469-8137.2003.00950.x
28. Hopkins R, Guerrero RF, Rausher MD, Kirkpatrick M (2014) Strong reinforcing selection in a Texas wildflower. *Curr Biol* 24:1995–1999. doi: 10.1016/j.cub.2014.07.027
29. Huang Y-S, Nong S-Y, Li X-K, Xie G, Tong Y-H (2022) *Vaccinium bangliangense*, a new species of Ericaceae from limestone areas in Guangxi, China. *PhytoKeys* 194:23–31. doi: 10.3897/phytokeys.194.81018
30. Huang Z-H, Liu H-L, Huang S-Q (2015) Interspecific pollen transfer between two coflowering species was minimized by bumble fidelity and differential pollen placement on the bumblebee body. *J Plant Ecol* 8:109–115. doi: 10.1093/jpe/rtv015
31. Jankauski M, Ferguson R, Russell A, Buchmann S (2022) Structural dynamics of real and modelled *Solanum* stamens: implications for pollen ejection by buzzing bees. *J Royal Soc Interface* 19:1–10. doi: 10.1098/rsif.2022.0040
32. Jauker F, Speckmann M, Wolters V (2016) Intra-specific body size determines pollination effectiveness. *Basic Appl Ecol* 17:714–719. doi: 10.1016/j.baae.2016.07.004
33. Johnson SD, Anderson B (2010) Coevolution between food-rewarding flowers and their pollinators. *Evol : Educ Outreach* 3:32–39. doi: 10.1007/s12052-009-0192-6
34. Kaiser-Bunbury CN, Vázquez DP, Stang M, Ghazoul J (2014) Determinants of the microstructure of plant – pollinator networks. *Ecology* 95:3314–3324
35. Kelemen EP, Skyrn K, Dornhaus A (2022) Selection on size variation: more variation in bumble bee works and in the wild. *Insectes Soc* 1–6. doi: 10.1007/s00040-022-00850-y
36. Klumpers SGT, Stang M, Klinkhamer PGL (2019) Foraging efficiency and size matching in a plant–pollinator community: the importance of sugar content and tongue length. *Ecol Lett* 22:469–479. doi: 10.1111/ele.13204
37. Knapp S, Sagona E, Carbonell AKZ, Chiarini F (2017) A revision of the *Solanum elaeagnifolium* clade (Elaeagnifolium clade; subgenus *Leptostemonum*, Solanaceae). *PhytoKeys* 84:1–104. doi: 10.3897/phytokeys.84.12695
38. Koch L, Lunau K, Wester P (2017) To be on the safe site – . ungroomed spots on the bee’s
39. body and their importance for pollination. *PLoS ONE* 12:1–16doi: 10.1371/journal.pone.0182522
40. De Kock C, Minnaar C, Lunau K, Wester P, Verhoeven C, Schulze MJ, Randle MR, Robson C, Bolus RH, Anderson B (2018) The functional role of the keel crest in *Polygala myrtifolia* (Polygalaceae) and its effects on pollinator visitation success. *S Afr J Bot* 118:105–111. doi: 10.1016/j.sajb.2018.06.011

41. Konzmann S, Hilgendorf F, Niester C, Rech AR, Lunau K (2020) Morphological specialization of heterantherous *Rhynchanthera grandiflora* (Melastomataceae) accommodates pollinator diversity. *Plant Biol* 1–8. doi: 10.1111/plb.13102
42. Lavery TM (1994) Bumble bee learning and flower morphology. *Anim Behav* 47:531–545
43. Li J-K, Song Y-P, Xu H, Zhang Y-W, Zhu J-Y, Tang L-L (2015) High ratio of illegitimate visitation by small bees severely weakens the potential function of heteranthery. *J of Plant Ecol* 8:213–223. doi: 10.1093/jpe/rtv021
44. Lichtenberg EM, Irwin RE, Bronstein JL (2018) Costs and benefits of alternative food handling tactics help explain facultative exploitation of pollination mutualisms. *Ecology* 99:1815–1824. doi: 10.1002/ecy.02395
45. Macior LW (1968) Pollination adaptation in *Pedicularis groenlandica*. *Am J Bot* 55:927–932
46. Magnusson A, Skaug H, Nielsen A, Berg C, Kristensen K, Maechler M, Bentham K von, Bolker B, Sadat N, Lüdecke D, Lenth R, O'Brien J, Brooks M (2018) *glmmTMB: generalized linear mixed models using template model builder*. R package version 1.0.2.1. Available from: <https://cran.r-project.org/web/packages/glmmTMB/index.html>
47. Mesquita-Neto JN, Viera AL, Schlindwein C (2021) Minimum size threshold of visiting bees of a buzz-pollinated plant species: consequences for pollination efficiency. *Am J Bot* 108:1–10. doi: 10.1002/ajb2.1681
48. Minnaar C, Anderson B, Jager MLd, Karron JD (2018) Plant–pollinator interactions along the pathway to paternity. *Ann. Bot.* doi: 10.1093/aob/mcy167
49. Moreira-Hernández JI, Muchhala N (2019) Importance of pollinator-mediated interspecific pollen transfer for angiosperm evolution. *Annu Rev Ecol Evol Syst* 50:191–217. doi: 10.1146/annurev-ecolsys-110218-024804
50. Muchhala N, Thomson JD (2012) Interspecific competition in pollination systems: costs to male fitness via pollen misplacement. *Func Ecol* 26:476–482
51. Nevard L, Russell AL, Foord K, Vallejo-Marin M (2021) Transmission of bee-like vibrations in buzz-pollinated plants with different stamen architectures. *Sci Rep* 11:1–10. doi: 10.1038/s41598-021-93029-7
52. Opedal ØH (2018) Herkogamy, a principal functional trait of plant reproductive biology. *J Integr Plant Biol* 179:677–687. doi: 10.1086/700314
53. Papaj DR, Buchmann SL, Russell AL (2017) Division of labor of anthers in heterantherous plants: flexibility of bee pollen collection behavior may serve to keep plants honest. *Arthropod-Plant Interact* 00:1–9. doi: 10.1007/s11829-017-9497-5
54. Parker AJ, Tran JL, Ison JL, Bai JDK, Weis AE, Thomson JD (2015) Pollen packing affects the function of pollen on corbiculate bees but not non-corbiculate bees. *Arthropod-Plant Interact* 9:197–203. doi: 10.1007/s11829-015-9358-z
55. R Development Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. (Austria)

56. Rasband WS, ImageJ USNational Institutes of Health, Bethesda, Maryland, USA, <https://imagej.nih.gov/ij/>,1997–2021
57. Russell AL, Golden RE, Leonard AS, Papaj DR (2016) Bees learn preferences for plant species that offer only pollen as a reward. *Behav Ecol* 27:731–740. doi: 10.1093/beheco/arv213
58. Russell AL, Buchmann SL, Papaj DR (2017a) How a generalist bee achieves high efficiency of pollen collection on diverse floral resources. *Behav Ecol* 00:1–13. doi: 10.1093/beheco/arx058
59. Russell AL, Morrison SJ, Moschonas EH, Papaj DR (2017b) Patterns of pollen and nectar foraging specialization by bumblebees over multiple timescales using RFID. *Sci Rep* 7:1–13. doi: 10.1038/srep42448
60. Russell AL, Fetters AM, James EI, Ashman TL (2021) Pollinator effectiveness is affected by intraindividual behavioral variation. *Oecologia*. doi: 10.1007/s00442-021-05016-4
61. Schiestl FP, Johnson SD (2013) Pollinator-mediated evolution of floral signals. *TREE* 28:307–315. doi: 10.1016/j.tree.2013.01.019
62. Silberglied RE (1979) Communication in the ultraviolet. *Annu Rev Ecol Evol Syst* 10:373–398
63. Solis-Montero L, Vallejo-Marin M (2017) Does morphological fit between flowers and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. *Ecol & Evol* 1–10. doi: 10.1002/ece3.2897
64. Sponsler D, Kallnik K, Requier F, Classen A, Maihoff AF, Sieger J, Steffan-Dewenter I (2022) Floral functional traits of mountain bumble bees are constrained but flexible through elevation and season. *Oikos* 1–12. doi: 10.1111/oik.05876
65. Stout JC (1999) Does size matter? Bumblebee behavior and the pollination of *Cytisus scoparius* L (doi: Fabaceae). *Apidologie* 31:129–139
66. Switzer CM, Russell AL, Papaj DR, Combes SA, Hopkins R (2019) *Curr Zool* 65:425–436. doi: 10.1093/cz/zoz013
67. Tong Z-Y, Huang S-Q Safe sites of pollen placement: a conflict of interest between plants and bees? *Oecologia*(2017)1–9doi: 10.1007/s00442-017-3999-9
68. Vallejo-Marín M (2019) Buzz pollination: studying bee vibrations on flowers. *New Phyt* 1–7. doi: 10.1111/nph.15666
69. Vallejo-Marín M, Nunes CEP, Russell AL (2022) Anther cones increase pollen release in buzz-pollinated *Solanum* flowers. *Evolution* 1–15. doi: 10.1111/evo.14485
70. van der Kooi CJ, Dyer AG, Kevan PG, Lunau K (2019) Functional significance of the optical properties of flowers for visual signalling. *Ann Bot* 123:1–14. doi: 10.1093/aob/mcy119
71. Vlašánková A, Padyšáková E, Bartoš M, Mengual X, Janečková P, Janeček S (2017) The nectar spur is not only a simple specialization for long-proboscid pollinators. *New Phyt* 215:1574–1581. doi: 10.1111/nph.14677
72. Vogel S(1978) Evolutionary shifts from reward to deception in pollen flowers. In *The pollination of flowers by insects* (ed Richards AJ), pp 89–96



73. Webb CJ, Lloyd DG (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. *N. Z. J Bot* 24:163–178. doi: 10.1080/0028825X.1986.10409726
74. Willmer PG, Finlayson K (2014) Big bees do a better job: intraspecific size variation influences pollination effectiveness. *J Pollinat Ecol* 14:244–254

## Figures

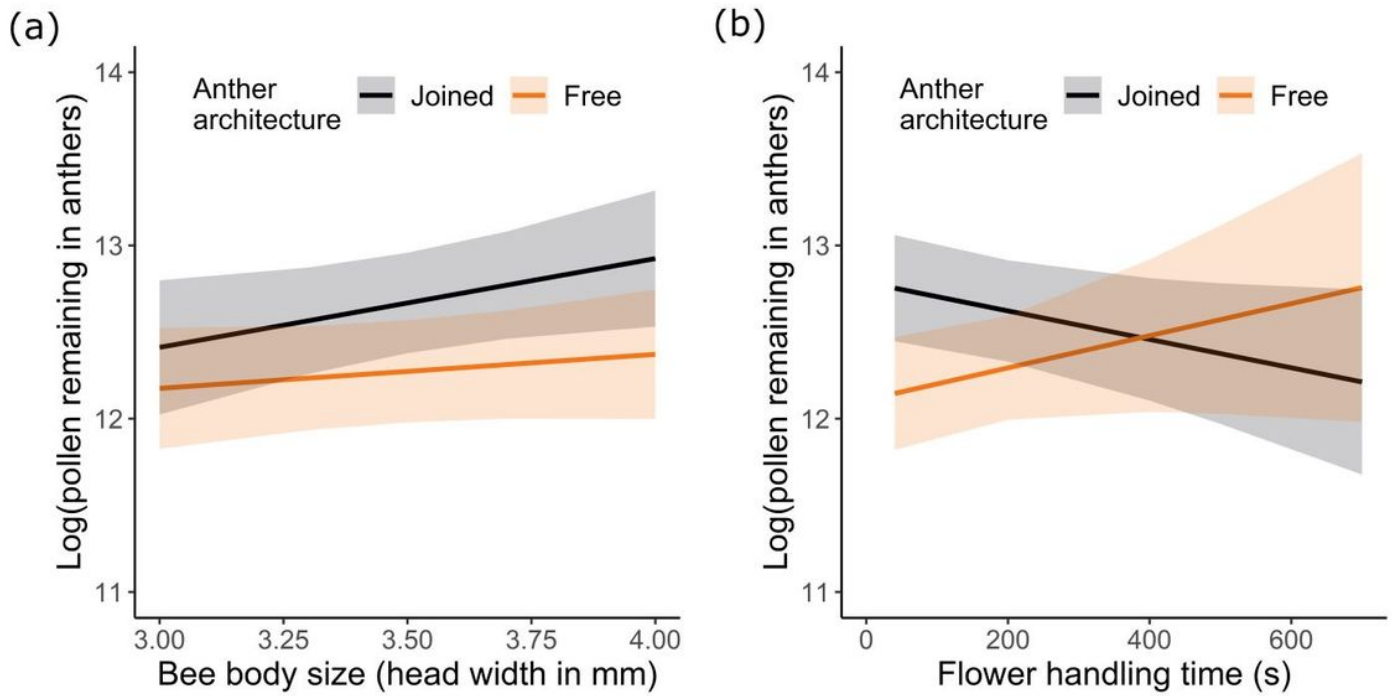
### Figure 1

Examples of poricidal plant species with (a, c, e) joined and (b, d, f) free anther configurations. (a) *Solanum ossicruentum*, (b) *Solanum sturtianum*, (c) *Dodecatheon conjugens*, (d) *Daniella revoluta*, (e) *Hodgsoniola junciformis*, (f) *Cheiranthra borealis*. Photographs: (a) Avery Russell, (b) Mark Marathon, (c) Kevin Thiele, (d) Michael Keogh, (e) Walter Siegmund, (f) Michael Jefferies. (d-f) licensed by CC BY-NC 2.0, (b-c) CC BY-SA 3.0 .



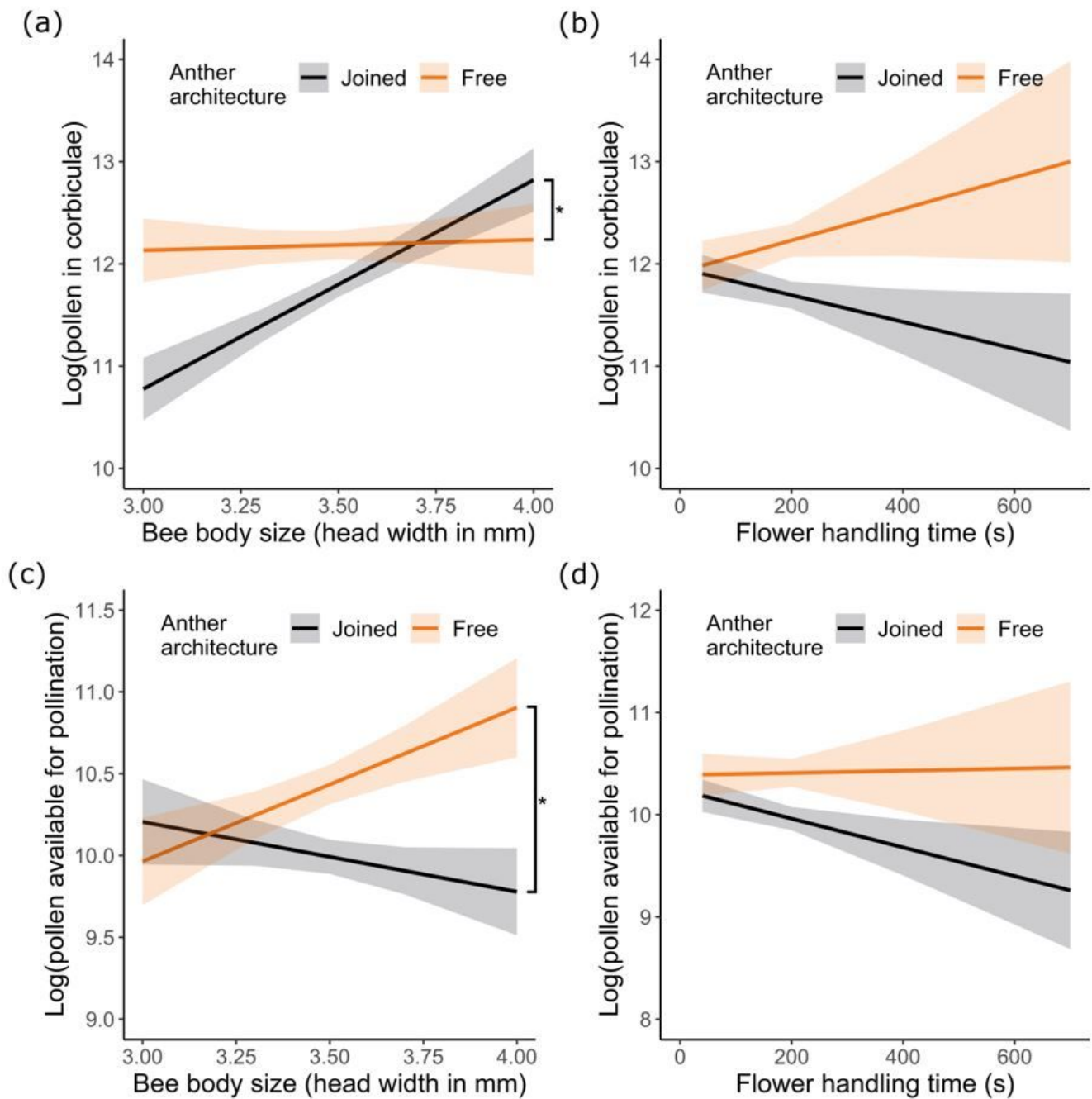
### Figure 2

Profiles of the two types of flowers used in treatments. (a) The natural “free” configuration of anthers and (b) the modified “joined” configuration of anthers. In both configurations the style is centrally located among the anthers. The background was digitally removed.



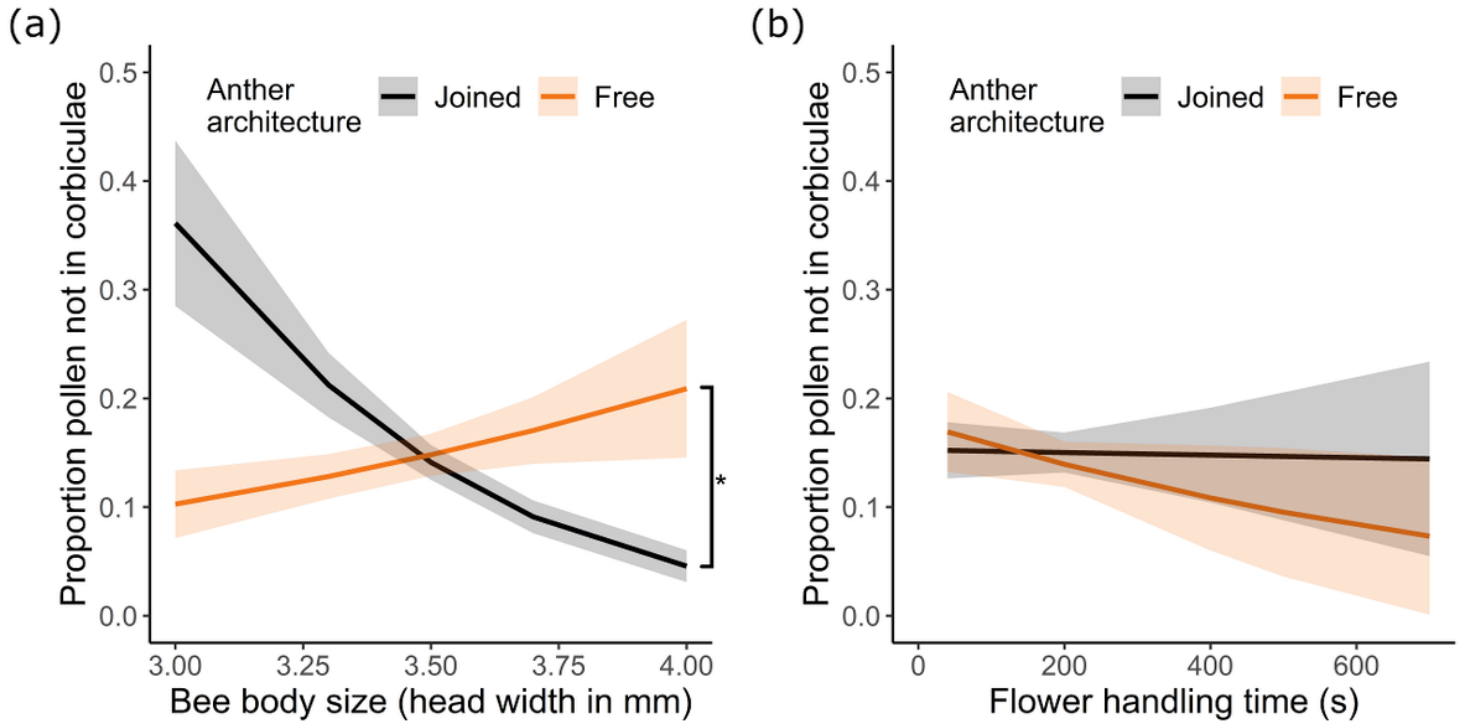
**Figure 3**

The quantity of pollen remaining in anthers as influenced by anther architecture and (a) bee body size or (b) total time spent handling flowers.  $N = 28$  and  $32$  bees for free and joined anther treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate standard errors.



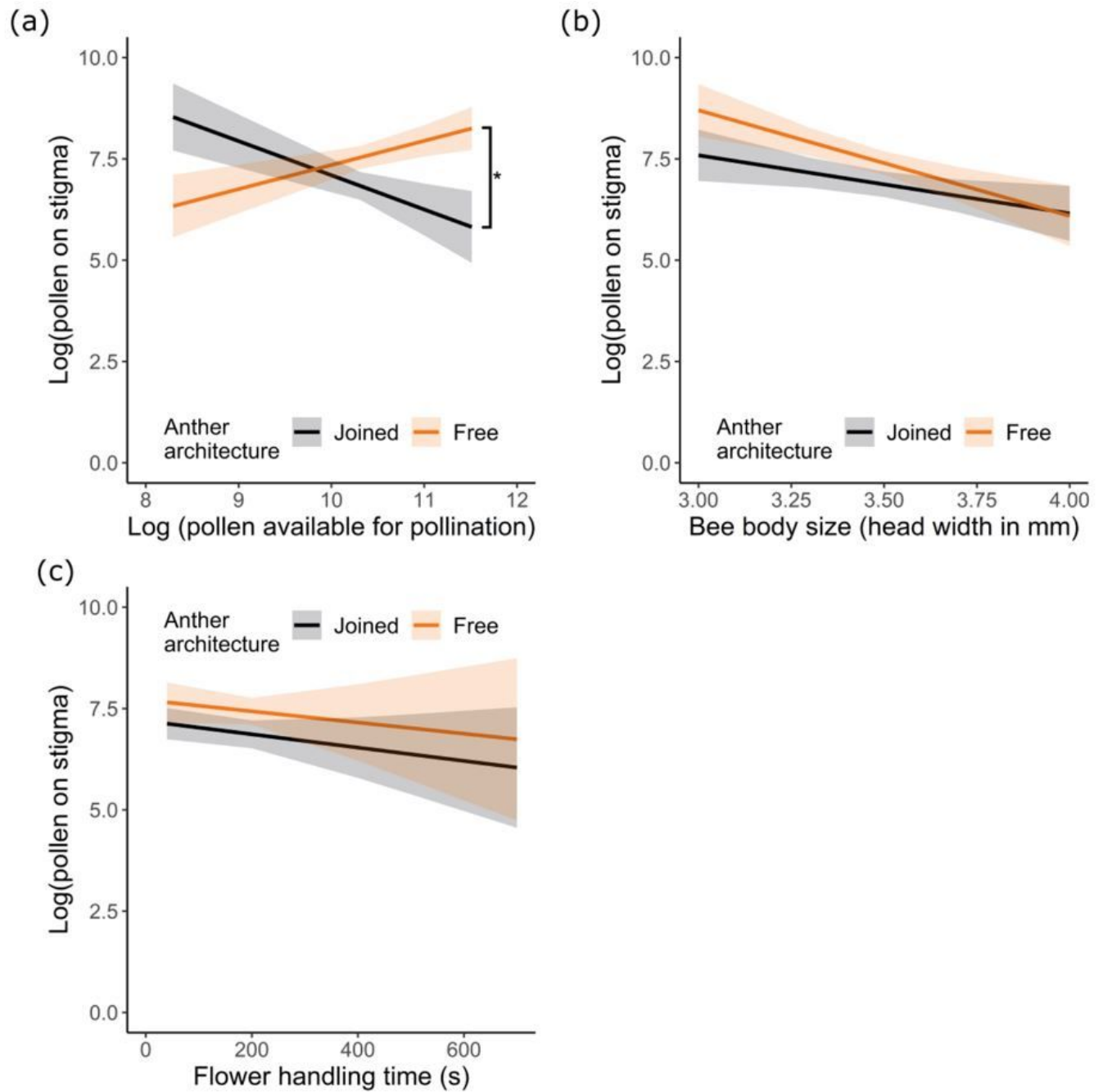
**Figure 4**

The quantity of pollen in the pollen baskets (corbiculae) or on the body (excluding corbicular pollen) as influenced by anther architecture and (a, c) bee body size or (b, d) total time spent handling flowers.  $N = 28$  and 32 bees for free and joined anther treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate standard errors. Asterisks indicate significant differences in the mean pollen in the corbiculae or available for pollination between treatments at  $P < 0.05$ .



**Figure 5**

The proportion of pollen in the pollen baskets (corbiculae) versus on the rest of the bee body as influenced by anther architecture and (a) bee body size or (b) total time spent handling flowers.  $N = 28$  and 32 bees for free and joined anther treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate standard errors. Asterisks indicate significant differences in the mean proportion of pollen not in the corbiculae between treatments at  $P < 0.05$ .



**Figure 6**

The quantity of pollen transferred to flower stigmas as influenced by anther architecture and (a) pollen on the body available for pollination (excluding corbicular pollen), (b) bee body size, and (c) total time spent handling flowers.  $N = 28$  and  $32$  bees for free and joined anther treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate standard errors. Asterisks indicate significant differences in the mean pollen transferred to recipient stigmas between treatments at  $P < 0.05$ .

## Supplementary Files

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- [AnalysisDataallestpollencountsround1182021.csv](#)
- [Antherarchbeemssupplementary4232022.docx](#)